



# AntiMal News



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Issue 3

## African Competitive Call



CNRFP team with Dr Souleymane Sanon, second left



UCT team with Professor Kelly Chibale, front row, first on left



AiBST team with Dr Collen Masimirembwa, second left

The AntiMal project has a long-term goal of improving research capacity in African institutions, and to this end has funding earmarked to strengthen malaria drug research in malaria-endemic countries in Africa. A competitive call was launched in May 2007, targeted at African scientists who are able to contribute to strengthening the research capacities and infrastructure of their host African institution. AntiMal's coordinating institution, the Liverpool School of Tropical Medicine (LSTM) worked in collaboration with the WHO and its malaria research capacity-building arm MIM/TDR to manage the competitive call. This initiative aims to develop original and innovative lines of research in drug discovery and development, establish or improve the required infrastructure, and generate strong collaborative links with European partners. The

goal is to build clearly sustainable capacity in the long term through support at the highest level within the collaborating institutions.

The call was advertised widely, and the applications were evaluated through a four stage process. As a result of this initiative, three new groups will join the consortium from 1st June 2008.

Dr Souleymane Sanon from the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) in Burkina Faso will lead a team investigating the antimalarial activity of medicinal plants used by traditional healers in Burkina Faso. A number of plants used to treat malaria by traditional healers have been identified by the CNRFP group. Antimalarial assays will be set up and extracts will be tested with a view to identifying the active molecules.

Professor Kelly Chibale from the

University of Cape Town (UCT) in South Africa and Dr Collen Masimirembwa from the African Institute of Biomedical Science & Technology (AiBST) in Zimbabwe will work together to improve preclinical pharmacology in the medicinal chemistry of lead drug discovery and optimisation. Testing of drug candidates to determine efficacy, mode of action, metabolism and safety is essential to the drug discovery process. Early testing of molecules can guide further chemical synthesis to select and improve potential new antimalarial drugs. These groups will improve facilities and expertise to test potential antimalarial molecules generated at UCT and AiBST, as well as within the AntiMal consortium.

We welcome these three new groups into the AntiMal consortium which will increase the number of partners to 37.

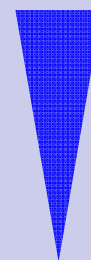
## Workshop on Good Laboratory Practice and Good Clinical Practice (GLP/GCP)



This workshop was run at the School of Tropical Medicine in Liverpool on the 24<sup>th</sup>, 25<sup>th</sup> and 26<sup>th</sup> July 2007. The one-day GLP course introduced delegates to the principles of Good Laboratory Practice and provided the opportunity for participants to understand the importance of GLP compliance, its relevance to clinical research and how to apply the requirements of GLP into their everyday work. The course covered topics such as roles and responsibilities, training, facilities, standard operating procedures, quality assurance, validation, reports and keeping records.

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## GLP/GCP Workshop continued

The two day GCP course provided delegates with a comprehensive and practical insight into the principles of GCP. Topics covered included the role of regulatory agencies and the clinical monitor, responsibilities of the investigator and the sponsor, documentation and data management. This

course was delivered from both an industrial and an academic perspective by presenters with experience of running clinical trials for malaria treatments.

The workshop was attended by 30 delegates from a wide range of backgrounds

and nationalities within Europe, Africa and Asia. Questionnaire feedback showed a positive response from delegates, with the interactive nature of the GLP course being particularly well received.

## PhD Student Projects Spotlight

**Archana Kaniti** is from India and is currently working in the laboratory of Dr Patrick Bray at the Liverpool School of Tropical Medicine. Archana is looking at the antimalarial mode of action and specific targeting of novel bis-cationic drugs.

