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Congress Abstracts
Holistic approach to malaria elimination

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Background: Malaria is a devastating infectious disease caused by parasites of the genus Plasmodium. It affects more than 200 million people worldwide and causes an estimated 700,000 deaths every year, primarily children in developing countries. Effective vaccines against malaria are not yet available and anti-malarial drugs are becoming less effective as the parasites develop resistance. In addition to the disease burden malaria has a severe impact on public health and economic welfare, hindering progress in countries where the disease is endemic. Urgent research is therefore required to holistically address the global burden of malaria.

Approach: Based on the funding of the German Fraunhofer Future Foundation a large multidisciplinary project consortium was formed to combine expertise from the infection biology, biotechnology, engineering and medical technology fields for the development of innovative and complementary approaches towards malaria elimination.

Results: We generated novel protein-based multi-stage malaria vaccine candidates against Plasmodium falciparum (PF) covering antigens from the pre-erythrocytic, erythrocytic and sexual stages. The vaccine candidates were expressed in yeast (Pichia pastoris) and plants (Nicotiana benthamiana) and elicited strong, balanced immune responses in mice and rabbits. Binding studies and immunofluorescence assays demonstrated the native conformation of the vaccine candidates. Affinity-purified pAbs showed strong inhibitory effects in functional assays for each stage.

In preparation for clinical testing, GMP-compliant production processes are being established for the yeast- and plant-based malaria vaccine candidates, the latter benefiting from a groundbreaking production facility with integrated vertical farming and 2D/3D-plant scanners. The facility is under construction at the IME in Aachen and will permit the automated large-scale manufacturing of the malaria vaccine candidates in plants according to GMP standards as well as the production of certified transgenic seeds.

In addition to the active vaccination, a potential passive vaccination approach against PF is being explored via a novel technology platform for the generation of human monoclonal antibodies from peripheral blood mononuclear cells of semi-immune donors. First inhibitory antibodies have been isolated and are being evaluated.

To complement the therapeutic approach and to strengthen malaria control a novel diagnostic platform for the automated microscopic acquisition and analysis of Giemsa-stained thin and thick blood smears is being developed. A prototype system has been set up, first segmentation and classification algorithms were generated and a training database was built including in total images of 4,195 annotated parasites and 2,881 artifacts. Preliminary evaluation of a non-overlapping test sets provided a detection accuracy rate of 94% for thick smears and 93% for thin smears, respectively.

Conclusion: The Fraunhofer Future Foundation Malaria Project has introduced a holistic concept to support the elimination of malaria by focusing not only on the generation of innovative vaccine candidates but also on GMP-compliant process development, novel enabling technologies for manufacturing and accurate, automated malaria diagnostics.


Recombinant human anti-malarial monoclonal antibodies – a technology platform

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Despite an enormous effort during the last years, a protective vaccine against malaria is still lacking. It becomes more and more evident that an exact profile of both the vaccine elicited and the natural immune response against Plasmodium falciparum infection is needed.

As part of a Fraunhofer Malaria Foundation Project, a Technology Platform for the isolation of human monoclonal antibodies from semi-immune donors living in a holoendemic setting has been established at the Fraunhofer IME in Aachen, Germany.

The technology is based on a five step process including: (1) the characterization of the antibody source by screening the serum of blood donors for their reactivity against Plasmodium falciparum antigens as well as for their functional inhibitory potential; (2) the antibody isolation by either the immortalization of B-cells via transformation with Epstein-Barr virus, or the construction of a phage display library and subsequent panning process; (3) the rescue of antibody genes by RT-PCR; (4) the recombinant production of human
monoclonal antibodies and recombinant fragments thereof in heterologous expression systems and (5) the characterization of the isolated antibodies for binding activity and parasite inhibition.

Based on this platform, both by phage display and B-cell transformation, antibodies specific for merozoite surface antigens have been isolated and characterized for their inhibitory potential. The presented Antibody Isolation Technology Platform allows the ongoing rescue, recombinant production and characterization of multiple human monoclonal antibodies against *Plasmodium falciparum* antigens and can be flexibly extended by including further donor material. In the future, this technology might allow to rescue and characterize whole specific antibody repertoires.

