This three day event will discuss aspects of Malaria control, infection and treatment in an informal academic setting. This year there are three main topics for discussion

Vector Control: Research, Economics and Policy
Immunology and Vaccination
Malaria Drug Development and Resistance Control

With plenty of opportunity for networking and debate, this informal international meeting will bring you up to date with current research and thinking regarding Malaria.

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Day 1: Vector Control: Research, Economics and Policy

Invited Speakers Abstracts

Availability and Affordability of Artemisinin Combination Therapies: do the subsidies work?

*Miss Giulia Boselli*, Global Health Specialist- Consultant, UK

Even if malaria is a preventable and treatable disease, the death toll caused by the *Plasmodium* parasites is still tremendously high: the WHO estimates 216 million malaria cases and 655,000 deaths in 2010, of which more than 90% and 80% respectively in sub Saharan Africa. WHO recommends ACTs for the treatment of uncomplicated malaria caused by *Plasmodium Falciparum* but costs and lack in availability still prevent million people in accessing them. The Global Fund launched in 2009 the Affordable Medicines Facility-malaria with the aim of lower the price for the final buyers: analysis of pros and cons of this approach.

Possible new controlling measures for the pyrethroid-resistant malaria vectors

*Dr Hitoshi Kawada*, Associate Professor, Institute of Tropical Medicine, Nagasaki University, Japan

LLINs might not be effective in case that mosquito blood feeding takes place when people are active outside of LLINs. Screening of eaves with pyrethroid-impregnated net was found to be effective in reducing human exposure to malaria vectors in such conditions. The use of exicto-repellency of pyrethroids as a spatial repellent which acts as a spatial barrier to invasion of mosquitoes or reduces feeding motivation of mosquitoes might be another countermeasure. Combinational use of the chemicals with different modes of action in LLINs such as IGRs which have sterilizing effects to flood-fed females might be one of the future selection.

Malaria control in coastal areas - special research and policy needs

*Professor Ranjan Ramasamy*, Visiting Professor, University of Jaffna, Sri Lanka

Salinity-tolerant *Anopheles* species that transmit malaria are present in many coastal locations worldwide. Recent findings also show that the dominant South Asian fresh water malaria vector *Anopheles culicifacies*, has adapted to lay eggs and undergo pre-imaginal development in brackish water near human habitations. The significance of salinity-tolerant vectors in malaria transmission at the present time and in the context of likely future environmental changes in coastal areas, including a rise in sea levels due to global warming, is discussed. Specific policies and research needed to control malaria now and in the future along tropical coasts are outlined.

Education in the successful elimination of malaria in Palestine begun 90 years ago.

*Mr Anton Alexander* LL.B, London, UK

100 years ago, Palestine was drenched in malaria. It was either uninhabitable in many areas or thinly populated as the disease had decimated the population. But 90 years ago, Palestine became the first place in the world where a successful national malaria-eradication campaign began. Dr Kligler, a public health scientist and architect of this successful malaria anti-larval campaign, emphasised the importance of education in such a campaign, but this aspect has been forgotten and overlooked. The lesson to be learnt is that probably without education, malaria elimination is unlikely.

Oral Presentation Abstracts

**MALE FERTILITY FOLLOWING OCCUPATIONAL EXPOSURE TO DDT.**

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Dichloro-diphenyl-trichloro ethane (DDT) has shown reproductive toxicant properties in experimental and epidemiological studies. We explored the hypotesis of an impaired male fertility among subjects occupationally exposed to DDT during an anti-malarial campaign which took place in Sardinia, Italy in 1946-50. **Methods.** Among a cohort of 4,552 male workers employed in the company conduting the anti malarial campaign, we identified 1310 men born and resident in Sardinia for their lifetime. Study subjects were divided into three groups: DDT applicators (N = 464), further divided according to whether their estimated cumulative DDT exposure was below or above the median (2.25 grams), bystander (N = 399), and unexposed (N = 358). Date of marriage up to 31 12 1979 and date of birth of the first child was obtained from the population registrars at the municipality of residence. Time to pregnancy (TTP) in months for the first successful conception was based on the date of marriage, as a surrogate of date of starting unprotected intercourses, and nine months before the birth date of the first child. The fecundability ratio (FR) among spouses of DDT workers was calculated with Cox's proportional hazard modelling, adjusting by father's age at marriage, and cutting the analyses at 12 months, in four subgroups.
are generated, it is not clear when they appear and how resistant strains of the parasite reduce its effectiveness. Although there are explanations of how resistant strains of the parasite can be due to the mosquitoes being infected by infected individuals, it is necessary that they be quarantined as is done in some countries and as for some diseases that have almost zero percent incidence these days, it is clear that a lot of efforts still need to be made on combating malaria by these interventions.

BEATING MALARIA: FIVE YEARS RETROSPECTIVE STUDIES FROM A HOSPITAL IN ENUGU, EASTERN NIGERIA DISAGREES

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In Nigeria, like most of the Third World endemic regions of malaria, the actual incidence and mortality rates are unknown due to incomplete reporting, and the necessity of this data is important on monthly and yearly basis in order to monitor more closely the progress on malaria control measures. But, that has not been the case. One of the factors militating against this is the displeasing and irritating nature of the inevitable sampling technique; which is the uncomfortable means of specimen collection (finger prick, vein puncture, etc) which are repulsing to the poorly enlightened volunteers – that then becomes reluctant despite the greater importance of the research need. There is then the need for a more accurate estimate of the incidence through clinical malaria. A total of 27,100 medical records of in-and out-patients of a hospital in Enugu, Eastern Nigeria were sent to the laboratory for investigations between January 2005 and December 2009 were critically studied, whereby ages, sexes, occupations, locations and monthly plus yearly incidences of diagnosed cases of malaria were analyzed. The result revealed a yearly statistically significant steady increase in number of diagnosed cases of malaria from 2005 to 2009 (38%, 39%, 42%, 43% and 42%, in ascending order, respectively). Also, an average of 41% (11,119) incidence over the 5 years, as against 19.1% (mere 489) for all the other parasitic diseases, was also recorded. Prevalence rates were highest during the drier periods of the 5 years. > 35 years age-group has the highest rate of incidence. Likewise, females (52.17%) were diagnosed more of malaria than males (47.83%). So also were the farmers and cattle herdsmen most diagnosed of the disease. In conclusion, there is a big, serious lag in the efforts on combating malaria by these indications. When compare to so many other diseases like smallpox, measles, etc that have almost zero percent incidence these days, it is clear that lot of efforts still need to be put in place against this tropical and sub-tropical menace called malaria. Since bad habits of the semi-enlightened communities is one of the major factors unfavourable against this war, the custodians of national health should put up more efforts, even sanctions and penalties against offenders, in order to discourage their negative attitudes. Machineries should also be put in place to protect those at higher risk, who might even be the major “reservoir” of the parasites. And, it is necessary that malaria patients should be quarantined as is done in some countries and for some diseases, until they are parasitaemia-negative and not discharged when they are only physically fit. Most of the present efforts had been overconcentrated on only the mosquitoes-vectors control; it is high time this battle be also focused squarely on the Plasmodium etiologic agents too. Then, and only then, these authors are sure the world can put a full-stop to malaria.

