Advances in Plasmodium vivax Malaria Research

May 28 - 29, 2013

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Gao Qi, PhD
Jiangsu Institute of Parasitic Diseases

Barbara Sina, PhD
Fogarty International Center

Neena Valecha, MBBS, MD
National Institute of Malaria Research, India
Acknowledgement of Support

Grant Support

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iVAX Travel Scholarships were provided by the iVAX consortium with support from the Bill & Melinda Gates Foundation.

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iVAX Travel Scholarships were provided by the iVAX consortium with support from the Bill & Melinda Gates Foundation.
The Scientific Organizing Committee and presenting organizations, the New York Academy of Sciences, “la Caixa” Foundation, B-DEBATE International Center for Scientific Debate, Barcelona Institute for Global Health (ISGlobal), and the Barcelona Centre for International Health Research (CRESIB), are pleased to welcome you to our conference **Advances in *Plasmodium vivax* Malaria Research**.

This conference has brought together leading and emerging investigators and clinicians working in the fields of *P. vivax* biology and genomics, host-parasite interactions, novel research techniques to overcome barriers to *in vitro*, *in vivo*, and clinical study of *P. vivax*, drug resistance and drug discovery, and recent clinical trial and in-field efforts in *P. vivax* prevention, treatment, control, and elimination. Our goal is to provide you with the most conducive environment for a lively, informed, and synergistic conversation about the latest advances and remaining challenges in this field.

Please see our conference agenda to learn more about the speakers featured in the plenary sessions. Day 1 of this conference will primarily address the latest advances in bench research. Day 2 will primarily focus on the translation of this research from the bench to the field. A series of **Hot Topic** presentations have been selected from late-breaking abstract submissions. In addition, a selection of rapid fire **Turbo Talks** will highlight exciting Poster Presentations, as selected by the Scientific Organizing Committee. We hope that you will take full advantage of the special features of this conference by joining us for the poster sessions, networking reception, and lunchtime workshop on **Editor’s Guide to Writing and Publishing Your Paper** (Day 1).
We are proud to announce that highlights of this conference will be published as an open-access multimedia Academy eBriefing report, which will include an archived selection of slides and audio from the conference presentations. These materials will be available on the New York Academy of Sciences’ website (www.nyas.org) shortly following the conference.

We hope this conference meets your expectations. Please do not hesitate to notify us and our staff of any questions or concerns.

Ivo Mueller, PhD
Chairman, Scientific Organizing Committee

Ellis Rubinstein
President and Chief Executive Officer, The New York Academy of Sciences

Pedro Alonso, PhD
Professor and Director, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)

Enric Banda, PhD
Director of Science, Research and Environment, "la Caixa" Foundation

Montserrat Vendrell, PhD
Chief Executive Officer, BIOCAT

TRANSPORTATION

Bus Schedule:
May 28th
Leave Hotel Jazz to CosmoCaixa @ 7:45 am
Leave CosmoCaixa to Hotel Jazz @ 8:00 pm

May 29th
Leave Hotel Jazz to CosmoCaixa @ 7:45 am
Leave CosmoCaixa to Hotel Jazz @ 5:40 pm
All faculty participating in this activity are required to disclose to the audience any significant financial interest and/or other relationship with the manufacturer(s) of any commercial product(s) and/or provider(s) of commercial services discussed in his/her presentation and/or the commercial contributor(s) of this activity.

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<tr>
<th>Faculty Name</th>
<th>Position</th>
<th>Financial Interest/Relationship</th>
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<tr>
<td>John H. Adams, PhD</td>
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<td>Pedro Alonso, PhD</td>
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<td>J. Kevin Baird, PhD</td>
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<td>Azucena Bardaji, MD, PhD</td>
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<td>Quique Bassat, MD, PhD</td>
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<td>Nicholas Cammack, PhD</td>
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<td>GlaxoSmithKline (GSK)</td>
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<td>Brice Campo, PhD</td>
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<td>Hernando del Portillo, PhD</td>
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<td>Rhoel R. Dinglasan, PhD, MPH</td>
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<td>Mary R. Galinski, PhD</td>
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<td>Brooke Grindlinger, PhD</td>
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<td>David C. Kaslow, MD</td>
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<td>Christopher L. King, MD, PhD, MPH</td>
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<td>Marcus V. Guimaraes Lacerda, MD, PhD</td>
<td>Research Support</td>
<td>GSK and Sanofi-Aventis</td>
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<td>Alan J. Magill, MD</td>
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<td>Dominique Mazier, PhD</td>
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## Faculty Disclosures

