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## AFRICAN MALARIA NETWORK TRUST

The AMANET Workshop on Molecular Biology and Immunology in Malaria Vaccine Development and the Makerere University-University of California at San Francisco (MU-UCSF) Malaria Symposium

*26-30 June 2006 Kampala, Uganda*

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### Workshop Report

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#### **Introduction**

The Faculty of Medicine, Makerere University and the African Malaria Network Trust (AMANET) held a five day malaria conference at Hotel Africana, Kampala, Uganda on 26-30 June 2006. The conference comprised of a two-day Symposium on Malaria organized by Makerere University-University of California at San Francisco (MU-UCSF), Malaria Collaboration and Uganda Malaria Surveillance Program (UMP). This was followed immediately by a three-day AMANET workshop on Molecular Biology and Immunology on Malaria Vaccine Development.

The MU-UCSF/UMP symposium focused on malaria treatment, control and research priorities while the AMANET workshop discussed current state-of affairs vis-à-vis immunological aspects of malaria vaccine development. Over 100 individuals participated in the event. Thirty-four (34) of these participants were sponsored by AMANET representing Burkina Faso, Cameroon, Gabon, Gambia, Ghana, Kenya, Mali, Nigeria, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe.

Also present were various partners and stakeholders in the field of malaria control including officials from the Uganda Ministry of health, Researchers from Universities/ Institutes, Malaria Consortium, World Health Organization, US President's Malaria Initiative (PMI), the US National Institutes of Health (NIH), and Epicentre (France).

#### **Day 1**

The workshop was officially opened by the Dean of the Faculty of Medicine, Makerere University who was represented by Professor Elly Katabira, also standing in for Vice Chancellor. Prof Katabira welcomed all participants from the various parts of the world and highlighted the plight of malaria in Uganda and Africa at large. He also encouraged useful deliberations between researchers and policy makers in view of promoting research policies that will translate into better control tools. Official opening of the workshop was followed by introductory remarks from the MU-UCSF Director, Dr Moses Kanya, and presentations by Drs Albert Kilian (Malaria burden

and control) and Phil Rosenthal (MU-UCSF Results and ongoing studies). In his presentation, Dr Kilian revealed the increasing trend in the malaria cases in Uganda over the years with more than 70,000 children dying from malaria every year. Dr Rosenthal talked on MU-UCSF achievements in research, training and capacity building as evidenced by the number of papers published and individuals trained both in Uganda and the U.S.

The Symposium continued with more presentations by Dr Wabwire Mangen and Dr Adoke Yeka on MU-UCSF Results and ongoing studies. Besides highlighting current projects, the presentations discussed published data on malaria drug resistance in Uganda. The data showed increasing resistance to Chloroquine as well as Fansidar with a conclusion that risk of treatment failure with CQ + SP was unacceptably high at all sites. It was also noted in this talk that a combination of Amodiaquine (AQ) + SP was much more efficacious than CQ + SP. It was further revealed that artemether/lumefantrine and artesunate/amodiaquine have been found to be more efficacious than other drug combinations. The presentation concluded that for ACTs to have maximum desired effect, a combination with other malaria control measures for reducing transmission intensity is vital.

An overview of the Malaria Consortium activities was presented by Ms Nakanwagi. The vision of Malaria Consortium is to become a recognized international resource, creating regional centres of expertise in malaria endemic areas, working together with partners to control malaria and other diseases to achieve better health. The consortium was said to be actively involved in operational research, malaria control through indoor residual spraying (IRS) and long lasting treated nets research, and research in other diseases like leishmaniasis, diarrhoeal and pneumonia.

Subsequent presentations were on *malaria drug policy in Uganda* (JB Rwakimari); *the challenges in making drug policy changes* (Kato), *home based management of fever strategy* (HBMF, by Sarah Staedke); *update on the US President's Malaria Initiative* (PMI, by Linda Quick), and an *Official opening of the MU-UCSF/UMSP Malaria Symposium overview of malaria research priorities in Uganda* (Grant Dorsey).

## **Day 2**

Day two kicked off with a presentation by Dr Ambrose Talisuna on *Training Health Workers, Operational Research: from policy makers to good medical practice using ACTs*. This talk discussed the need for training, the process and time it takes to change policy comparing and contrasting Uganda's experience to that of other countries in Africa.

Dr Rosenthal then presented and discussed preliminary findings from a study that compared *combination antimalarial therapies among Ugandan children*. As part of these findings, Dr Njama Denise looked at *presumptive anti-malarial treatment of febrile episodes among children*, a practice widely advocated in Africa.