MATHEMATICAL MODEL FOR THE SPREAD OF PYRIMETHAMINE RESISTANCE IN PLASMODIUM FALCIPARUM

Mario Cañon Ayala*, Hernando Díaz*, Vladimir Corredor Espinel** and Andrés Olarte*  
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Pyrimetamine is one of the most widely used drugs for malaria treatment, but the generation of resistant parasites reduces its effectiveness. Although there are explanations of how resistant strains of the parasite are generated, it is not clear when they appear and how the various epidemiological factors are involved.
For this reason, mathematical models are used as a tool to help evaluate these factors and to propose strategies of treatment implementation in a specific population.

In this study, a dynamic mathematical model of malaria was formulated to determine the population size for several resistance-related parasite genotypes in a population of humans and mosquitoes. The model measures the population of 16 genotypes, associated with 4 mutations of the DHFR (dihydrofolate reductase) gene. These new genotypes may appear due to mutation or genetic recombination. Furthermore, the model includes the effect of drug coverage in the population of infected humans as well as the effect of the transmission conditions (between humans and mosquitoes), on the generation of resistant strains.

Additionally, high and low transmission conditions have been simulated, since parasite genotypes are generated at different frequencies depending on these conditions. In consequence, the model takes wild parasite population (without mutations) as an initial condition and, depending on the transmission conditions, new particular genotypes are generated. The results show that in high transmission conditions resistant strains will only appear under high coverage drug treatment. On the other hand, in low transmission conditions, a low coverage drug treatment is enough to generate resistance. This approach also allows the estimation of the time when the appearance of resistant genotypes is imminent and of how it is related to different epidemiological parameters. In this way, the simulation shows the time when the Pyrimethamine treatment becomes ineffective. According to these results, it appears that the use of this treatment only is not enough to eliminate malaria and other control measures are necessary.

Finally, this model can be used as a decision tool to evaluate policies of disease control with medication, by estimating the long-term effect of the introduction of a drug in a specific population.

THE 'PADDY PARADOX' REVISITED: HOW RICE FARMING IMPACTS ON HOUSEHOLD ECONOMIC STATUS AND MALARIA RISK IN EASTERN RWANDA
A. Rulisa, F. Kaatera, C. Ingabire, E. Hakizimana, M. Vugt, L. Mutesa, L. Kempen, P.O. Box 6655 Kigali, Rwanda

Abstract

Introduction: Economic activities may entail negative externalities for public health, which is particularly problematic in poverty-stricken areas with few alternative livelihood options. The case of rice farming in eastern Rwanda fits this description, as it provides breeding sites for malaria-infested mosquitoes but at the same time generates cash income and improves nutritional standards locally. These economic benefits may in turn reduce malaria incidence through channels such as better housing and higher investment in prevention, even though people are at a higher risk due to an expansion of the mosquito population. This so-called 'paddy paradox' has been observed in a number of studies, but its prevalence is disputed.

Methods: The study unpacks the impact of rice cultivation on malaria incidence by comparing households that differ in their involvement in rice cultivation and proximity to the marshlands that host the rice fields. To this purpose, a large-scale survey was conducted among more than 4,000 households (comprising 17,000 individuals) in the area from June to December 2013. Data on household demographics, economic status, malaria prevention efforts as well as health-seeking behaviour has been collected. All household members have also been screened for malaria parasitemia and anaemia, and a malnutrition assessment was carried out for under-five children. In addition, qualitative data was collected through nine focus group discussions.

Results: It is shown that rice farming is positively and significantly associated with households’ wealth, food security, health insurance status, and protection against malaria. At the same time, it is confirmed that rice farming practices increase the risk of malaria transmission through expanded mosquito populations. Rice fields are the main breeding site in the area. Households located nearby the marshlands where rice is cultivated are the most affected by malaria. For those households who generate income from rice production directly, the income effect dominates, resulting in a lower disease burden from malaria. By contrast, households in communities that are located close to the rice cultivation areas but who do not participate in this economic activity, face a higher malaria burden.

Conclusion: Rice farming leads to private benefits in the economic domain, which spills over into the health domain, but at the same time creates a public health risk. As a result, the 'paddy paradox' hypothesis is confirmed at the level of rice-producing households, but rejected at the wider community level. Hence, strategies need to be developed that are able to tap the private benefits of rice cultivation and re-direct these to fund collective action against malaria. The paper explores various modalities that are potentially capable of doing so and discusses their financial and organizational feasibility.

Key words: malaria, rice farming, externalities, food security, mixed methods
EPITOPE MAPPING OF *Plasmodium knowlesi* MEROZOITE SURFACE PROTEIN-142 (MSP-142) USING SYNTHETIC PEPTIDE LIBRARY AND PHAGE DISPLAY LIBRARY

Fei Wen Cheong, Mun Yik Fong and Yee Ling Lau

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*Plasmodium knowlesi* is now known as the fifth *Plasmodium* species that can cause human malaria and its infection is widely distributed in Southeast Asia. The *Plasmodium* merozoite surface protein-142 (MSP-142) appears as a potential target blood stage vaccine, and for diagnosis of malaria. Potential antigenic determinants (epitopes) within *P. knowlesi* MSP-142, which are recognised by raised anti-pkMSP-142 antibodies, were identified using overlapping synthetic peptide library and phage display library. Screening of anti-pkMSP-142 antibodies with synthetic peptide library and random phage display library isolated nine and 14 peptides, respectively. Anti-pkMSP-142 antibodies exhibited high reactivity towards peptides in two regions on *P. knowlesi* MSP-142 (residues 37-95 and residues 240-289) by using both epitope mapping techniques, suggesting that the two regions could be possibly the dominant epitope regions on *P. knowlesi* MSP-142. Two of the most prominent peptides (P10 and P31) were evaluated using mouse model. Level of cytokines interferon-gamma and interleukin-2 was significantly higher in P31-immunized mice as compared to the negative control mice. Both peptide-immunized mice sera were able to recognise and react with pkMSP-142, and the IgG isotype distribution was IgG2b > IgG1 > IgG2a > IgG3. Antibodies raised against both peptides were able to recognise *P. knowlesi* blood stage parasites in immunofluorescence assays, indicating that P10 and P31 are immunogenic and could be the potential epitopes which serve as the binding sites on the blood stage parasites.

THE ROLE OF GENDER IN MALARIA PREVENTIVE BEHAVIOUR: EVIDENCE FROM RURAL HOUSEHOLDS IN MALINDI AND NYABONDO PROVINCES IN KENYA

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Malaria remains a major health and development challenge in the Sub-Saharan economies including Kenya, yet it can be prevented. Technologies to prevent malaria are available but are not universally adopted. While a number of studies have analyzed malaria preventive behavior in many developing countries, rigorous empirical work on its determinants in Kenya is scarce. We thus, examine the determinants of adoption of malaria prevention practices using a recent data-set collected in 2013 by the International Center for Insect Physiology and Ecology (ICIPE). Our study contributes to existing literature by exploring the role of gender in adoption of malaria prevention practices. We estimate separate models for male and female headed households to determine if the drivers of adoption differ between the two categories of households.

