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Congress Abstracts

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Address of the editor:
PD Dr. Gabriele Pradel
Institute of Molecular Biotechnology
RWTH Aachen University
Worringerweg 1
52074 Aachen

01

Holistic approach to malaria elimination

Alexander Boes[1], Holger Spiegel[1], Véronique Beiss[1], Güven Edgü[1], Helga Schinkel[1], Marcel Houdelet[1], Stefan Schillberg[1], Markus Sack[2], Thomas Rademacher[1], Pia Dahm[1], Robin Kastilan[1], Johannes Buyel[1], Tanja Holland[1], Daniel Blessing[1], Nadja Vöpel[1], Stephan Hellwig[1], Jürgen Drossard[1], Rolf Fendel[1], Stephanie Kapelski[1], Dominika Maskus[1], Otchere Addai-Mensah[1], Melanie Seidel[1], Stefan Barth[1], Markus Geese[3], Kristian Reiss[3], Christian Wenzel[3], Christian Hügel[4], Oliver Scholz[4], Günther Kostka[4], Michaela Benz[4], Erik Haßlmeyer[4], Christian Münzenmayer[4], Christian Weigand[4], Matthias Scheuermayer[5], Gabriele Pradel[2], Edmond Remarque[6], Bart Faber[6], Rolf Horstmann[7], Egbert Tannich[7], Rainer Fischer[1,2], Andreas Reimann[1]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany
[2]Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany
[3]Fraunhofer Institute for Production Technology (IPT), Aachen, Germany
[4]Fraunhofer Institute for Integrated Circuits, Erlangen (IIS), Germany
[5]Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany
[6]Biomedical Primate Research Centre, Rijswijk, the Netherlands
[7]Bernhard Nocht Institute For Tropical Medicine, Hamburg, Germany

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 *Pf* 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 malaria vaccine candidates but also on GMP-compliant process development, novel enabling technologies for manufacturing and accurate, automated malaria diagnostics.

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02

Recombinant human anti-malarial monoclonal antibodies – a technology platform

Rolf Fendel, Stephanie Kapelski, Dominika Maskus, Melanie Seidel, Otchere Addai-Mensah, Andreas Reimann, Rainer Fischer, Torsten Klockenbring, Stefan Barth

Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany

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

Veronique Beiss[1], Markus Sack[1], Matthias Scheuermayer[2], Alexander Boes[1], Gabriele Pradel[3], Rainer Fischer[1,3]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany
[2]Research Center for Infectious Diseases, University of Wuerzburg, Wuerzburg, Germany
[3]RWTH Aachen University, Institute for Molecular Biotechnology, Aachen, Germany

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

Clemens Frank[1], Ralf Krumkamp[1], Nimako Sarpong[2], Peter Sothmann[1], Ellis Owusu-Dabo[2], Jürgen May[1], Benno Kreuels[1,3]

[1]Research Group Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Kumasi Centre for Collaborative Research, Kumasi, Ghana
[3]Section for Tropical Medicine, 1st Department of Internal Medicine, University Medical Centre Hamburg-Eppendorf, Germany

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: –4.5 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.

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05

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

Kim E. Schmidt[1], Janina M. Küpper[1], Judith Alferink[2,3], Beatrix Schumak[1], Andreas Limmer[4,5], Sabine Specht[1], Achim Hörauf[1]

[1]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany
[2]Department of Psychiatry and Psychotherapy, University Hospital Muenster, Muenster, Germany
[3]Institute of Molecular Psychiatry, University Hospital Bonn, Bonn, Germany
[4]Department of Orthopaedics, University Hospital Bonn, Bonn, Germany
[5]Institutes of Molecular Medicine and Experimental Immunology, University Hospital Bonn, Bonn, Germany

We investigated the impact of doxycycline treatment on *Plasmodium berghei* ANKA (PbA) induced experimental cerebral malaria (ECM). The complex inflammatory networks triggerd 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.