The human monoclonal antibodies derived from this platform do not only inherit a great potential as a tool for basic and applied research in malariology but also might deliver alternatives for malaria therapy for multi resistant strains. In a further aspect, these antibodies might be suitable as travelers' vaccines conferring immediate protection.


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03

Evaluating PfGAP50 as a component of novel recombinant subunit vaccines derived from plants

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One key objective in the Malaria Project of the Fraunhofer Future Foundation is the development and production of novel malaria vaccine candidates using plant-based systems. Plants are ideal manufacturing platforms for these purposes because their quality control and subcellular targeting capabilities allow the efficient production of novel, multi-stage, multi-component subunit vaccines in the optimal biochemical environment.

The 44.6-kDa transmembrane protein PfGAP50 of *Plasmodium falciparum* has previously been described as part of the inner membrane complex, anchoring the actin-myosin motor of the invasive stages. Recently, we showed that during gametogenesis in the gut of the mosquito vector, PfGAP50 relocates from the inner membrane complex to the plasma membrane. Here it functions as a receptor for the complement regulatory protein Factor H, which the newly formed gametes bind from the mosquito blood meal to evade lysis by human complement. Because antibodies against PfGAP50 can synergistically activate the classical and the alternative complement pathway, the transmembrane protein represents a promising novel candidate for transmission blocking vaccines.

We have now successfully produced His6-tagged variants of PfGAP50 variants by transient expression in Nicotiana benthamiana leaves, which are targeted to the ER, the apoplast and the plastids of the plant cells. The corresponding yields were 60 mg/kg (ER variant), 80 mg/kg (apoplast variant) and up to 120 mg/kg (plastid variant). All three proteins were harvested 5 days after infiltration and were efficiently extracted by blending with two volumes of PBS containing 2 µM CoSO4 and 500 mM NaCl. After centrifugation to remove debris, the proteins were recovered from the supernatant by Ni2+ IMAC, reaching 85% purity. The ER and plastid variants were tested for their ability to bind Factor H and used for the immunization of rabbits. The resulting sera will then be tested for transmission-blocking activity using a standard membrane feeding assay.

Our results demonstrate that functional PfGAP50 can be produced efficiently in plants, further adding to our portfolio of *Plasmodium* spp. antigens that can be used to develop the next generation of combination vaccines against malaria.


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04

Effects of urbanicity on malaria and the development of immunity – results from a hospital based study in Ghana

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Introduction: Malaria incidence has declined considerably over the last decade. This is partly due to a scale-up of control measures but may also be driven by a decline of vector populations due to urbanisation. The aim of this study was to analyse, on a micro-epidemiological scale, the association between malaria and urbanisation and a possible effect of urbanicity on the acquisition of immunity.

Methods: In 2012, children <15 years presenting to St. Michael’s Hospital in Pramso/Ghana with fever (≥38.0°C) were recruited. Malaria was defined as fever with a parasitaemia of ≥5000/µl. We used an innovative urbanicity-scale to measure the level of urbanicity of our study settlements to examine an association between urbanicity and malaria risk. The proportion of malaria cases to all fever cases (“Malaria-Positive-Fraction of Fevers” (MPF)) was calculated for each settlement as a proxy for malaria risk. To obtain
stable estimates of the MPF villages with less than 20 participants were excluded. The median age of all malaria cases was calculated for each settlement as a proxy for how quickly immunity is acquired. Associations between MPF and urbanicity, MPF and median age, and urbanicity and median age were displayed using scatterplots with a regression line and corresponding 95% confidence interval and modelled using a linear regression.

**Results:** Of 2,567 recruited children, 2,274 (89%) from 28 settlements could be included in this analysis. There were 728 malaria cases (overall MPF 0.32). The MPF varied from 0.19 to 0.54 between settlements and was negatively associated with the level of urbanicity (coefficient: –0.5 per 10 points on the urbanicity-scale, \( R^2 = 0.63, p<0.001 \)). Median age of children presenting with malaria varied from 31 to 64 months between settlements and was negatively associated with the MPF (coefficient: –3.7 per 0.1 MPF, \( R^2 = 0.19, p=0.03 \)).

**Discussion:** Our results confirm an association between urbanisation and a declining prevalence of malaria. We also demonstrate that the acquisition of immunity is heterogeneous on a micro-epidemiological scale and that it is associated with transmission intensity.