We find that access to health information, residing in villages with higher experience in malaria prevention, having knowledge on malaria transmission increase the number of practices adopted in a household. Our results further show that determinants of adoption are generally comparable between the two categories of households, except for one variable–household participation in community group, which significantly increases adoption among female headed households but has no effect among male headed households. These findings suggest that universal policy tools can be used to promote uptake of integrated malaria prevention practice, for female and male headed households. In particular, policies which increase flow of health information into households, knowledge of households about the disease (causes, and control), and interaction with adopting neighbors, will have the most pronounced effect on reducing increasing adoption of malaria prevention practices in both male and female headed households. In addition, policies that promote participation in community groups will have augment the effect on increasing adoption among female headed households.

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The malaria vector begins life as an embryo. Understanding the embryonic development of this insect may ultimately open new avenues for vector control. One of the earliest and most important steps in the development of any embryo is the process of gastrulation. It is during gastrulation that the prospective mesodermal and endodermal cells are brought onto the inside of the embryo. This process has been particularly well studied at the cellular and genetic level in the insect Drosophila melanogaster. In particular, the internalisation of mesodermal cells in D. melanogaster requires precise spatial and temporal regulation of folded gastrulation (fog) and T48 gene expression. The fog and T48 gene products then activate Rho mediated cell signalling pathways. This in turn leads to constriction of the apical side of the cells, thereby initiating the internalisation of the prospective mesoderm. This process of apical constriction and many of the molecular components involved are conserved in other morphogenetic events and in other species. However, direct homologs of fog and T48 have not been identified. We are interested in understanding the evolution of this morphogenesis pathway and have begun by identifying fog and T48 homologs in other dipterans. Sequence analysis of the identified fog homologs shows fog to be a rapidly evolving gene while T48 is evolving less rapidly. We have also analyzed the expression of fog and T48 in D. pseudoobscura embryos. Our results show T48 expression to be conserved in the prospective mesoderm of D. pseudoobscura and D. melanogaster at the onset of gastrulation. The temporal and spatial expression of fog is also conserved. However, the level of fog expression appears to be lower in D. pseudoobscura than in D. melanogaster and morphological analyses highlight differences in the dynamics of gastrulation between these two species. We have now also identified homologs of fog and T48 in the mosquito Anopheles gambiae. This insect has been reported to gastrulate differently to Drosophila and was previously thought to lack a fog homolog. We are therefore examining the cell shape changes underlying gastrulation, and the expression of fog and T48 in A.gambiae. Ultimately we hope these studies will provide insight into the evolutionary processes that shape the developmental pathways of morphogenesis including those of this important malarial vector.

MOLECULAR DIAGNOSIS AND PHYLOGENY OF MSP1 GENE OF PLASMODIUM FALCIPARUM IN HUMANS IN DEMOCRATIC REPUBLIC OF SAO TOME AND PRINCIPE

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Malaria incidence in Democratic Republic of Sao Tome and Principe (DRSTP) is decreasing after multiple intervention strategies, and the anti-malaria mission has moved forward to pre-elimination stage. This study applied methods of polymerase chain reaction (PCR) and sequencing to provide molecular evidence on malaria epidemiology. The merozoite surface protein 1 (MSP1) is the principal surface antigen of the blood stage form of the Plasmodium parasite. It has been considered a major candidate for the development of an antimalaria vaccine. In this study, blood films as well as dry blood spot papers were collected from suspected malaria cases (N=514) from year of 2011 to 2013 in Sao Tome and malaria mass screening (N=6,855) on whole island of Principe. These clinical human samples were analyzed by light microscopy (LM) and PCR. Our molecular data showed that about 3.2% (15/462) of malaria-free febrile patients in DRSTP were diagnosed as malaria positive according to gold standard method of LM. In Principe, result of nested PCR showed identical positivity rate with LM. Among these positive samples, their MSP1 genes were amplified and sequenced. Results showed that at least 3 distinct isolates were circulated in DRSTP. In conclusion, this study showed that the method of PCR plays an important role when malaria mission has
Day 2: Immunology and Vaccination

Invited Speakers Abstracts

Citrulline: A novel therapeutic for the cerebral malaria
Dr Irene Gramaglia, Associate Professor, La Jolla Infectious Disease Institute, USA
Irene Gramaglia received her Ph.D from King's College London and is currently an Associate Professor at the La Jolla Institute for Infectious Disease in San Diego, California. Her research focuses on the mechanisms of cerebral malaria pathogenesis using the mouse model of malaria. Her findings that nitric oxide bioavailability is critical for cerebral malaria pathogenesis forms the basis for much of her research. Her current research extends into pathways that can be manipulated to restore bioavailable nitric oxide and delves into alternative mechanisms by which therapeutics can protect against severe malaria in addition to effects on nitric oxide bioavailability.

Prospects for Pregnancy-Associated Malaria Vaccination Predicated on Antibody-Mediated Immunity
Professor Andrew Taylor-Robinson, Professor of Immunology/Haematology & Deputy Dean Research | School of Medical & Applied Sciences, CQ University Rockhampton, Queensland, Australia
Women with pregnancy-associated malaria exhibit severe infection and increased risk of low birth weight may cause infant death. With subsequent pregnancies and further exposure to the variant P. falciparum parasites which bind to receptors that are expressed uniquely on placental endothelium, antibodies develop earlier and women experience milder infections. A vaccine to stimulate development of antibodies would be protective against infection with wild-type parasites. This aim is hampered by significant antigen diversity and available vaccine candidates may only protect women living in malaria-endemic areas. Incremental progress is being made towards producing an effective vaccine.

Etiology and treatment of cerebral malaria
Jacob Golenser, Kuvin, Centre for the Study of Infectious and Tropical Diseases, The Hebrew University of Jerusalem-Hadassah Medical School, Israel
We suggest a possible role for brain endothelial cells (HBEC) in supporting P. falciparum development and inducing pro-inflammatory responses that are crucial in the pathogenesis of CM. Experimental CM (ECM) was diagnosed by neuroimaging using liposomal Indocyanine green. Administration of liposome-encapsulated glucocorticoids, β-methasone hemisuccinate (nSSL-BMS) resulted in selective accumulation of BMS in the brains of sick mice and reduced ECM rates. The treatment led to lower levels of cerebral inflammation, reduced hemorrhage and edema, in correlation with corresponding cytokines and chemokines. Administration of the novel anti-plasmodial artemisone, following nSSL-BMS treatment, resulted in complete cure of the malaria symptoms. Overall, because of the immunopathological nature of CM, combined immunomodulator/anti-plasmodial treatment should be considered for prevention/treatment of human CM and long-term cognitive damage.

Dendritic cells during malaria infection
Dr Pierre Guermonprez, Center for Molecular and Cellular Biology of Inflammation, King's College London, United Kingdom
Innate sensing mechanisms trigger a variety of humoral and cellular events that are essential to adaptive immune responses. Here we describe an innate sensing pathway triggered by Plasmodium infection that regulates dendritic cell homeostasis and adaptive immunity through Flt3 ligand (Flt3l) release. Plasmodium-induced Flt3l release in mice requires Toll-like receptor (TLR) activation and type I interferon (IFN) production. During infection, Flt3l preferentially stimulates expansion of the CD8α(+) dendritic cell subset or its BDCA3(+) human dendritic cell equivalent and has a substantial impact on the magnitude of T cell activation, mostly in the CD8(+) compartment. Our findings highlight a new mechanism that regulates dendritic cell homeostasis and T cell responses to infection.