**Archana explains the rationale behind her project** "Work from the Vial laboratory in Montpellier has demonstrated very potent activity of a variety of bis-cationic molecules against malaria. Recently, the Vial group has focussed on bis-quaternary ammonium molecules although other bis-cations such as alkyl amidines and guanidines are also under development. Although the exact target is not known at present, all these compounds are known to inhibit the synthesis of phosphatidyl choline in the parasite. This prevents the synthesis of new membranes in the rapidly growing trophozoite and therefore kills the parasite. In Liverpool we have developed a range of similar compounds based on the bis-benzyl amidine structure. Interestingly, these compounds appear to work in a very different way to the bis-cations developed by the Vial group. The Liverpool compounds act more like quinoline drugs i.e. by binding to heme and interfering with the production of hemozoin crystals in the parasite. I am interested to find out why these drugs, which although structurally very similar, appear to have such a different mode of action to that of the Vial compounds".

In fact, there is some evidence that bis-quaternary ammonium compounds can also bind to heme, a fact that has not escaped Archana: "Part of my project involves investigating the heme-binding properties of bis-quaternary ammonium compounds and alkylamidines from the Vial group. We will measure accurately the strength of the interaction with heme and measure the ability of these compounds to inhibit the production of hemozoin crystals. These results will be compared to results I have already obtained with the Liverpool compounds



At the moment there are a couple of possibilities; the Vial compounds may have a dual mode of action, targeting both hemozoin crystallization and phosphatidyl choline synthesis or alternatively the heme binding may just act as a drug reservoir mechanism to drive the drug uptake to very high levels in the parasite. Preliminary work seems to support the latter explanation because the Vial compounds do not always inhibit the crystallization of heme even though they may bind to it".

**What about the possibility of drug resistance?** One of the big problems with developing drugs that target heme is the possibility of cross-resistance developing with existing heme-binding drugs such as chloroquine. "I am not really concerned about that" said Archana: "I have looked at the activity of our new compounds against genetically modified parasite lines engineered to carry either the mutations responsible for chloroquine resistance or the wild-type genes for comparison. Rather than getting cross-resistance I find that the chloroquine-resistant lines are actually more sensitive to the new compounds. Part of my project involves investigating the mechanism behind this and I have obtained some evidence that mutant PfCRT (a transporter protein) from chloroquine resistant parasites is effectively transporting our new diamidine drugs into the digestive vacuole of the parasite, where they can interact with the heme target. Actually I am con-

vinced that heme is still an excellent target, it is not altered in chloroquine resistant parasites and my work shows that it may be possible to hijack the resistance mechanism to target more drugs against resistant parasites".

**What about bioavailability?** Another potential problem with doubly charged drugs is poor transmembrane permeability and poor oral bioavailability as exemplified by existing diamidines such as pentamidine. Again, Archana is confident that these problems can be overcome; "The Vial group has been experimenting with the prodrug approach, using chemical substitutions to mask the charge and make the compounds more lipophilic. Once absorbed the compounds are metabolised to the active drug by enzymes in plasma. We are taking the same approach in Liverpool, using a number of well-characterized prodrug substitutions and I am just about to start testing some of the new compounds *in vivo*, it's an exciting time. I am also interested in the transport of these highly charged compounds into the parasite itself. A further objective of my project is molecular characterisation of a putative *Plasmodium falciparum* organic cation transporter that we suspect may be acting as a conduit for bis-cationic drugs. I have identified a gene in *Plasmodium falciparum* based mainly on bioinformatic comparison with organic cation transporters in other organisms. Currently I am trying to clone the gene into *E.coli* to confirm the sequence and then I am planning to do functional studies in *Xenopus* oocytes. Hopefully these studies might give us some information on how these relatively impermeable drugs are accumulating to high levels, specifically inside malaria parasites."

Archana particularly enjoys working in the new "state of the art" laboratories at the Liverpool School of Tropical Medicine and is looking forward to further collaboration with Dr Henri Vial at the Centre National de la Recherche Scientifique, in Montpellier France.