Recommendations from this study were that in the era of ACT, resources to expand the use of microscopy or other validated diagnostic methods may provide a cost effective measure for promoting rational use of antimalarial therapy.

Two presentations focused on *HIV/Malaria interactions*. The first talk was given by Dr Gasasira Anne who reviewed the current literature in this field. Dr Achan Jane then discussed findings of a study on *interactions* between HIV and malaria in children.

The study shows that the use of ITNs and septrin prophylaxis is effective in lowering the incidence of malaria in HIV positive patients. The findings also hinted on the fact that providing integrated interventions in areas heavily infected by malaria and HIV is crucial for reducing the burden of the two diseases.

The afternoon session covered topics on molecular biology and malaria vaccine immuno-epidemiology. A series of presentations were made each with a key take home message. Dr Dorsey presented a paper on the use of molecular genotyping to improve estimates of anti-malarial treatment. Dr Hakim Ssendagire gave an overview of molecular studies done to monitor levels of resistance to Chloroquine and *Fansidar* at a site in central Uganda. Dr Sam Nsobya discussed the molecular markers for drug resistance and concluded that studies of molecular markers may complement in vivo studies by helping policy makers in the planning of anti-malarial treatment guidelines. Other presentations in this session were on chemokine biomarkers and polymorphisms in falciparum malaria in Ugandan children (Dr Tom Egwang), and immuno-epidemiological studies of natural IgG responses to *P. falciparum* vaccine candidates in Apac (Dr Brenda Okech).

The final session had two presentations; Dr Heidi Hopkins discussed the increasing importance of RDTs in the diagnosis of malaria especially in rural areas. Dr Gisela Schneider presented an *Overview of the Joint Malaria Training Programme (JMTP)*, detailing the goal, objectives and envisaged contributions of the programme in the fight against malaria in Uganda through improved case management and effective use of intervention tools such as ACT, IPT, ITN and IRS.

The MU-UCSF symposium was wrapped up by Dr Kanya who reviewed the presentations made over the two days and emphasized the need for continued research to guide policy.

### **Day 3**

The AMANET workshop on Molecular Biology and Immunology in Malaria Vaccine development began with the introduction of all participants and facilitators. Dr Roma Chilengi then briefly explained the goal and objectives of the workshop and the scope of activities being undertaken by AMANET. This was followed by a lively discussion with the participants calling on AMANET to extend its activities to include

other sites. After the warming introduction, Professor Mats Wahlgren (Karolinska Institute, Sweden) presented an overview of malaria immunity. Dr Adrian Luty (Radboud University, Netherlands) then gave a talk on *T-cell immunity*. T-cell immunity was said to play an important role in the elimination of exo-erythrocytic parasites via CD8+ T-cells with help from CD4+ T-cells. CD4+ T-cells were also said to be effective in controlling asexual blood stage parasite multiplication possibly through cytokine mediated hyper-regulation of phagocytic cell activity.

A presentation on *B-Cell immunity* was then given by Dr Fred Kironde (Makerere University, Uganda). Dr Kironde said that the degree of immunity is believed to be correlated with antibody titres and IgG subclasses. The mechanisms behind this action was said to involve blockage of merozoite invasion, enhancement of parasite clearance through opsonisation, parasite toxin neutralisation, inhibition of cell attachment (cytoadherence), antibody dependent cell mediated cytotoxicity (ADCC) and inhibition of rosetting of parasites. Success in the development of malaria vaccine was said to be hampered by parasite ability to evade and suppress immune response (immunesuppression/immunomodulation) through antigenic diversity, variation, sequestration, etc. Later on, Prof. Wahlgren discussed Malaria immunity to severe and pregnancy malaria. It was noted that pregnancy reduces a woman's immunity to malaria, making her more susceptible to severe malaria than other adults and that malaria is a significant contributor to anaemia in pregnancy. Malaria infection of the placenta and anaemia were said to be major contributors to low birth weight and premature delivery.

The lecture on Malaria vaccine development strategy was give by Dr Qijun Chen (Karolinska Institute, Sweden). Dr Chen said malaria vaccine strategies are targeted to the prevention of infection, prevention of anti-disease manifestation and transmission blocking. The working hypothesis is to block parasite invasion pathways, disrupt parasite development process, neutralize parasite toxin, and inhibit adhesion. Several vaccines candidates were said to be undergoing some form of trials at different stage and these included attenuated parasite-irradiated sporozoites, recombinant proteins with AMA-1, MSP-1, 2, CSP, synthetic peptide (Sp66, LSP), virus-like protein particles, DNA – (plasmid, virus) and RNA (virus particle).