TAntibody longevity to malaria vaccine candidate antigens in immuno-epidemiology studies
Dr Freya Fowkes, Head of Malaria and Infectious Disease Epidemiology, Burnet Institute, Australia
It is widely said that anti-malarial antibodies are very short-lived, but there are little published data that address this question. How antibodies to malaria vaccine candidate antigens are acquired, maintained and persist in field settings is therefore unclear. In our program of research we examine antibody levels to antigens expressed by various life-cycle stages of P. falciparum and P. vivax in multiple population-based studies. Data generated are valuable for predicting the duration of responses induced by candidate malaria vaccines and identifying issues to address in vaccine development if vaccines are to provide extended protection over many years.
Platelets do not kill blood-stage Plasmodium parasites but function in experimental cerebral malaria pathogenesis.

Dr Joyce M. Velez, Post-doctoral fellow, La Jolla Infectious Disease Institute, USA

Clinical studies indicate that thrombocytopenia correlates with the development of severe falciparum malaria. Recent studies reported that platelets kill blood-stage Plasmodium parasites, and propose that aspirin will be contra-indicated in malaria patients. We observed that thrombocytopenia does not affect parasite replication, and platelets binding to apoptotic infected RBCs were minimal. We propose that platelets are not components of the innate immune system killing malarial parasites and that the clinical use of aspirin should not be altered based on this potential mechanism. Platelets are, however, required for pathogenesis and platelet CD40 is a key molecule in the pathogenic process.

Etiology and treatment of cerebral malaria

Jacob Golenser, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University of Jerusalem-Hadassah Medical School, Israel

Cerebral malaria (CM) is a severe complication and a leading cause of death by *Plasmodium falciparum* infection. CM is likely the result of interrelated events: mechanical obstruction due to parasite sequestration in the microvasculature and up-regulation of Th1 immune responses. In parallel, blood-brain-barrier (BBB) breakdown, and damage or death of microglia, astrocytes and neurons occur. The sequestration may benefit the parasite by avoiding clearance by the spleen and allowing a preferable microaerophilic condition.

We found in a co-culture system of human brain endothelial cells (HBEC), *P. falciparum* parasitised red blood cells and peripheral blood mononuclear cells (PBMC) a novel pro-inflammatory role of HBEC operating through potentiation of production of IFNγ by PBMC, and concurrent reduction of interleukin-10. IFNγ increased the expression of CXCL10 and intercellular adhesion molecule (ICAM)-1, both of which are considered to be crucial in the pathogenesis of CM. These results support an active role for HBEC in the pathogenesis of CM. Inhibition of HBEC and PBMC interactions may either reduce the occurrence or improve the prognosis, of CM. In addition, the HBEC promoted *P. falciparum* development by releasing growth factors that are heat stable, partly chloroform stable and <3kDa MW.

CM is diagnosed in general by the presence of *P. falciparum* and behavioral changes in the host. Typical physiological alterations exist but are not used for diagnosis. Neuroimaging has been used only in elucidation of CM mechanisms. Using the *P. berghei* ANKA mouse model for experimental cerebral malaria (ECM), based on the BBB breakdown, we attempted using Indocyanine green (ICG) imaging for developing diagnostic method for CM. ICG is the only FDA-approved near-infrared (NIR) fluorescent dye for intravenous injection. However, its poor aqueous stability and short half-life in plasma limit its utility in diagnosing disease. We found enhanced emission intensity of liposomal ICG in comparison with the free ICG, probably, owing to increased stability and to the dye’s embedding within the liposomal bilayer in its monomeric form. The Liposomal ICG’s emission was greater in the brains of sick mice compared to naïve mice and drug treated mice (where CM was prevented). The liposomal ICG characteristics, together with its known biocompatibility, make liposomal ICG a valuable delivery form for *in vivo* NIR imaging of CM.

We found that several immunomodulators may decrease ECM, including a novel formulation of liposome-encapsulated glucocorticoids, β-methasone hemisuccinate (nSSL-BMS). Administration of nSSL-BMS resulted in selective accumulation of BMS in the brains of sick mice, reduced ECM rates and created a survival time-window needed for administration of an anti-plasmodial drug before severe anemia is developed. The treatment led to lower levels of cerebral inflammation, expressed in corresponding cytokines and chemokines, reduced hemorrhage and edema. nSSL-BMS effectively prevented the cerebral syndrome even if treatment was started at late stages of the disease when clear signs of neurological impairment were present. Administration of the anti-plasmodial artemisone, following nSSL-BMS treatment, resulted in complete cure of the malaria symptoms. Overall, because of the immunopathological nature of CM, combined immunomodulator/anti-plasmodial treatment should be considered for prevention/treatment of human CM and long-term cognitive damage.
Malaria is a vector-borne infectious disease caused by protozoan parasites with an estimated 300 million clinical cases every year. The most standard detection method; Giemsa-stained blood smears to detect malaria parasite in erythrocytes has low sensitivity (0.002-0.01%), time-consuming (~ 60 min) and need to technical handling. The other detection methods, immunochromatography and PCR, also have some problems in sensitivity and rapidity. Here we report a novel cell microarray chip system to detect malaria-infected erythrocytes with the high-sensitivity in a short time. The cell microarray chip is made from polystyrene with over 20,000 microchambers, which can accommodate 127 ± 6 erythrocytes. Over 2,700,000 erythrocytes were dispersed with mono-layer formation on a microarray. We could detect malaria-infected erythrocytes as low as 0.0001% with spreading of human erythrocytes on the microarray followed by staining of malaria nuclei with fluorescent dye using a microarray scanner within 15 min. Now, we are developing the system based on cell microarray chip technology including erythrocytes isolation column and exclusive fluorescence detector in cooperation with electrical appliance manufacturer. We examined 17 blood samples which were obtained from malaria patients in St. Mary's Hospital Lacor at Uganda using developing diagnosis system. Good correlation was observed between cell microarray chip method and conventional light microscopy according to simple linear regression analysis (R²=0.9222) below 2.0% parasitemia. Our developing technology based on cell microarray chip for rapid, accurate and high sensitive detection of malaria parasites can be used in the early diagnosis of malaria as well as in monitoring/surveillance of malaria control efforts in the future.