**05**

**Doxycline inhibits experimental cerebral malaria by altering T cell responses and reducing inflammatory and tissue-degrading mediators**

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We investigated the impact of doxycycline treatment on *Plasmodium berghei* ANKA (PbA) induced experimental cerebral malaria (ECM). The complex inflammatory networks triggered by the parasite leads to the destruction of the blood brain barrier (BBB). Administration of doxycycline prevented neuropathology in PbA infected mice. Local inflammation was reduced to a minimum and BBB damage was prevented.

Other tetracyclie derivatives show similar protective effects, but only those with known immune-regulatory properties.

Our results provide evidence that the inhibition of ECM is to a large extend by anti-inflammatory actions of doxycycline, despite observed anti-parasitic effects. Protection is observed in high dose infected animals and likewise in animals receiving additional parasites later infection.

Analysing brain tissue by RNA-array and on protein levels, we found that in treated animals, the expression of CCL5, an important T cell recruitment factor in inflammation was reduced. Accordingly, T cell infiltration was impaired. The T cells accumulated in the spleen and despite similar activation compared to PbA infected controls, these cells showed reduced parasite-specific cytotoxicity after doxycycline treatment. In addition, ECM development could be associated with increased expression and activity of gelatinolytic MMP2, and cytolytic granzyme B in the brain, which were both reduced after doxycycline.

Our results suggest that during ECM in addition to known anti-parasitic effects several systemic and local inflammatory processes are targeted by doxycycline, inhibiting BBB disruption and neuropathology. Thus we provide theoretical support for retaining doxycycline in the treatment of severe human malaria.


**06**

**Isoprenoid biosynthesis as target for new antimalarial drugs**

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The biosynthesis of isoprenoids is achieved in *P. falciparum* via the 1-deoxy-d-xylulose 5-phosphate (DXP) pathway, which is typically used by plastids of plants and by most bacteria. In *P. falciparum* the enzymes of the DXP pathway are localized inside the plastid-like organelle (apicoplast). Fosmidomycin (FR-31564), an antimicrobial compound originally isolated from the culture broth of *Streptomyces lavendulae*, as well as some related compounds are highly specific inhibitors of DXP reductoisomerase. A total of 9 clinical sites later infection.

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Hypusine in Plasmodium: a molecule determining the severity of malaria

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Hypusination in eukaryotic initiation factor 5A (EIF5A) is a unique posttranslational modification which so far has only been discovered in this protein. Hypusine was first isolated by Shiba et al. (1971) from brain cells and identified as a 4-amino-2-hydroxybutyllysine-derivative. This posttranslational modification is formed within two consecutive enzymatic steps i.e. deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase. Deoxyhypusine synthase transfers the aminobutyl moiety from the triamine spermidine to a specific lysine residue in EIF-5A while deoxyhypusine hydroxylase catalyzes hydroxylation in the side chain.

Over recent years we have identified both enzymes in the human malaria parasites P. falciparum and P. vivax. Both enzymes were evaluated as drug targets [1]. Currently, investigations on the biological function of this unique posttranslational modification are performed. In recent experiments we showed that downregulation by silencing elf-5A, dhs and doth genes with shorthairpin RNAs leads to an impaired hypusine biosynthesis and to growth retardation of the parasite. Infection of NMRI mice with schizonts from the lethal P. berghei ANK strain transgenic for plasmodial elf-5A-specific shRNA or DHS-specific shRNA resulted in low parasitemia 2-9 days post infection before animals succumbed to hyperparasitemia similar to infections with the related but non-lethal phenotype P. berghei strain NK65. RT-PCR and Western blot experiments performed with blood from the transfected erythrocytic stages showed that both genes are important for the proliferation of the parasite. Moreover, these experiments clearly demonstrate that the hypusine pathway in Plasmodium is linked to human iNos induction.

Preliminary results of an in vivo knockdown show that elf-5A and dhs genes are essential for the proliferation of the parasite thus revisiting an old acquaintance for their importance in cell proliferation. This findings were even more supported in phenotyping experiments. To exclude any resistance association with the gene locus of elf-5A and dhs genes “knock in” experiments will be performed.