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06

Isoprenoid biosynthesis as target for new antimalarial drugs

Jochen Wiesner

Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Project Group Bioresources, Gießen, Germany

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 phase II studies on the efficacy of fosmidomycin for the treatment of *P. falciparum* malaria have been conducted. Fosmidomycin monotherapy resulted in fast parasite and fever clearance but was inefficient to eliminate the parasites completely. Higher efficacy was achieved by combining fosmidomycin with clindamydin, which had been shown in vitro to act synergistically. A combination of fosmidomycin (30 mg/kg) and clindamycin (10 mg/kg) administered orally twice daily for 3 days resulted in a cure rate of approximately 90% in patients older than 3 years. The fosmidomycin derivative FR-900098, which can be isolated from the culture broth of *S. rubellomurinus* or produced synthetically, exhibits low toxicity and approximately 2-fold improved antimalarial activity, thus representing a promising new development candidate.

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07

Hypusine in Plasmodium: a molecule determing the severity of malaria

Annette Kaiser, David Kersting, Mirco Krüger

Institute for Pharmacogenetics, Medizinisches Forschungszentrum, Essen, Germany

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-hydroxybutyl)lysine-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 druggable targets [1]. Currently, investigations on the biological function of this unique posttranslational modification are performed. In recent experiments we showed that downregulation by silencing *eiF-5A*, *dhs* and *dohh* 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* ANKA wildtype strain transgenic for plasmodial eIF-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 *eif-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 *eif-5A* and *dhs* genes “knock in” experiments will be performed.

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08

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

Carine F. Djuika[1], Sabine Fiedler[2], Martina Schnölzer[2], Cecilia Sanchez[1], Michael Lanzer[1], Marcel Deponte[1]

[1]Department of Parasitology, Ruprecht-Karls University, Heidelberg, Germany
[2]Genomics & Proteomics Core Facility, German Cancer Research Center (DKFZ), Heidelberg, Germany

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 thiolperoxidases, which utilize one or two cysteine residues to reduce hydroperoxides. For most peroxiredoxins, the electron donor is thioredoxin. One of the five *P. falciparum* peroxiredoxins, *Plasmodium falciparum* Antioxidant Protein (*Pf*AOP), has been partially described *in vitro*. Although its physiological role remains unknown, *Pf*AOP was shown to preferentially use an electron donor system consisting of glutaredoxin and glutathione (GSH) [1]. We now dissected the unusual mechanism of *Pf*AOP *in vitro* [2]: Our data clearly reveal that *Pf*AOP acts via a ping-pong mechanism using *P. falciparum* glutaredoxin (*Pf*Grx) and GSH as true substrates. Only one *Pf*AOP cysteine residue and one *Pf*Grx cysteine residue are sufficient for catalysis. Thus, *Pf*Grx is not just a simple replacement of thioredoxin. Our data also point to a GSH-dependent enzyme activation and a negative subunit cooperativity of *Pf*AOP. In silico analyses furthermore suggest that *Pf*AOP 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.

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09

Protein S-nitrosylation in Plasmodium falciparum

Lihui Wang, J. H. Prieto, E. Fischer, S. Rahlfs, K. Becker

Biochemistry and Molecular Biology, Justus-Liebig University Giessen, Giessen, Germany

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 excets 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 (PfGAPDH) was found to be inhibited by S-nitrosylation on tis 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)

Jörg J. Möhrle

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

Thomas Spangenberg

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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12

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

Béatrice Gréco[1], Philip Hewitt[1], Ralf Schmidt[1], Thomas Spangenberg[2], Jeremy Burrows[2]

[1]Merck Serono, Darmstadt, Germany
[2]Medicines for Malaria Venture, Geneva, Switzerland

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 treatment, 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 $t_{1/2}$ life >100 hrs. It provides complete remission in the *P. berghei* mice model and a slow paracitocidal 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

Markus Wolf[1], Ingrid B. Müller[2], Bernhard Ellinger[1], Oliver Keminer[1], Philip Gribbon[1], Carsten Wrenger[3]

[1]European ScreeningPort GmbH, Hamburg, Germany
[2]Biochemical Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[3]Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Science, University of São Paulo, São Paulo, Brasil

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 *Pf*Pdx1 and *Pf*Pdx2 (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 ^{3}H-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.