## PhD Student Projects Spotlight (Continued)



**Abdirahman Abdi** from Kenya is working in the laboratory of Professor Christian Doerig at the Wellcome Centre for Molecular Parasitology, University of Glasgow on a project to evaluate *Plasmodium falciparum* protein kinases enzymes as potential drug targets. This project is run in collaboration with Professor Keith Wilson at the University of York.

Abdi explained "My host laboratory has been interested for a number years in protein phosphorylation in *P. falciparum*. In addition to fundamental research objectives aimed at understanding the role of protein kinases in the parasite's life cycle, the group also has an "applied" objective, which is to identify *Plasmodium*-specific protein kinase inhibitors that could represent leads for novel antimalarials. The rationale for targeting the parasite protein kinases is based on the facts

(i) that protein kinases (PKs) are prime targets for chemotherapy in cancer and other diseases, and (ii) that the divergence between plasmodial PKs and

those of their human host suggests selective inhibition might be achieved".

*In silico* studies of the entire complement of PK-coding genes in the *P. falciparum* genome revealed that although the *P. falciparum* kinome includes members of most major groups of the eukaryotic protein (ePK) kinase families, tyrosine kinases (TKs) are not represented. This family of PKs play important roles as receptors and upstream regulators in several signalling cascades in metazoans. They are a major drug target for diseases such as cancer, with several TK inhibitors already in clinical use as chemotherapeutic agents. The parasite possesses, however, a family of five protein kinases that cluster within the tyrosine kinase-like kinase (TKL) group of ePKs; these enzymes may fulfil roles similar to those played by TKs in higher eukaryotes in the sensor/upstream part of signalling pathways. The *Tetrahymena thermophila* and *Dictyostelium discoideum* kinomes include TKLs but no TKs, which is consistent with the view that TKLs evolved in unicellular organisms, perhaps serving as antecedent to the TKs.

The aims of the PhD project are at characterising the parasite TKLs at three levels:

**Biochemical level:** we will attempt to clone and express the TKLs as soluble and active recombinant proteins.

This will enable us (i) to gather information on the biochemical regulation of their activity *in vitro*; (ii) to generate

tools for protein-protein interaction studies aimed at identifying binding partners in parasite extracts (pull-downs); and (iii) to develop inhibition assays that may serve as the basis of a high throughput screening procedure to identify inhibitors in chemical libraries.

**Function level:** we will attempt to determine the role of these enzymes in the parasite's life cycle through a reverse genetics approach, and thus validate them (or not) as potential drug target.

**Structural level:** we will attempt to solve the 3-D structure for those TKLs that can be expressed as soluble, active recombinant enzymes and play an essential role in the parasite's life cycle, in order to provide a basis for rational drug design.

In the first 18 months of his PhD, Abdi has successfully managed to produce two of the enzymes as soluble and active recombinant proteins, and to show that the activity of one of these is dependent on a SAM ("Sterile Alpha Motif") domain located on a N-terminal extension of the enzyme. He also obtained evidence that at least two of the PfTKLs are dispensable for asexual growth.

Abdi is continuing his work with Professor Doerig at Glasgow looking at the biochemical and functional studies before going to the University of York to focus on the structural aspects of the project under the supervision of Professor Wilson.

## Project Management Workshop

A project management workshop was held in Nairobi, Kenya on the 19<sup>th</sup> to 21<sup>st</sup> September 2007. The aim of the workshop was to introduce the principles, process and tools of project management in order to manage projects more effectively. The workshop was delivered in an interactive format by professional trainers with a unique and effective style. Topics included effective decision making, influencing skills, managing team meetings and personal effectiveness. A project involving the construction of a rollercoaster (see photo right) was used as a theme throughout the course to reinforce the principles of managing projects as a team.

A total of 22 participants attended the workshop including consortium members from Kenya (IPR, ACCT, the University of Nairobi, COSMOS and KEMRI), Italy, France and the UK. The wide scope of delegates from different institutions allowed the opportunity to network and foster new links. Questionnaire feedback demonstrated that a valuable learning experience was had by all, with most delegates giving top marks to the content, delivery and the ability of the workshop to meet objectives and expectations.