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<td>Ivo Mueller, PhD</td>
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<td>Daniel E. Neafsey, PhD</td>
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<td>Robert D. Newman, MD, MPH</td>
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<td>Jetsumon Sattabongkot Prachumsrisri, PhD</td>
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<tr>
<td>Ric Price, MD</td>
<td>Consultant</td>
<td>Member of data safety monitoring board</td>
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<td>Gao Qi, PhD</td>
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<tr>
<td>George Dennis Shanks*, MD, MPH</td>
<td>Consultant</td>
<td>Unpaid consultant to GSK’s tafenoquine development project for which travel reimbursement was received</td>
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<td>Barbara Sina, PhD</td>
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<td>Paul Sharp, PhD</td>
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<td>Melanie Brickman Stynes, PhD, MSc</td>
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<td>Marcel Tanner, PhD, MPH</td>
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<tr>
<td>Neena Valecha, MBBS, MD</td>
<td>Research Support</td>
<td>Clinical trials; support by organizations like Medicines for Malaria Venture (MMV)</td>
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<td>Consultant</td>
<td>Expert Scientific Advisory Committee member of MMV, Member Advisory Board of MMV – GSK for development of tafenoquine</td>
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An * after the speaker’s name indicates that the speaker intends to discuss unlabeled uses of commercial product, or an investigational use of a product not yet approved for this purpose. The speaker will disclose this information during his/her presentation.
DAY 1: TUESDAY, MAY 28, 2013

8:00 AM  Registration, Continental Breakfast, and Poster Session I Setup

8:45 AM  Welcome Remarks
Enric Banda, PhD, "la Caixa" Foundation
Miquel Marti, B-DEBATE
Ivo Mueller, PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB), and Walter and Eliza Hall Institute
Pedro Alonso, PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)
Brooke Grindlinger, PhD, The New York Academy of Sciences

9:15 AM  Keynote Lecture
The Need for a Global Strategic Plan for Plasmodium Vivax Control and Elimination
Robert D. Newman, MD, MPH, World Health Organization
The Keynote Lecture is generously sponsored by Fundación Ramon Areces

SESSION 1: FROM THE BENCH: ‘OMICS CONTRIBUTION TO OUR UNDERSTANDING OF P. VIVAX BIOLOGY
Co-Chairs: Jane M. Carlton, PhD, New York University and Daniel E. Neafsey, PhD, Broad Institute

10:00 AM  Global P. vivax Diversity: From Genome to Populations or a War in Progress
Jane M. Carlton, PhD, New York University

10:20 AM  Recent Advances in Vector Genomic Resources
Daniel E. Neafsey, PhD, Broad Institute

10:40 AM  Multi-OMICS Approaches for Dissecting P. vivax-Vector Interactions
Rhoel R. Dinglasan, PhD, MPH, Johns Hopkins University

11:00 AM  Hot Topic Talk selected from Late-breaking Abstract Submissions

11:15 AM  Hot Topic Talk selected from Late-breaking Abstract Submissions

11:30 AM  Networking Coffee Break
SESSION 2: FROM THE BENCH: UNDERSTANDING HOST-PARASITE-VECTOR INTERACTIONS
Co-Chairs: Hernando del Portillo, PhD, ICREA at Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB) and Fabio M. Costa, PhD, Instituto de Biologia Universidade Estadual de Campinas

12:00 PM Systems Biology Approaches to P. vivax Research
Mary R. Galinski, PhD, Emory University

12:20 PM From Host-Parasite Biology to Antigen Discovery
Hernando del Portillo, PhD, ICREA at Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)

12:40 PM Hot Topic Talk selected from Late-breaking Abstract Submissions

12:55 PM Turbo Talks 1–4
Brief presentations highlighting exciting Poster Presentations

1:25 PM Networking Lunch and Poster Viewing

2:15 PM Early Career Investigator Mentoring Workshop
(Concurrent with Lunch)
(For graduate students, post-doctoral fellows, and junior faculty)

Editor's Guide to Writing and Publishing Your Paper:
Brooke Grindlinger, PhD, The New York Academy of Sciences
Former Editor, Journal of Clinical Investigation
In this 45-minute workshop, participants will gain an inside look into the editorial review process and how to best present the results of their work for publication. Travel Fellowship awardees are required to attend.