Poster Presentation Abstracts

A MOUSE MODEL OF HETEROGENEOUS IMMUNOLOGICAL RESPONSE TO MALARIA

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Malaria infection in humans elicits a wide range of immune responses that can be detected in peripheral blood, but we lack detailed long-term follow-up data on the primary and subsequent infections that lead to naturally acquired immunity. Studies on antimalarial immune responses in mice have been based on models yielding homogenous infection profiles. Here, we present a mouse model in which a heterogeneous course of Plasmodium yoelii lethal malaria infection is produced in a non-congenic ICR strain to allow comparison among different immunological and clinical outcomes. Three different disease courses were observed ranging from a fatal outcome, either early or late, to a self-resolved infection that conferred long-term immunity against re-infection. Qualitative and quantitative changes produced in leukocyte subpopulations and cytokine profiles detected in peripheral blood during the first week of infection revealed that monocytes, dendritic cells and immature B cells were increased in highly-parasitized mice dying in the first week after infection. Besides, CD4+CD25high T cells expanded at an earlier time point in early deceased mice than in surviving mice and expressed higher levels of intracellular Foxp3 protein. In contrast, survivors showed a limited increase of cytokines release and circulation of innate cells. From the second week of infection, mice that would die or survive showed similar immune profiles, although CD4+CD25high T cells number increased earlier in mice with the worst prognosis. In surviving mice is remarkable the expansion of activated circulating T cell and switched-class B cells with a long-term protective humoral response from the second infection week. In addition, correlating with the parasitemia level, it was observed an increase in total cellularity of spleen during the first week of infection that remained after 16 months of the infection in surviving animals. B cell
The Receptor for Advanced Glycation Endproducts (RAGE) is a newly identified multi-ligand receptors and a member of the immunoglobulin superfamily. It involves in the signal transduction from pathogen substrates to cell activation during the onset and perpetuation of inflammation. The activation of RAGE can initiate a series of intracellular signal transduction pathways that leads to a sustained inflammatory reaction. Many previous studies suggest that RAGE perpetuates and amplifies inflammatory reactions and targeting this receptor might help curbing the hyperinflammatory responses that occur in inflammation-associated conditions. Since malaria infection is associated with severe systemic inflammation which is characterized by massive release of pro-inflammatory cytokines such as TNFα, IFNγ, IL-1 and IL-6 etc. in the systemic circulation, we therefore investigated whether modulation of RAGE would produce any beneficial outcome during the infection.

*Plasmodium berghei* ANKA infection in ICR mice were used as a severe model of malaria infection. RAGE was modulated *in vivo* by means of treating the infected mice (i.v injection) with an antagonist (recombinant RAGE Fc chimera) and neutralizing antibody against RAGE. Effects on cytokines release and histopathological conditions of major organs (brain, liver, spleen, lungs, kidney) during the infection were evaluated upon treatment. Results showed that RAGE was upregulated especially during the late critical stage of the infection. The elevated levels of RAGE in the circulation was also found to be positively correlated with parasitaemia development. Inhibition and neutralization of RAGE in malarial mice resulted in a significant decrease of the elevated level of pro-inflammatory cytokine IFNγ as compared to the untreated malarial mice, but no significant changes were observed in the elevated levels of TNFα and IL-6. The release of anti-inflammatory cytokine IL-10, IL-4 and IL-2 levels were found to be further augmented in the treated malarial mice. Histopathological analysis on major organs revealed that inhibition and neutralization of RAGE in malaria-infected mice resulted in an improved tissue architecture, decrease in parasitized red blood cells (PRBC) sequestration, and also reduced pigmentation and inflammatory response in all organ tissues.

Findings from this study suggest that RAGE plays an important role during malaria infection. Its modulation during the infection has led to a significant impact on the profile of major cytokines release during the infection and also positive outcomes on the histopathological features of infection. Based on the current findings, RAGE may have the beneficial potential to be a target for immunotherapeutic development for malaria treatment.
EXPLORING THE PREVENTION OF MALARIA USING INSECTICIDE TREATED NETS (ITNS) THROUGH THE EXPERIENCE OF MOTHERS IN RURAL NIGERIA

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The effect of Malaria attack on maternal and child health in Nigeria is high compared with other countries in sub-Saharan Africa. This problem has been a persistent issue in Nigeria and many researchers have tried to proffer solutions. Insecticide treated nets (ITN) have been identified as providing approximately 80% protection against malaria attack. In my practice as a practicing nurse working in the hospital and in the community, it was apparent that women were particularly vulnerable to malaria infection. Malaria deaths are still estimated to 660 000 people worldwide, 10,000 maternal and 200,000 neonatal deaths yearly. In Nigeria, malaria accounts for 60% of outpatient’s visit of health facilities, 30% of childhood death, and 11% of maternal deaths. All the measures put in place to control malaria have failed to meet up with the set target of the Millennium Development Goals. It should be noted that malaria has resulted in increase in maternal and infant morbidity and mortality rate. Constant loss of pregnancy and infant death exposes a woman to emotional and psychological trauma which could eventually result in mental health problems. This study aims at enhancing understanding of the perceptions and experiences of malaria and associated care seeking and treatment, and the barriers and challenges faced by pregnant women and mothers in accessing and utilizing ITNs in Eastern Nigeria. High variation exists in awareness and utilization of ITN in Nigeria compared with increased awareness and usage in countries such as Rwanda. Strong political will and leadership as well as solid support from partners contributed significantly to their success. However, paying closer attention to the lived experience and meaning making processes of those who are among the most vulnerable could bring an additional dimension to prevention practices and to the body of qualitative research related to health and wellbeing in Nigeria.

IMMUNOGENICITY OF BACTERIAL-EXPRESSED RECOMBINANT PLASMODIUM KNOWLESI MERozoIte SURFACE PROTEIN-142 (MSP-142)

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Plasmodium knowlesi is the fifth Plasmodium species that can infect humans. The Plasmodium merozoite surface protein-142 (MSP-142) is a potential candidate for malaria vaccine. However, limited studies have focused on P. knowlesi MSP-142. A ~42 kDa recombinant P. knowlesi MSP-142 (pkMSP-142) was expressed using an Escherichia coli system. The purified pkMSP-142 was evaluated with malaria and non-malaria human patient sera (n = 189) using Western blots and ELISA. The immunogenicity of pkMSP-142 was evaluated in mouse model. The purified pkMSP-142 had a sensitivity of 91.0% for detection of human malaria in both assays. Specificity was 97.5 and 92.6% in Western blots and ELISA, respectively. Levels of cytokine interferon-gamma, interleukin-2, interleukin-4, and interleukin-10 significantly increased in pkMSP-142-immunized mice as compared to the negative control mice. pkMSP-142-raised antibody had high endpoint titres, and the IgG isotype distribution was IgG1 > IgG2b > IgG3 > IgG2a. pkMSP-142 was highly immunogenic and able to detect human malaria. Hence, pkMSP-142 would be a useful candidate for malaria vaccine development and seroprevalence studies.

Day 3: Drug Development and Resistance Control

Invited Speakers Abstracts

Malaria Drug Discovery: From genome to drug lead
Dr Glenn McConkey, Senior Lecturer, School of Biology, University of Leeds, UK
Based on genome analysis and metabolic network analysis, novel targets for malaria drugs are being discovered. Several examples of targets will be presented. Potent inhibitors of one target that we have shown to be essential for parasite growth have been developed using a computational rational drug design approach. The target is dihydroorotate dehydrogenase, required for pyrimidine biosynthesis. These inhibitors are active against parasites at levels equal to current antimalarial drugs.
Natural products as a source of new drugs and/or herbal treatments for malaria

**Dr Colin Wright**, Reader in Pharmacognosy, Bradford School of Pharmacy, University of Bradford, UK

Many plant species are used traditionally for the treatment of malaria. Since the important antimalarials, quinine and artemisinin are obtained from Cinchona species and Artemisia annua respectively, it is not unreasonable to expect that plant species may yield further potent antimalarials. Using the West African species Cryptolepis sanguinolenta as an example, the potential of natural sources to yield new antimalarials will be explored. A limitation of antimalarials such as artemisinin is that they are often not available and/or affordable to many of those who need them, especially in Africa and this has led to the promotion of herbal treatments for malaria. In this presentation the effectiveness of *A. annua* has a herbal treatment for malaria will also be discussed.