References


Plasmodium falciparum antioxidant protein as a model enzyme for a special class of glutaredoxin/glutathione-dependent peroxiredoxins

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The growth of the human malaria parasite Plasmodium falciparum within red blood cells is postulated to depend on the ability to remove reactive oxygen species. In the absence of catalase and classical glutathione peroxidases, peroxiredoxins have been suggested to play a crucial role for the parasite’s antioxidant defense [1]. Peroxiredoxins are a heterogeneous family of thioredoxin-like proteins, which reduce hydroperoxides. For most peroxiredoxins, the electron donor is thioredoxin. Only two cysteine residues are sufficient for catalysis. Thus, PfGrx is not just a simple replacement of thioredoxin. Our data also point to a physiological role remains unknown, PFAOP was shown to preferentially use an electron donor system consisting of glutaredoxin and glutathione (GSH) [1]. We now dissected the unusual mechanism of PFAOP in vitro [2]: Our data clearly reveal that PFAOP acts via a ping-pong mechanism using P. falciparum glutaredoxin (PfGrx) and GSH as true substrates. Only one PfGrx cysteine residue is sufficient for catalysis. Thus, PfGrx is not just a simple replacement of thioredoxin. Our data also point to a GSH-dependent enzyme activation and a negative subunit cooperativity of PFAOP. In silico analyses furthermore suggest that PFAOP is a member of a special subclass of glutaredoxin/GSH-dependent peroxiredoxins. This subclass might be the result of a peculiar molecular evolution that helped malaria parasites and other pathogens to cope with reactive oxygen species.

References
09

Protein S-nitrosylation in Plasmodium falciparum


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The human malaria parasite Plasmodium falciparum is exposed to oxidative and nitrosative stresses due to the rapid proliferation and multiplication of the parasites and the host immune response. Nitric oxide (NO) and NO-derived reactive nitrogen species (RNS) constitute major nitrosative stress involved in the control of malaria parasites. NO has both cytostatic and cytotoxic effects on the cell growth of Plasmodium parasites. However, the action mode and the intraparasitic targets of NO in P. falciparum remain largely unexplored. Protein S-nitrosylation (protein-SNO), a nitrosative stress-induced ubiquitous modification of protein cysteine thiols by NO, has emerged as a principal mechanism by which NO excerts biological functions. Although studied intensively in higher eukaryotes and bacteria, protein-SNO has not been systematically studied in human malaria parasites. By using a biotin-switch assay-based proteomic approach, we identified 319 potential S-nitrosylation targets in P. falciparum. Functional profile analysis of the identified proteins suggested S-nitrosylation may influence a variety of cellular metabolic processes in P. falciparum, among which glycolysis appears to be a major targeted pathway. Particularly, glycolytic activity of P. falciparum glyceraldehyde-3-phosphate dehydrogenase (PGAPDH) was found to be inhibited by S-nitrosylation on its active-site cysteine. Additionally, we found that P. falciparum thioredoxin 1 (PfTrx1), a central protein disulphide oxidoreductase, can be site-specifically S-nitrosylated at its non-active site cysteine (Cys43). More importantly, we reported that PfTrx1 possesses both denitrosylating and transnitrosylating activities mediated by its active site cysteine residues and Cys43, respectively. A redox status-based model of PfTrx1 in the regulation of protein-SNO in the parasites was proposed.

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10

Introduction to Medicines for Malaria Venture (MMV)

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Medicines for Malaria Venture (MMV)

Apart from Artemisinin Combination Therapies (ACTs) there are relatively few effective treatments for Plasmodium falciparum malaria and only one complete treatment for Plasmodium vivax.

This talk will summarize the work of Medicines for Malaria Venture (MMV) and its mission to reduce the burden of malaria in disease-endemic countries by discovering, developing and facilitating delivery of new, effective and affordable antimalarial drugs.

MMV manages a significant antimalarial pipeline and this has been strengthened in recent years with the delivery of new artemisinin combination therapies, promising new clinical candidates and early stage discovery projects. The talk will explain both the challenges that need to be overcome and the strategy adopted to eradicate the disease, including definitions of target product profiles necessary for asexual blood stage cures (including single dose combination treatment), transmission blocking, vivax and chemoprotection.