SESSION 3: FROM BENCH TO FIELD: ADVANCES IN UNDERSTANDING PROTECTIVE IMMUNITY AND VACCINES
Co-Chairs: John H. Adams, PhD, University of South Florida and Luzia Helena Carvalho, PhD, Centro de Pesquisas René Rachou, Fundacao Oswaldo Cruz

3:00 PM Mechanisms of Naturally Acquired Immunity to P. vivax Malaria
Christopher L. King, MD, PhD, MPH, Center for Global Health and Diseases, Case Western Reserve University

3:20 PM Strategic Approaches to Developing a P. vivax Vaccine
David C. Kaslow, MD, PATH Malaria Vaccine Initiative
SESSION 4: FROM BENCH TO FIELD: BIOLOGY AND EPIDEMIOLOGY OF RELAPSE IN P. VIVAX
Co-Chairs: J. Kevin Baird, PhD, Eijkman-Oxford Clinical Research Unit and Mary R. Galinski, PhD, Emory University

5:10 PM The Epidemiology of P. vivax Relapses
J. Kevin Baird, PhD, Eijkman-Oxford Clinical Research Unit

5:30 PM Relapse in vitro/ex vivo: Where Are We?
Dominique Mazier, PhD, National Institute of National Health and Medical Research (INSERM) and University of Pierre & Marie Curie

5:50 PM Hot Topic Talk selected from Late-breaking Abstract Submissions

6:05 PM Panel Discussion

6:35 PM Networking Reception and Poster Session I

8:00 PM Day 1 Concludes
9:40 AM  Discovery Pipeline for Relapse Prevention  
Brice Campo, PhD, Medicines for Malaria

10:00 AM  Turbo Talks 8–10  
Brief presentations highlighting exciting Poster Presentations

10:15 AM  Hot Topic Talk selected from Late-breaking Abstract Submissions

10:30 AM  Networking Coffee Break

SESSION 6: FROM BENCH TO BEDSIDE: PATHOPHYSIOLOGY OF SEVERE P. vivax MALARIA
Co-Chairs: Marcus V. Guimaraes Lacerda, MD, PhD, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado and Nicholas Anstey, PhD, Menzies School of Health Research

11:15 AM  An Overview on Severe P. vivax and its Pathogenesis  
Nicholas Anstey, PhD, Menzies School of Health Research

11:35 AM  Fatal P. vivax and Implications for Understanding its Pathophysiology  
Marcus V. Guimaraes Lacerda, MD, PhD, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado

11:55 AM  A Multicenter Descriptive Study of Plasmodium vivax–associated Admissions to Reference Hospitals in Brazil and India: Is Severe Disease the Same Everywhere?  
Quique Bassat, MD, PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)

12:15 PM  Hot Topic Talk selected from Late-breaking Abstract Submissions

12:30 PM  Hot Topic Talk selected from Late-breaking Abstract Submissions

12:45 PM  Turbo Talks 11–13  
Brief presentations highlighting exciting Poster Presentations

1:00 PM  Networking Lunch and Poster Session II

SESSION 7: FROM BENCH TO THE FIELD: P. vivax IN AFRICA, INCLUDING HOST GENETICS ADAPTATION, AND EPIDEMIOLOGY
Chair: Ivo Mueller, PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB), and Walter and Eliza Hall Institute

2:30 PM  P. vivax in African Apes  
Paul Sharp, PhD, University of Edinburgh
AGENDA

2:50 PM  
**Burden of P. Vivax Infection During Pregnancy: Preliminary Results from the PregVax Multicenter Collaborative Project**  
Azucena Bardají, MD, PhD, Barcelona Centre for International Health Research (CRESIB)-Hospital Clínic, University of Barcelona