Fast tracking antimalarial drug discovery through repositioning

**Nirmalan Niroshini**, University of Salford, Manchester, UK

Drug repositioning, whereby FDA approved drugs already used in other diseases are screened for antimalarial activity, could prove a fast-tracked route to discover new antimalarial options and synergistic partner drugs. Fluorescence-based in vitro drug susceptibility assays optimized in the laboratory are used in the preliminary screens. A second research strategy focuses on the investigation of a range of natural product options for antimalarial efficacy with potential leads taken forward for bioassay guided fractionation and active compound isolation. Fractionation end points will be guided by proteomic/mass spectrometric parameters to define early perturbations secondary to drug effects.

Treating malaria at the expense of public health?

**Dr Mohga Kamal-Yanni**, Senior health & HIV policy advisor, Oxfam GB

Development of enantiomerically pure aminoalcohol quinoline derivatives to improve their antimalarial efficiency and assessment of their activity against *Plasmodium falciparum* in combination with dihydroartemisinin

**Dr Catherine Mullié**, Assistant Professor, Faculté de Pharmacie, Université de Picardie Jules Verne, France

In an attempt to reduce side-effects induced by mefloquine and try to avoid resistance, a series of enantiomerically pure amino alcohol quinoline derivatives structurally close to mefloquine but with a single asymmetric carbon has been synthesized. The activity of (S)-derivatives was compared to that of their (R)-counterparts on mefloquine-sensitive and –resistant strains of *Plasmodium falciparum*. The (S)-derivatives were found to be 2 to 10 times more active and retained their activity on a strain with a reduced sensitivity to mefloquine. As the current policy to avoid the increase in *P. falciparum* resistant strains is to combine drugs, the in vitro activity of the more promising compounds of this series in combination with dihydroartemisinin will also be discussed.

Artemether Loaded Nanoparticles: Development using Quality by Design approach, evaluation of antimalarial efficacy and uptake specifically by malaria-infected erythrocytes

**Kamalinder K Singh**, Professor of Pharmaceutical technology and drug delivery

School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston, PR1 2HE, United Kingdom

Malaria is a global health priority with more than 3 billion people at risk of acquiring the disease. Treating malaria has become greatest challenge despite of all the advances in technology and innovations. The main reason for failure of the current conventional chemotherapy is development of multiple drug resistance and non-specific targeting to intracellular parasite which result in high doses of therapeutic agents and their related toxicities. Targeting approach for malaria-infected erythrocytes using nanosystems open new doors for the treatment of the disease. The goal of the malaria therapy is targeting the infected RBCs to achieve high intra cellular drug concentration. In order to reach the set goal the carrier system should be able to cross multiple membrane barriers to access the intraparasitic target. The proposed talk will discuss development and optimisation of biodegradable Artemether loaded human serum albumin based (HSA) nanoparticles (AAN) for targeting parasitized RBCs (pRBCs) for treatment of malaria. Quality by Design (QbD) approach led to development of robust and reproducible product. The formulations have been characterised for morphology, entrapment efficiency, DSC, XRD, FTIR analysis and *in-vitro* drug release. The Artemether nanoparticles were evaluated for their antimalarial efficacy using *P. falciparum* and *P. berghei* species *in-vitro* and *in-vivo* respectively. While the *in-vitro* susceptibility assay showed five-fold lower IC<sub>50</sub> than plain drug, Pharmacodynamics studies showed antimalarial activity at 50% of therapeutic
Antimalarials that improve immune the response  
**Professor José M. Bautista**, Complutense University of Madrid, Madrid, Spain  
Blood-stage Plasmodium parasites are the main cause of disease and death during malaria infection, and important inducers of naturally acquired immunity. However, these asexual stages do not seem to elicit efficient long-term immune responses. In our lab we have observed that anti-malarial compounds with a “delayed-death” effect promote effective immune responses in preclinical studies. Whereas no vaccine for this disease has yet been licensed, combined therapy including a parasitostatic agent to promote -term immune protection in infected patients could be a candidate strategy to control malaria.

Malaria control: the nutraceutical potential of natural cocoa powder  
**Professor Frederick Addai**, Academic Researcher, University of Ghana Medical School  
Historical and extant anecdotal evidence and limited empirical validation evince antimalarial activity of natural cocoa powder. Persuasive literature abound on how dietary nutrients in cocoa powder may act individually and synergistically to afford protection from clinical malaria. The tropical regions that produce cocoa roughly overlap those that are malaria endemic. Echoing the reasoning that effective malaria control strategies should combine interventions adapted to local needs based on specific ecological, epidemiological, economic and social conditions, this talk advocates natural cocoa as diet-mediated antimalarial prophylaxis. Multi-nutrient and multiple mechanistic potential makes parasite resistance to natural cocoa very unlikely.

Oral Presentation Abstracts

**TRES CANTOS OPEN LAB: CATALYZING INNOVATION IN DISEASES OF THE DEVELOPING WORLD DRUG DISCOVERY**  
**Elena Fernández-Álvaro** and **Lluís Ballell-Pages**  
*GlaxoSmithKline, Diseases of the Developing World-Tres Cantos Medicines Development Campus. Tres Cantos 28760. Madrid, Spain*

Innovative strategies are presently required to discover and develop new pharmaceuticals against Diseases of the Developing World (DDW). Since 2010, GlaxoSmithKline Tres Cantos Medicines Development Campus (TC-MDC) has adopted an open innovation strategy that fosters collaboration and transfer of knowledge with both academia and biotech, with the final aim to deliver new effective medicines for DDW (malaria, tuberculosis and kinetoplastid diseases). As part of this approach, the Open Lab at TC-MDC provides visiting scientists funding and access to GSK facilities, compound collections and drug discovery expertise, in an attempt to exploit a novel model of collaboration for DDW medicine discovery and development. Projects are supported by the Tres Cantos Open Lab Foundation ([www.openlabfoundation.org](http://www.openlabfoundation.org)) after evaluation and approval by a governing board constituted by renowned experts in the DDW field.

The Open Lab has funded more than 36 different project partnerships with academia, which to date have focused on the development of novel drug discovery tools, the exploration of new molecular targets and phenotypic assays in high throughput screening programs and the optimization of novel lead molecules. So far, 10 projects have supported different groups working on malaria drug discovery. The program is currently open and on the lookout for a range of exciting drug discovery opportunities with the potential to deliver tools and compounds that can dramatically affect our capacity to discover novel promising drug candidates against malaria and other DDW.

**DISCOVERY OF PROMISING NOVEL ANTIMALARIAL THIENOPYRIMIDINONE DERIVATIVES**  
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Malaria is a devastating pathology which in 2010 affected 219 million people worldwide (range 154 – 289 million) and caused 660 000 deaths attributable mainly to *P. falciparum* (range 610 000 – 971 000) [1].
A large series of original thienopyrimidinones was synthesized by an innovative and efficient process. More than 100 compounds were tested and best ones showed promising in vitro antiplasmodial activity (IC$_{50}$ between 45 nM and 800 nM towards K1 P. falciparum multidrug resistant strain) and low in vitro cytotoxicity (CC$_{50}$ between 15 µM and 68 µM towards HepG2 and CHO cell lines). Selectivity indexes were included between 9 and 533 and structure-toxicity and structure-activity relationships were precisely defined. Encouraging results were obtained from a preliminary in vivo evaluation of the best compound also identified very active against Plasmodium liver stages (P. yoelii IC$_{50} = 11.7$ nM). A mechanistic study of these non mutagenic compounds antiplasmodial activity has important consequences for their future therapeutic profile since they do not use the already described mechanisms of action of commercialized drugs, an indication of their potential synergistic effect [3,4].