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14

A PHIST protein interacts with the intracellular ATS domain of PfEMP1 and localizes to knob structures in P. falciparum

Alexander Oberli[1], Leanne Slater[2], Erin Cutts[2], Françoise Brand[1], Ioannis Vakonakis[2], Hans-Peter Beck[1]

[1]Swiss Tropical and Public Health Institute and University of Basel, Switzerland
[2]Department of Biochemistry, University of Oxford, UK

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 localize 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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15

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

Makoah N. Aminake, Florian Apitius, Rainer Fischer, Gabriele Pradel

Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany

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 mitgut. 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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16

A multimeric protein complex in the sexual stages of Plasmodium falciparum

Andreas von Bohl[1], Andrea Kuehn[2], Nina Simon[2], Vanesa Nkwouano Ngongang[2], Stefan Baumeister[3], Maike Gieseke[2], Marie Adrienne Dude[2], Rainer Fischer[1], Gabriele Pradel[1]

[1]Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany
[2]Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany
[3]Institute for Parasitology, University of Marburg, Marburg, Germany

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 PfWLP-1, which is expressed in gametocytes and here interacts with selected PfCCp proteins. WD40 domains are highly abundant in eukaryotic proteomes and posses a general scaffolding function by mediating protein-protein interactions. We hypothesize that PfWLP1 is involved in the assembly and folding of the PfCCp-based MPCs. We further show that the MPC is linked to the gametocyte surface via the protein interaction of PfCCp4 with Pfs230, which then binds to the GPI-anchored Pfs48/45. Lack of Pfs230 in gene-disruptant parasites results in a destabilization of the parasitophorous vacuolar space in the gametocytes. Previous studies showed that Pfs230 is cleaved at its N-terminal end, once the gametocytes are taken up by the blood-feeding mosquito and gametogenesis is initiated in the mosquito midgut. We demonstrate that Pfs230 processing results in its increased interaction with the MPC components, and impaired Pfs230 processing causes the partial release of the PfCCp proteins from the macrogamete surface. Furthermore, the GPI-anchored Pfs25 locates to the macrogamete surface during gametogenesis, and here also interacts with MPC components. While Pfs25 stays on the surface following zygote formation, the other MPC proteins disappear from the zygote surface within 3 h post-feeding. Noteworthy, several PfCCp proteins can subsequently be detected in intracellular condensed structures during ookinete formation. These structures might resemble the recently identified crystalloids, cell structures that function as protein repositories.

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17

Combined var gene and microsatellite genotyping of Plasmodium falciparum strains to study the inheritance of variant surface antigens

Ellen Bruske, Joana Volk, Matthias Frank

Institute of Tropical Medicine, University of Tuebingen, Tuebingen, Germany

In *Plasmodium falciparum* malaria, cytoadherence of infected red blood cells to host endothelial receptors is mediated by a polymorphic family of surface proteins collectively referred to as *Plasmodium falciparum* membrane protein 1 (PfEMP1). This protein family is considered the primary target of the humoral immune response. Each parasite harbours approximately 60 different variants of the 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* strains 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*. On this account, we characterized the 3D7 strain and its sibling parasite E5. 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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18

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

Dominika J. Maskus, Susanne Bethke, Alexander Boes, Holger Spiegel, Torsten Klockenbring, Rainer Fischer, Stefan Barth, Rolf Fendel

Fraunhofer Institute for Molecular Biology and Applied Ecology, Aachen, Germany

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 Ghanaian 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 IgG1κ1 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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19

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

Priscila Thihara Rodrigues[1,2], Marcelo Urbano Ferriera[1], Leonie Raijmakers[3], Ivo Mueller[3], Ingrid Felger[4]

[1]Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
[2]Scholarship from CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico
[3]Infection & Immunity Division, Walter & Eliza Hall Institute, Parkville, Australia
[4]Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland

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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20

Effect of DPAP-specific inhibitors on gametogenesis in Plasmodium falciparum

Tim Weißbach[1], Ludmilla Sologub[2], Rainer Fischer[1], Gabriele Pradel[1]

[1]Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany
[2]Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany

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 then 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 PfDPAP1 and PfDPAP3 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 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 PfSUB1, which in consequence processes the egress molecule PfSERA5 [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 PfSUB1/PfSERA5-independent pathway.