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11

The Malaria Box: An Open Access Catalyst for Drug Discovery

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Medicines for Malaria Venture (MMV)

The discovery of new chemotypes to feed the pipeline of antimalarial drugs remains a constant challenge, particularly in light of emerging resistance to current therapies. Recently, phenotypic screenings have been successfully used for antimalarial hit generation where the biological target(s) may often not be clearly identified. To catalyse malaria research by both filling the pipeline and having a better understanding of ligand-target relationships, a unique screening tool has been elaborated: the Malaria Box.

The Malaria Box is a set composed of 400 commercially available chemical entities derived from a selection of more than 20,000 hits from the screening of corporate and academic libraries. The originality of the Malaria Box relies in its composition of 200 lead-like and 200 probe-like compounds that have confirmed activity on blood-staged Plasmodium falciparum and that have been assessed for cytotoxicity. Lead-like compounds commensurate with oral absorption and the presence of known toxicophores has been reviewed. Conversely probe-like compounds are intended to represent the broadest cross-section of structural diversity.
Significantly, the scope of the Malaria Box goes beyond the Malaria field as active compounds may have utility in other parasitic or neglected diseases. It is well-documented that artemisinin was initially discovered from helminth research and is currently a gold standard drug against Malaria. Also, the presence of orthologues of various molecular targets may lead to new therapeutic applications in orphan diseases or for example oncology. Ultimately, the data collection resulting from the Malaria Box would enable the community to better understand similarities and differences between parasite diseases or orphan diseases by mining data sets that were previously considered separately.

Herein we disclose the selection process applied to assemble the Malaria Box as well a preliminary results.


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Filling the gap in anti-malarial drugs with the development of novel long-duration compounds: a Merck Serono and Medicines for Malaria Venture partnership

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The recent partnership between Merck Serono, the pharmaceutical branch of Merck KGaA, and Medicines for Malaria Venture (MMV), a non-for-profit organization, focuses on a drug discovery program aiming at developing new anti-malarial compounds with long duration characteristics. With the increasing threat of emerging resistance to current anti-malarial treatments, it is critical to expand the variety of new therapeutic options. This includes compounds providing long term protection to reduce the potential of re-infection and allowing for treatment combination to decrease the risk for triggering resistance mechanisms. This is currently a major gap in the target compound profiles being currently explored by various organizations.

Mefloquine (Lariam®) remains today the standard long duration standard, with a half-life of a few weeks, however its severe CNS side effects highlights the need for the development of a therapeutic alternative. A new chemical class has recently been discovered at Merck Serono that presents key characteristics to fill this gap. This carbazole derivative series has in vitro efficacy on blood stage P. falciparum (NF54) in the nanomolar range. The lead compound is metabolically stable in rodents and humans, allowing for an extended PK profile in mice with a t½ life >100 hrs. It provides complete remission in the P. berghei mice model and a slow parasitocidal onset in immunosuppressed P. falciparum infected mice, without recrudescence 60 days after infection. Additionally, it shows in vitro efficacy on an extended panel of P. falciparum strains presenting various degree of resistance to current anti-malarial treatments.

Although the program is still in its early phase, the overall profile of this series is very promising and may fill the gap in long-duration, safe anti-malarial treatments. Finally, it is noteworthy that the particular set-up of the Merck Serono-MMV partnership, where the project team is composed of members from both organizations and the resources and networks are shared, is a clear example of an innovative partnership between a pharmaceutical company and a not-for-profit organization allowing for an efficient and truly integrated program.


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13

A novel high throughput screening approach targeting the vitamin B6 biosynthesis in Plasmodium falciparum

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Pyridoxal phosphate (PLP), the respective active molecule of vitamin B6, is de novo synthesised in Plasmodium falciparum by the PLP-synthase which consists of the enzymes PFDx1 and PFDx2 (Wrenger et al., 2005). Due to the lack of this synthase complex in humans the enzymes provide an ideal starting point for medicinal chemistry programs. In a fluorescent medium throughput screen different lead series were identified and their activity was confirmed on cellular level utilising a 96-well 3H-hypoxanthine incorporation assay established at the Wrenger group using P. falciparum cultured in human red blood cells.