References

KHAYA GRANDIFOLIOLA: POTENTIAL SOURCE OF ANTI-MALARIAL DRUG
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Malaria is endemic in tropical Africa south of Sahara. The incidence of resistance to available anti-malaria drugs made the search for newer drugs especially from medicinal plants inevitable. As a result of this K. grandifoliola was investigated for its anti-malaria properties. activity even against chloroquine resistant P. falciparum strain in vitro. Grandifolin a mexicanolide type of limonoid was isolated from the active NH-2 fraction of K. grandifoliola gave 1.C$_{50}$ of 1.4 µg/ml against the multi-drug resistant strain. The anti-malarial principle of this plant gave 91% chemosuppression of plasmodial parasite and an I.C$_{50}$ value of 1.7 µg/ml for the multidrug resistant clone W2 strain and 0.8 µg/ml for the Nigerian Plasmodium falciparum isolates. From the toxicological and biochemical studies carried out and reported it was found out that the plant was generally safe at low doses with LD$_{50}$ > 1000 mg/kg, but could be toxic at high doses if administered sub-chronically at doses above 500mg/Kg. Morphological abnormalities were found only within the white matter of the cerebral cortex. It also showed moderate anti-inflammatory activity 69.43% at 200mg/kg.

The effects of the plant on red blood cells and bones have been studied. It gave increase in Red Blood Cells count (RBC), Packed Cell Volume (PCV), haemoglobin and plasma iron content in rats. There was a general trend of reduction in bone minerals (Ca, P, Mg and Cu.) at 500mg/Kg dose. However there was a dose dependent increase in bone potassium and iron (K and Fe) content. The alkaline phosphatase (ALP) decreased. K. grandifoliola showed positive effect on erythropoesis and no significant effect on bone mineral content at therapeutic doses of 100mg/kg. K. grandifoliola had also been shown to have hypoglycaemic, hypoproteinaemic and hypocholesterolaemic effects on rats there was reduction in the Liver protein content and glutathione (GSH). The concentration of free fatty acid in the plasma was not significantly reduced nor was there any significant increase in the liver malondialdehyde (MDA) in the extract treated rats. Combination therapy is one of the ways through which resistance is combated as such in a pharmacodynamic evaluation, the interaction of K. grandifoliola with two standard antimalarial drugs – chloroquine and halofantrine using murine mammalian models were evaluated. It was observed that K. grandifoliola: halofantrine combination elicited synergistic effect at sub- optimal doses. The mean survival period of the parasitized animals was also enhanced by the combination. Very low dosage of halofantrine would be required to potentiate parasite clearance when the two drugs were combined. This would constitute great advantage to halofantrine which is associated with cardiotoxicity at high doses.
A ROLE FOR AMP DEAMINASE IN MALARIA RESISTANCE
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There is strong evidence that host genetic polymorphisms can convey malaria resistance. Most of the known mutations cause alterations to red blood cell (RBC) cytoskeletal proteins, membrane proteins or hemoglobin. Here we aim to uncover novel host factors involved in malaria resistance. Using a mutant mouse line carrying a gain of function in Ampd3, generated by ENU mutagenesis, we propose a novel mechanism of host resistance to malaria infection.

The line Ampd3MRi49372 carries a dominant mutation in the erythrocyte specific isoform of AMP deaminase. This enzyme regulates a step in purine metabolism crucial for maintaining the balance between ATP and GTP within the RBC. The mutation – T689A – causes a gain of function in the enzyme, resulting significantly elevated GTP levels, up to 73 fold higher in homozygotes. Mutants also exhibited marked reticulocytosis, with the percentage of immature RBCs reaching 60%, compared to 3% in wildtype. This is likely due to dramatically increased red cell turnover; RBCs from homozygous mutants were found to have a half-life of just 2 days, compared to 18 days for wildtype.

When challenged with P. chabaudi, Ampd3MRi49372 proved extremely resistant, with both homozygous and heterozygous mutants showing a dramatic increase in survival, by 100% and 60% respectively. However, when challenged with P. berghei, no difference in survival was observed. Interestingly, significantly more gametocytogenesis was observed in the mutants during both P. chabaudi and P. berghei infection. We propose that the difference in Ampd3MRi49372 susceptibility between the two Plasmodium spp is mediated by the short RBC halflife. P. chabaudi, which has a tropism for mature RBCs, has far fewer cells in which to grow within Ampd3MRi49372 mice, making it harder for an infection to be established; P. berghei has a tropism for reticulocytes, and so the opposite is true.

This study provides the first evidence that a mutation in Ampd3 can lead to malaria resistance in mice, and shows that balanced host purine metabolism is essential for supporting the growth of Plasmodium spp within RBCs.

ROLE OF HOST IMMUNE SYSTEM IN THE CLEARANCE OF RODENT Plasmodium DURING ARTESUNATE MONOTHERAPY
Carla Claser, Zi Wei Chang, Hutt Wang Teo, Laurent Renia.
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Artemisin is a potent antimalarial drug whose mechanism of action is still unknown. When used as monotherapy to treat uncomplicated cases of Plasmodium falciparum, treatment failure and recrudescence is observed. In particular, it is not known, as for other antimalarial drug, whether there is the participation of the immune system in parasite clearance. Using two rodent malaria models, P. berghei ANKA (PbA) and P. yoelii YM (PyYM), we have first shown that in immune-deficient mice (Rag2 KO) infected with rodent Plasmodium, artesunate (ART) monotherapy led to a drastic reduction of parasite development but not clearance, as an exponential growth of the parasite was observed in 100% of deficient mice after cessation of treatment, suggesting the involvement of adaptive immune system in ART efficacy. Using knockout (KO) mice in C57BL/6 background, we have demonstrated that ART efficacy is B cell-dependent in PyYM infection, and CD4+ and IFNγ dependent in PbA infection. In both models, ART efficacy was shown to be independent of CD8+ T cells. Despite IFNγ level in the sera of treated PbA-infected C57BL/6 was below the limit of detection, ART didn’t affect the immune function, as no difference was found in the total number of spleen leukocytes, CD8+ and CD4+ T cells, NK1.1+CD3- cells, as well as IFNγ+ secreting CD8+ and CD4+ T cells. Interestingly, in PbA-infected splenectomized C57BL/6 and transgenic MAFIA mice (conditional knockout for myeloid cells), ART efficacy was impaired. These data suggest that while spleen is important to accelerate the parasite clearance, macrophages are making the link between innate and adaptive response needed for ART efficacy.