The first evidence of the protective role of sickle cell trait against severe malaria was by Allison in 1954 as described by Dr Francine Ntoumi (EDCTP, Netherlands) in her talk on Host genetic factors and protection from malaria: Sickle cell. The sickle cell trait was said to result from a mutation of the b-chain of the globulin due to a single gene base pair mutation (A to T) in haemoglobin genome sequence. The talk further reported that this results into reduced solubility of the deoxyhemoglobin molecule and erythrocytes assume irregular shapes. The sickled erythrocytes become trapped in the microcirculation and cause damage to multiple organs resulting in HbSS (homozygote) having a high mortality rate and HbAS (heterozygote) having a

selective advantage against mild and severe malaria. The mechanism behind the conferment of protection, as she said, with sickle cell trait is still unclear although higher levels of cellular and humoral immunity to crude or neo *P. falciparum* antigens in carriers of haemoglobin AS have also been shown with contributions to protective effects.

#### Day 4

Day four started with a talk by Dr Ntoumi on Characterization of malaria parasites. Dr Ntoumi said that parasite diversity has been described by two phenomenon; Allelic polymorphism -polymorphism within a gene (number and/or size of repeats), and Antigenic variation – which occurs during the course of an infection where the parasite expresses different forms of the same gene, for instance the var genes used to escape human host immune responses. – Importance in evaluation of malaria vaccines. It was further revealed that the malaria parasite *P. falciparum* exhibits the largest polymorphism in chromosomes structure. This calls for a need to characterize parasite genetic markers.

The talk concluded that interpretation of genotyping markers has to take into account the study design and the fluctuations of parasite populations. Relationships between infecting genotypes and malaria control interventions also need to be further investigated.

Dr Michael Theisen (Statens Serum Institut, Denmark), in his talk on the *Development of candidate malaria vaccines: Experience with synthetic peptides*, mentioned six candidate malaria vaccine targets: antisporezoite stage vaccines, antihepatic stage vaccines, anti-erythrocytic stage vaccines, antitoxic vaccines, anti-gamete ookinete stage and live attenuated stage. The peptide synthesis vaccine approach was said to be relatively inexpensive and convenient from a regulatory point of view, it lacks contamination by irrelevant foreign proteins or DNA and improves the focus of the immune system upon specific domains. Issues on purification by RP-HPLC, fast sequence analysis by mass spectrometry, improved chemistry; scaling up production, antigenicity and immunogenicity were also discussed as challenges in the process.

In his second presentation on *Development of candidate malaria vaccines: Experience with recombinant proteins*, Dr Thiesen said that recombinant proteins are used for the assessment of antigenicity using ELISA, T cell assays; Immunogenicity in mice, rabbits, monkeys; can be demonstrated for purification of specific antibodies and in clinical trials. Clinical development plans for the recombinant candidate malaria vaccine GMZ2 were also presented, as an example of a vaccine based on recombinant technology.

Dr Chen then examined *PfEMP1 as an anti-severe malaria vaccine*. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) was said to be the parasite's virulence factor associated with severe malaria. It was also noted that PfEMP1

molecules are both functionally specialised and differentially recognized. Dr Chen then discussed research on var genes responsible for the production of mediators that bind the infected red blood cells to CD36 domain of endothelial cells of blood vessels. It was also noted that research in Aotus monkeys has shown promising results as the monkeys developed only severe anaemia after infection with *P.falciparum* but not cerebral malaria. Even better results have been shown with Rhesus monkey which developed no *P. falciparum* infection.

In a presentation on *Assays for Evaluating Immune Responses*, Dr Adrian Luty discussed the importance, methods and timing of immunoassays in malaria vaccine development. Dr Luty said that the stage- and antigenspecificity of vaccine-induced responses will determine the choice and design of immunoassays. Vaccines targeting pre-erythrocytic stages of *P.falciparum* may be designed to induce both antibody (anti-sporozoite) and CMI (anti-liver stage) responses while vaccines targeting asexual or sexual stages may be designed primarily to induce antibody responses. It was also said that assays may need to take account of functional attributes of specific 'protective' responses. Regarding time, Dr Luty said that immunogenicity is usually assessed at least once after each immunization e.g. 2 or 4 weeks later and/or on the day of each subsequent boosting, as well as some months after the last boost to assess longevity of responses. Qualitative and quantitative assays and their evaluation methods were also discussed.

In his presentation on *Immunological correlates in the evaluation of malaria vaccines* Dr Daniel Dodoo (Noguchi Memorial Institute for Medical Research, Ghana) presented lessons learnt from passive transfer of IgG experiments which have shown IgG to play an important role in protection against *P. falciparum* blood stage infection. It was also said that studies have unequivocally supported a crucial role for antibodies in naturally acquired protection, the specificities and mechanism(s) of action of these protective antibodies remains largely unknown.