The AntiMal management staff at the Liverpool School of Tropical Medicine moved in February 2008 to the Centre for Tropical and Infectious Diseases (CTID), a new state of the art, environmentally designed building.



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## AntiMal Project Review

The goal of the AntiMal project is to establish a portfolio of new antimalarial drugs from within the European scientific community and to select candidates from within the portfolio for progression to "first into man" studies within the 5 year programme. The original portfolio contained 25 scientific workpackages arranged in 5 thematic clusters:-

Cluster 1 - Novel quinolines

Cluster 2 - Novel peroxides

Cluster 3 - Lipid targets

Cluster 4 - Other targets

Cluster 5 - Post candidate selection

An external scientific advisory committee (ESAC) was established to assess the scientific progress of the individual workpackages on an ongoing basis. The committee consists of nine experts in the field of malaria and drug development who are independent of the scientific research funded by AntiMal.

A review meeting was held in Liverpool in September 2007 to assess the progress of the scientific workpackages over the first 22 month period of the project in line with the goal of achieving progression to "first into man" studies. The result of the review was the ongoing funding of 12 of the original 25 scientific workpackages as follows:-

### Cluster 1 – Novel quinolines

WP 4.1.1.1 – Novel 4-aminoquinolines 1 - Professor Steve Ward, LSTM.

WP 4.1.1.2 – Novel 4-aminoquinolines 2 - Professor Donatella Taramelli, UMIL.

### Cluster 2 – Novel peroxides

WP 4.1.2.1 – Synthetic peroxides - Professor Paul O'Neill, UOL.

WP 4.1.2.4 – Natural endoperoxides from marine organisms - Professor Giuseppe Campiani NatSyn.

### Cluster 3 – Lipid targets

WP 4.1.3.1 – Discovery and selection of an orally-potent bithiazolium antimalarial - Dr Henri Vial, CNRS.

WP 4.1.3.2 – Prodrugs of alkyl amidine as orally potent antimalarials - Dr Henri Vial, CNRS.

WP 4.1.3.3 – Orally available diamidines - Dr Pat Bray, LSTM.

### Cluster 4 – Discovery

WP 4.1.4.1 – Protein kinase targets: validation, screening and structure - Professor Christian Doerig, INSERM.

WP 4.1.4.5 – Development of novel bisarylurea compounds with high efficacy against *Plasmodium falciparum* - Professor Michael Lanzer, UOH.

WP 4.1.4.6 – Transporters - Professor Sanjeev Krishna, SGHMS.

WP 4.1.4.7 – Novel chalcones as antimalarials - Dr Arsalan Kharazmi, LICA Pharmaceuticals.

### Cluster 5 – Platform Activities

WP 4.1.5.8 – Platform for efficacy studies and potential drug combinations to delay resistance development – Dr Livia Vivas, LSHTM.

During 2007 an additional three African partners joined the AntiMal consortium with the aim of increasing the volume and diversity of the AntiMal portfolio. This resulted in the addition of 2 new workpackages which were not reviewed by ESAC in September 2007. These workpackages will continue for the duration of the AntiMal project.

Additional workpackages:-

WP TTC1 – novel antifolates - Dr Alexis Nzila, KEMRI, Kenya

WP TTC2 – natural product derived antimalarials - Dr Chris Parkinson, CSIR in South Africa.

The new rationalised portfolio of 14 workpackages will focus the resources more specifically on achieving the aim of the project. The role of ESAC has been crucial to this process and a further review is planned in March 2009 (see events below).

## Coming Events

**AntiMal Management Committee Meeting, 22nd October 2008, Montpellier, France**

**Second AntiMal ESAC Review Meeting, 3-4 March 2009, Liverpool, United Kingdom**