These promising data encouraged us to develop a high throughput assay in 384-well plate format. After analysing the assay in terms of stability, variability and DMSO tolerance 256,000 compounds were screened on 9 consecutive days using 945 384-well plates. The median z’ of the primary screen was 0.72 and the assay yielded 3607 Hits corresponding to a Hit rate of 1.4% (50% activity cut off). Hits were analysed in two consecutive counter screenings leading to active molecules which are now analysed in vivo. The data presented will cover some aspects of the high throughput screening performed at European ScreeningPort.
A PHIST protein interacts with the intracellular ATS domain of PfEMP1 and localizes to knob structures in *P. falciparum*

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The most severe form of malaria is caused by the asexual development of the apicomplexan parasite *Plasmodium falciparum* within the red blood cell (RBC) and leads to almost one million deaths each year. In order to avoid splenic clearance the parasite transports adhesion molecules like PfEMP1 to the surface of the infected red blood cell (iRBC). PfEMP1 is responsible for endothelial binding of the iRBC, which in turn accounts for all pathology observed in malaria.

Recently, the conserved intracellular domain of PfEMP1 (ATS) was suggested to comprise conserved protein interaction epitopes (Mayer et al., 2012). Moreover it was shown that this domain interacts with the PHIST domain of PFI1780w, a member of the recently discovered *Plasmodium* helical interspersed sub-telomeric (PHIST) family (Sargeant et al., 2006). Therefore, we proposed that PHIST domains facilitate protein interactions, and that disruption of this interaction could interfere with the parasite’s cytoadherence.

In order to elucidate the localization of a subset of PHIST proteins including PFI1780w we performed a GFP/3xHA tagging approach. Besides PFI1780w which was found at the iRBC surface and membrane associated, another PHIST protein (PFE1605w) has been shown to localize to the knobs. We could show that the PHIST domain of PFE1605w binds with a 10x higher affinity (Kd ~3 µM) to the intracellular ATS domain of PFEMP1 than the previously reported PFI1780w. In contrast, no binding has been observed for MAL8P1.4, a PHIST protein which we have shown to have localized to a subset of Maurer’s clefts.

Various localizations and binding properties of PHIST proteins suggest that diverse functions can be identified within this protein family and the PHIST domains of PFI1780w and PFE1605w are so far the only proteins found to bind to the intracellular ATS domain of PFEMP1. Moreover the cellular localizations of these two proteins are consistent with the binding of ATS.


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Development of a Novel ELISA-based Assay for the detection of mature gametocytes

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Malaria caused by *Plasmodium falciparum* remains one of the most devastating diseases in the world. According to the WHO, the disease claimed close to 650,000 lives in 2011 with 90% of the victims coming from the African region. Due to the persistent lack of an effective vaccine, the fight against malaria relies mostly on chemotherapy and chemoprophylaxis. However the treatment of the disease is complicated by the parasite ability to develop resistances against almost all the current antimalarial drugs and the vectors of the disease are resistant to most insecticides, a situation which further slows down the elimination strategies. Moreover, most drugs used to treat malaria kill the asexual stages of the parasite, but do not prevent gametocytes formation and maturation. Developing therapies which contain gametocytocidal antimalarial drugs are predicted to be the best way to further limit malaria transmission since asexual stages or non-viable gametocytes can’t develop inside the mosquito midgut. The goal of our study was to develop an enzyme-linked immunosorbent assay (ELISA) to quantify the gametocyte load in drug-treated *P. falciparum* cultures, in order to investigate the gametocytocidal activity of antimalarial compounds. For this purpose, the surface protein of *P. falciparum* namely Pfs230 was chosen as the target for ELISA development. Pfs230 localizes to the surface of the parasite plasma membrane of the gametocyte stages III, IV and V. Polyclonal antibodies capable of recognizing this protein were successfully generated for this study. However, for the purpose of drug screening in 96 well plates, achieving an acceptable detection signal depends on a successful gametocytes growth in 96 well plates. To further improve the sensitivity of the assay, a monoclonal antibody directed against the above mentioned protein is currently being developed.