Professor Wen Kilama talked on the Role of public-private-partnership (PPP) in malaria vaccine development. He mentioned that progress in malaria control has been achieved elsewhere but not in Sub Saharan Africa, and as of late, malaria morbidity and mortality is on the increase. The problem has been associated with drug and insecticide resistance, weakened health systems and poor environmental management to mention but a few. Malaria vaccine research and development was said to be a lengthy and costly exercise attracting no interest to current market forces. It was also noted that recently there has been increase in funding malaria R&D from governments, bilateral and multilateral donors, philanthropy and NGOs. This now calls for a careful and determined public-private partnership throughout, and the involvement and recognition of responsibilities by all stakeholders (scientists, politicians, the media, funders, pharmaceutical agent, NGOs, etc) and at all stages.

## Day 5

Day five kicked off with lectures towards human use. In his lecture on Clinical evaluation of candidate malaria vaccines, Dr Chilengi said that basic pre-clinical guidelines should aim at defining the characteristics, safety and immunogenicity of the product in appropriate animal models. He further said that adequate animal studies should be done and there must be close collaboration between preclinical and clinical teams. Good manufacturing practice (GMP) principles were also discussed. Regarding evaluation of malaria vaccines, Dr Chilengi said that the current convention is to take the products through a series of phases to assess safety first in non immune populations, then to endemic-areas followed by assessment of immunogenicity and efficacy. Clear go-no-go criteria must be set up. The vaccine development roadmap was also presented and said to be a long and costly process.

In a presentation on *Standardized assessment of immunogenicity/immune responses in preclinical studies and vaccine trials in Africa* Dr Daniel Dodoo said that the primary goal of malaria vaccine development is to reduce morbidity and mortality caused especially by *P. falciparum* among children in Africa. It was said that traditional techniques exist for the evaluation of specific immunological responses i.e. ELISA, IFA, Cytokine CTL assay, ELISPOT assay, etc. In many studies/trials today, there have been differences in methodologies. These differences in immunological assay protocols have made data comparison difficult hence the need for standardization of antibody and T-cell assays to enable comparison of immunological measurements in immunoepidemiological studies and vaccine trials. To address this, the AMANET sponsored Afro-immuno Assay (AIA) develops standardized assays using the same reagents and statistical tools to assess the relationship between acquisition of malaria specific antibody responses to four potential malaria vaccine candidate antigens. The objectives of AIA were also presented and these included development and introduction of standardized immunological assays that could form part of a set of criteria for the validation of promising malaria vaccine candidate antigens, provision of essential baseline information for clinical trials and enhancement of quality assured laboratory capacity and capability.

Dr Luty later gave a lecture on Adjuvants: giving the right boost to malaria vaccines. In this talk an adjuvant was said to be a substance that, in combination with a specific antigen, produces more immunity than the antigen alone i.e. an immuno-potentiating agent. Examples of licensed adjuvants used in the US (Aluminium salts [AlOH; AlPO<sub>4</sub>], MF59, oil-in-water emulsion, component of Chiron's flu vaccine, comprising squalene & surfactants- Tween 80/Span85) and Europe (Virosomes; reconstituted influenza virosomes, IRIV, Berna Biotech and Cholera toxin B subunit-used in a cholera vaccine licensed by PowderJect, UK) were presented and discussed. Their mechanism of action were also presented and discussed. The following adjuvants were said to be currently in use for malaria vaccines in development; Alum (alhydrogel, various commercial suppliers e.g. Superfos, Denmark); ASO<sub>2</sub> (GSK proprietary adjuvant; oil-in-water with MPL (Corixa Corp) & QS- 21 (Antigenics Inc.); ISA 720 (SEPPIC,

Montanide); water-in-oil with vegetable oil & emulsifier [mannide monooleate]; ISA 51 (SEPPIC, Montanide); water-in-oil with mineral oil & emulsifier [mannide monooleate]; CpG (manufactured by Coley Pharma); different types of CpG for stimulation of e.g. plasmacytoid DC, B cells, NK cells, etc.

The last presentation was made by Prof Kilama who discussed *Ethical* perspectives on malaria research for *Africa*. Prof Kilama said that research is needed to sharpen existing blunt antimalarial tools, to discover, develop and deploy new antimalarial tools, and to bridge the 10/90 gap. However, research must answer to the health needs of study populations. It was further said that historically researchers have abused research participants hence the need to protect research them. The presentation then discussed the three pillars in health research ethics which are informed consent (in the African settings), standards of care and its dilemmas, and benefits and risks.