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21

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

Jannika Katharina Wierk[1], Maria Kamper[1], Heidrun von Thien[1], Anna Bachmann[1], Egbert Tannich[1], Volker Theo Heussler[2], Christina Deschermeier[1]

[1]Department of Molecular Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Institute of Cell Biology, University of Bern, Bern, Switzerland

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-dependant 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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Author index
(referring to abstract number)

Addai-Mensah, Otchere 01, 02
Alferink, Judith 05
Aminake, Makoah N. 15
Apitius, Florian 15
Bachmann, Anna 21
Barth, Stefan 01, 02, 18
Baumeister, Stefan 16
Beck, Hans-Peter 14
Becker, K. 09
Beiss, Véronique 01
Beiss, Veronique 03
Benz, Michaela 01
Bethke, Susanne 18
Blessing, Daniel 01
Boes, Alexander 01, 03, 18
Brand, Françoise 14
Bruske, Ellen 17
Burrows, Jeremy 12
Buyel, Johannes 01
Cutts, Erin 14
Dahm, Pia 01
Deponte, Marcel 08
Deschermeier, Christina 21
Djuika, Carine F. 08
Drossard, Jürgen 01
Dude, Marie Adrienne 16
Edgü, Güven 01
Ellinger, Bernhard 13
Faber, Bart 01
Felger, Ingrid 19
Fendel, Rolf 01, 02, 18
Ferriera, Marcelo Urbano 19
Fiedler, Sabine 08
Fischer, E. 09
Fischer, Rainer 01, 02, 03, 15, 16, 18, 20
Frank, Clemens 04
Frank, Matthias 17
Geese, Markus 01
Gieseke, Maike 16
Gréco, Béatrice 12
Gribbon, Philip 13
Haßlmeyer, Erik 01
Hellwig, Stephan 01
Heussler, Volker Theo 21
Hewitt, Philip 12
Holland, Tanja 01
Hörauf, Achim 05
Horstmann, Rolf 01
Houdelet, Marcel 01
Hügel, Christian 01
Kaiser, Annette 07
Kamper, Maria 21
Kapelski, Stephanie 01, 02
Kastilan, Robin 01
Keminer, Oliver 13
Kersting, David 07
Klockenbring, Torsten 02, 18
Kostka, Günther 01
Kreuels, Benno 04
Krüger, Mirco 07
Krumkamp, Ralf 04
Kuehn, Andrea 16
Küpper, Janina M. 05
Lanzer, Michael 08
Limmer, Andreas 05
Maskus, Dominika J. 18
Maskus, Dominika 01, 02
May, Jürgen 04
Möhrle, Jörg J. 10
Mueller, Ivo 19
Müller, Ingrid B. 13
Münzenmayer, Christian 01
Ngongang, Vanesa Nkwouano 16
Oberli, Alexander 14
Owusu-Dabo, Ellis 04
Pradel, Gabriele 01, 03, 15, 16, 20
Prieto, J. H. 09
Rademacher, Thomas 01
Rahlfs, S. 09
Raijmakers, Leonie 19
Reimann, Andreas 01, 02
Reiss, Kristian 01
Remarque, Edmond 01
Rodrigues, Priscila Thihara 19
Sack, Markus 01, 03
Sanchez, Cecilia 08
Sarpong, Nimako 04
Scheuermayer, Matthias 01, 03
Schillberg, Stefan 01
Schinkel, Helga 01
Schmidt, Kim E. 05
Schmidt, Ralf 12
Schnölzer, Martina 08
Scholz, Oliver 01
Schumak, Beatrix 05
Seidel, Melanie 01, 02
Simon, Nina 16
Slater, Leanne 14
Sologub, Ludmilla 20
Sothmann, Peter 04
Spangenberg, Thomas 11, 12
Specht, Sabine 05
Spiegel, Holger 01, 18
Tannich, Egbert 01, 21
Vakonakis, Ioannis 14
Volk, Joana 17
von Bohl, Andreas 16
von Thien, Heidrun 21
Vöpel, Nadja 01
Wang, Lihui 09
Weigand, Christian 01
Weißbach, Tim 20
Wenzel, Christian 01
Wierk, Jannika Katharina 21
Wiesner, Jochen 06
Wolf, Markus 13
Wrenger, Carsten 13