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During their differentiation in the human host, the gametocytes of *Plasmodium falciparum* display a remarkable number of adhesive proteins on their surface. These include the PfCCp protein family, six secreted proteins that assemble to multimeric protein complexes (MPCs) and locate to the parasitophorous vacuole. We now identified another MPC component, the WD40 domain-repeat protein-like protein, encoded by 60 different members of the *var* multigene family. Antigenic variation – a mechanism to avoid the host’s immune response – is the result of mutually exclusive expression of one of the 60 *var* gene loci and switching of the active *var* gene. The *var* gene repertoire of individual *Plasmodium* isolates is almost completely different between individual isolates. However, it is not clear if this antigenic diversity is constantly expanded by recombination events or if it simply reflects a very large pool of individual variants. Recombination events have been found in subtelomeric regions of heterologous chromosomes, which harbour the majority of gene families encoding variant surface proteins such as PfEMP1. In these regions, single nucleotide polymorphisms (snps) are prevalent. It remains an open question if these snps represent recombination events of erroneous assembly due to repetitive regions and homopolymer runs. Here we aim to use microsatellite typing and locus specific *var* gene PCR as a tool to investigate the inheritance of *var* genes and other multicopy gene families in *P. falciparum*. In a first step, we tested 70 microsatellite primers across the genome in silico using bioinformatical tools and single nucleotide polymorphism maps for E5 and 3D7. In a second step, these primers were evaluated by PCR on genomic DNA and all fragments were characterized by Sanger sequencing. Together with a PCR analysis of the entire *var* gene family this allowed us to assess if *var* gene inheritance follows a Mendelian pattern. In a third step, microsatellite analysis based on fragment length polymorphism will be established in order to enable high throughput analysis of field isolates.


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Efficient antibody rescue from antigen-specific human B lymphocytes following Epstein-Barr virus-transformation

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Malaria tropica, caused by *Plasmodium falciparum* still poses a major challenge with regard to global health care as well as to the economy in endemic countries. There is no efficient vaccine at hand, and multiple drug-resistant parasite strains are emerging. As yet,
passive vaccination strategies with recombinant full-size human monoclonal antibodies are not pursued despite the fact that it has been shown early that these may confer immediate immunity toward as well as effectively cure malaria infections by *Plasmodium falciparum*.

Several technologies exist that allow the isolation and production of human full-size antibodies or antibody fragments, such as the techniques of Fab- or scFv-phage display, the use of transgenic mice possessing human immunoglobulin loci, as well as the transformation of B cells by Epstein-Barr Virus (EBV). Here, we would like to present a methodology that efficiently joins EBV transformation of peripheral B lymphocytes, V gene rescue, and recombinant antibody production in plants.

In an attempt to harness the protective immunoglobulin repertoire of Ghananian donors IgG+/CD22+ B lymphocytes from peripheral blood were selected by means of fluorescently labeled antigens and flow cytometry. Antigen-specific B cells were subsequently infected with Epstein-Barr virus. After 3–4 weeks ELISA and spectratyping served to assess secretion of desired IgG by and the clonality of the obtained lymphoblastoid cultures. Sequences of variable regions of promising candidates were amplified, cloned into plant-expression vectors, and expressed in the context of human full-size IgG1x1 in *N. benthamiana*. Integrity, binding characteristics, and functionality were verified by Western blots, ELISA, surface plasmon resonance (SPR) measurements, confocal immunofluorescence microscopy of parasites, and growth inhibition assays (GIA) in vitro.

Several human monoclonal antibodies directed against *Plasmodium falciparum* were successfully isolated which possess different affinities toward their epitopes and different characteristics of growth inhibition.

The recombinant production of these antibodies in plants allows the large scale production at moderate cost, which may build the base for affordable therapeutic anti-malarial antibodies.


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**The genetic diversity of Plasmodium vivax from Papua New Guinea inferred with mitochondrial genomes**