A RAPID IN VITRO BIOLUMINESCENCE-BASED RATE-OF-KILL (BRoK) ASSAY TO SCREEN THE MMV “MALARIA BOX”
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Massive screens of chemical libraries for antimalarial activity have identified thousands of compounds that exhibit sub-micromolar potency against the blood stage of the malarial parasite Plasmodium falciparum.
The laboratories working on new antimalarial drug discovery programs have mostly relied either on traditional approach of random screening or screening of targeted compounds libraries against specific molecular targets. Modern drug discovery programs have greatly benefited from a number of recent scientific and technological advancements, including technologies for in vitro phenotypic screening, identification of diverse new targets through genomics, bioinformatics and proteomics. Availability of annotated genome maps and large number of validated chemotherapeutic targets have fostered a paradigm shift in new drug discovery approaches. Overall, limited successes with HTS and combinatorial chemistry have renewed the interests in rational molecular-targets based drug discovery approach. However, the phenotypic screenings against parasite cell cultures have been more successful in discovery of new antimalarial drug leads.

A multidirectional screening paradigm has been adopted for new antimalarial drug discovery and probing the new molecular targets/pathways. This involves screening of compound libraries through a battery of validated target enzymes/functions, screening of targeted compound libraries likely to act on specific target enzyme/pathways and application of new antimalarial drug leads with novel pharmacophores to probe the chemotherapeutic targets. Terpenoids and isoprenoids are highly abundant in natural resources and may target the unique non-mavelonate pathway of isoprenoid biosynthesis & protein prenylation functions in the malaria parasite; screening of a selected set of isoprenoids led to identification of new antimalarial leads. Natural and semisynthetic organosulfur compound libraries, selected as the potential inhibitors of glutathione- and cysteine-dependent antioxidant defense functions, have also yielded potential new antimalarial leads. Discovery of apicoplast and plant-like metabolic pathways in apicomplexan parasites have led to assessment of phytotoxic molecules, phytoalexins and herbicides as potential antimalarial agents. Several antimalarial leads with novel pharmacophore structures namely, machaeriols, manzamines, indolizidines, budmanchiamines, ingenamines, epoxidones and aporphine alkaloids have also been identified. Manzamine A and artemisinin dimers provided single does cure for malaria in Plasmodium berghei rodent model. Screening of libraries of inhibitor of histone deacetylase, lactate & malate dehydrogenases generated through rational medicinal chemistry approach have also provided some new antimalarial drug leads. Recently, screening of a set of selected standard inhibitors of E3 ubiquitin ligase, which catalyzes the covalent attachment of ubiquitin to the substrate (the last step in the conjugation process), have also shown prominent antimalarial activity. A few selected leads have been followed further in preclinical model and further optimization through structure-activity-relationships. Multiple drug discovery approaches have complemented each other and accelerated the discovery of new antimalarial drug leads and new molecular targets.

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Blood-stage Plasmodium parasites are the main cause of disease and death during malaria infection, and important inducers of naturally acquired immunity. However, these asexual stages do not seem to elicit efficient long-term immune responses. Our recent research on the discovery of new antimalarials we observed that anti-malarial compounds with parasitostatic effect promote effective immune responses in mice. Through this effect, the natural product maslinic acid and the antibiotic borrelidin have been shown to induce reactive antibody responses in a mouse model of lethal P. yoelii malaria. Plasmodium-infected mice treated with maslinic acid [1, 2] or borrelidin [3] recovered from the disease and subsequently developed an immunity that protected them from re-infection upon further parasite challenge after the initial infection. This long-term immunity has the feature of near imperceptible parasitemia after re-infections and a large increase in total serum levels of anti-P. yoelii IgGs showing augmented avidity. Although IgGs displayed a wide molecular weight range of parasite antigens recognized during the course of re-infection, long-term memory IgGs mainly reacted against high and low (but not medium) molecular weight parasite antigens. Immunofluorescence microscopy revealed that circulating IgGs bound predominantly to late intracellular stage parasites, mainly schizonts. We conclude that the long-term immunoprotection conferred mainly by borrelidin or maslinic acid could be related to the parasitostatic inhibition of parasite growth at mature intraerythrocytic stages associated with an immunostimulatory action probably mediated by the polypharmacology of the drug itself. Whereas no vaccine for this disease has yet been licensed, combined therapy including a parasitostatic agent to promote long-term immune protection in infected patients could be a candidate strategy to control malaria.


Present Situation of Antimalarial Drugs in Japan
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Before the Second World War, Approximate 7000 malaria patients every year had occurred in Japan focusing on vivax malaria. The number of malaria patients had decreased dramatically with a peak of 28200 patients in 1946, and recently from 50 to 100 imported malaria patients has been reported in each year. From such a situation, approved antimalarial drugs has been only Quinine and Mefloquine in Japan. The medical treatment to the imported malaria patients was very difficult. Then, Dr.H.Tanaka(The Institute of Medical Science, The University of Tokyo ) and others established "Research group on Chemotherapy of tropical diseases", and this research group started to supply the medicine for tropical disease including 6 antimalarial drugs.

The 6 antimalarial drugs were Chloroquine, Mararon, Coartem, Artesunate, Primaquine, Quineine Inj.

Since 2010 to 2012 this research group supplied these antimalarial drugs to 152 imported malarial cases. From 2010 the Ministry of Health, Labour and Welfare started the non-approved drug review meeting where the necessity on medical treatment is high. In this meeting Maralone was recommended application for approval. Maralone was approved in 2012, using this research group’s data. Moreover, Primaquine also uses this research group data and is in preparation stage of application for approval. Now this review meeting are discussing regarding Coartem. I would like to report the present situation of the antimalarial drugs in Japan, and future prediction.
The Chinese medicinal plant, Artemisia annua (Sweet Wormwood or qing hao), supports the production of artemisinin combination therapies (ACTs), a highly effective treatment for malaria. With almost a million deaths from malaria each year and 40-50% of the world’s population living in areas with a high risk of the disease, there are renewed calls for malaria eradication. The global demand for ACTs is predicted to rise sharply over coming years.

Although A. annua has been used for over 400 years to treat malaria in China, it is only recently that it has been grown as a crop and any breeding strategies employed. There are currently few breeding tools available for improvement and conversion of the species into a predictable crop.

We performed deep sequencing on the transcriptome of A. annua to identify genes and markers for fast-track breeding. Extensive genetic variation enabled us to build a detailed genetic map with nine linkage groups. Replicated field trials resulted in a quantitative trait loci (QTL) map that accounts for a significant amount of the variation in key traits controlling artemisinin yield. At the same time, potential high yielding parent lines have been identified from a high throughput forward screen (FSHYs) for plants with increased artemisinin content over the industry leader variety, Artemis. Our breeding program was focused around developing hybrids from these parent lines. The high yielding hybrids are being validated in field trials in regions of existing commercial production. Markers derived from the map are used in the selection of elite hybrid parents and in quality control processes.

The new breeding tools will help to improve agricultural production of artemisinin and at the same time contribute to the economic sustainability of the many third world farmers of A. annua. Intensive and rigorous field trials program in 2009-2011 has identified two front runner hybrids that significantly outperformed artemisinin yields of Artemis as well as local varieties. We have established and continue to have an exclusive relationship with commercial seed producer East West Seed Ltd (EWS). Commercial quantitates of seed from CNAP varieties were provided by EWS to growers in Africa in 2013. Artemisinin extracted from the dried leaf has the potential to contribute to 70-120 millions ACTs.