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The *Plasmodium vivax* affects populations of Central America, South America, Middle East, South, Southeast and Central Asia, Oceania and East Africa, where 3.3 billion people are currently at risk of infection and 70–80 million clinical cases are reported each year. Previous studies have contrasted the genetic diversity of parasite populations in the Americas with those in Asia and Oceania, concluding that New World populations exhibit low genetic diversity consistent with a recent introduction. In this study we sequenced the complete mitochondrial genome of 52 isolates of *P. vivax* from Papua New Guinea to investigate the genetic diversity present in this region. The network of mtDNA haplotypes was constructed using the program Network, combining 721 genome sequences available in the GenBank database with 52 sequences from Papua New Guinea. We identified 360 distinct haplotypes, including 3 haplotypes not found in earlier surveys of global mitochondrial diversity in *P. vivax*. Our Network analysis suggests that isolates from Papua New Guinea are divided in sub-populations. The first PNG sub-population is closely related to isolates from South America, predominantly those from Venezuela, Peru, Colombia and Brazil, and also related to African isolates in particular from Madagascar. Another sub-population from PNG is dispersed in the network, related mitochondrial DNAs are of diverse origin, for example, from Central America, South America (Brazil predominately), Southeast Asia, South Asia, China and Korean. Up the present, there is no definitive conclusion about the arrival of *P. vivax* in Oceania, but genetic data suggest that this continent was probably colonized by *P. vivax* at multiple time points. This could explain the finding of two groups of haplotypes in PNG, each of these with distinct relationships within the haplotype network.


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**Effect of DPAP-specific inhibitors on gametogenesis in Plasmodium falciparum**

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The transmission of the malaria parasite *Plasmodium falciparum* from humans to mosquitoes is mediated by sexual precursor cells, the intraerythrocytic gametocytes, which become activated in the mosquito midgut by environmental stimuli and undergo gametogenesis. Egress of the gametocytes from the enveloping erythrocyte is a crucial step for the parasites to prepare for fertilization, but the molecular mechanisms of gametocyte egress are not well understood. Previous studies indicated that plasmodial proteases are involved in this process [1], [2], but these proteases have not yet been identified. A new study showed that inhibition of the dipeptidyl aminopeptidases PDPAP1 and PDPAP3 resulted in blocked transmission of *P. falciparum* to the mosquito [3]. We now aimed to investigate, if the DPAPs play a role in gametocyte egress. We studied the effect of two DPAP-specific inhibitors (ML4118S and JCP410) on blocking the egression of the gametocytes. Our results showed that the inhibition of both aminopeptidases significantly affects the transmission of the parasite to the mosquito. The findings are consistent with the idea that DPAPs play an important role in egress of the gametocytes from the erythrocyte, suggesting that these enzymes are potential targets for the development of new antimalarial drugs.

on gametogenesis via exflagellation inhibition assay and electron microscopy. Both inhibitors were able to completely inhibit gametogenesis by impairing the rupture of the parasitophorous vacuole membrane. Noteworthy, while the asexual replication cycle DPAP3 activates the subtilisin-like protease PISUB1, which in consequence processes the egress molecule PISERA5 [4] these two proteins cannot be detected in gametocytes via immunofluorescence assays. Our data indicate that plasmodial DPAPs mediate the egress of the gametocytes from the enveloping erythrocyte in a PISUB1/PISERA5-independent pathway.

References

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Live-cell imaging of mitogen-activated protein kinase localization in Plasmodium berghei liver stage parasites

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Mitogen-activated protein kinases (MAPKs) mediate a variety of cellular functions like stress response, proliferation and differentiation in eukaryotic cells. Two MAPK homologs designated as MAPK1 and MAPK2 have been identified in Plasmodium.

For Plasmodium berghei liver stage parasites, transcription of both genes was confirmed by RT-PCR. GFP-tagged PbMAPK1, expressed either under control of the strong constitutive EEF1a promoter or the endogenous pbmapk1 promoter, displayed a stage-dependent and dynamic subcellular localization pattern during liver stage development. In the early liver schizont, PbMAPK1 localized inside the parasite’s nuclei, whereas a distinct localization of PbMAPK1 in comma/ring-shaped structures in proximity to the parasite’s nuclei and the invaginating parasite membrane was observed during late liver stage development. In contrast, GFP-tagged PbMAPK2 showed a constitutive association with the dividing parasite nuclei. PbMAPK1 knock-out parasites displayed normal host cell invasion and completion of in vitro liver stage development.

To further characterize the PbMAPK1 localization in late liver stage parasites, subcellular compartments putatively related to the PbMAPK1 localization were visualized by live-cell imaging of transgenic parasites expressing GFP-tagged fusion proteins targeting phosphoinositides and structures involved in microtubular organization.

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