3:10 PM  
**Hot Topic Talk selected from Late-breaking Abstract Submissions**

3:25 PM  
**Hot Topic Talk selected from Late-breaking Abstract Submissions**

3:40 PM  
**Networking Coffee Break**

SESSION 8: FROM BENCH TO IMPACT: HOW DOES P. VIVAX FIT INTO THE GLOBAL ERADICATION AGENDA?  
Co-Chairs: Jetsumon Sattabongkot Prachumsri, PhD, Mahidol University and Gao Qi, PhD, Jiangsu Institute of Parasitic Diseases

4:10 PM  
**Successful Elimination of Malaria in P. falciparum and P. vivax Co-endemic Areas**  
Speaker to be announced

4:30 PM  
**The Need for a Specific Focus on P. vivax in the Malaria Elimination Science Agenda**  
Pedro Alonso, PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)  
Marcel Tanner, PhD, MPH, Swiss Tropical Health Institute

4:50 PM  
**Panel Discussion**  
P. vivax: The Last Parasite Standing  
Moderator: Barbara Sina, PhD, Fogarty International Center

5:20 PM  
**Closing Remarks**  
Alan J. Magill, MD, Bill & Melinda Gates Foundation

5:40 PM  
**Conference Adjourns**

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The Need for a Global Strategic Plan for Plasmodium Vivax Control and Elimination


While Plasmodium falciparum is responsible for the vast majority of cases and deaths from malaria worldwide, Plasmodium vivax, the most geographically widespread species, is responsible for a large number of cases; it is increasingly recognized as a cause of severe malaria and even death. There are an estimated 2.6 billion people at risk of P. vivax; and the World Malaria Report 2011 estimated 19.4 million P. vivax cases (range 13.4 to 24.6 million) in 2010, with the greatest number in Asia and Latin America. Many countries have exclusively P. vivax transmission. Abundant data show that transmission of P. falciparum is more responsive to control measures. The scale up of integrated malaria control measures generally results in a shift in balance between the two species such that P. vivax becomes dominant. P. vivax is increasingly becoming resistant to chloroquine, the primary drug used for treatment.

The prevention of P. vivax, especially in settings where vectors are exophilic and/or exophagic, has received inadequate attention. Although control strategies such as seasonal mass drug administration (MDA) with primaquine have been used successfully in some settings in Central Europe and Asia, inadequate documentation of safety and efficacy has prevented the wider uptake of such interventions. Parasitological diagnosis of P. vivax has been hampered by late development and slow roll out of highly sensitive and specific bivalent Rapid Diagnostic Tests.

Despite a WHO recommendation that confirmed P. vivax infection be radically treated with primaquine, it is not policy in all transmission areas; where it is policy, such treatment is sometimes not prescribed by health workers due to fears of primaquine-induced haemolytic anaemia among patients with G6PD deficiency, for which reliable field tests are still not available. Globally, there remains confusion and disagreement over dosages and treatment duration. While many WHO technical and strategy documents makes reference to P. vivax, there has never been a specific global strategy with time-bound objectives that articulates how to approach the problem of P. vivax at global, regional, and country levels. Such a strategy has now been called for by the WHO Malaria Policy Advisory Committee as a first step in developing a Global Technical Strategy for Malaria Control and Elimination 2016–2025. This P. vivax strategy will be based on: 1) a review of the most recent evidence on programmatic effectiveness of different prevention, control and surveillance interventions of vivax malaria; 2) a review of the current policy and practice on P. vivax service delivery at country and regional level; 3) a review of P. vivax-specific recommendations that are dispersed across various WHO guidance documents; 4) an analysis of on-going research with regard to P. vivax, and how results emerging from such work are likely to influence control and elimination strategies over the next decade, and what research gaps remain; and 5) an economic analysis of the requirements for P. vivax control and elimination.
Global P. vivax Diversity: From Genome to Populations or a War in Progress

Jane M. Carlton, PhD, New York University, New York, New York

Abstract not available at the time of printing.

Recent Advances in Vector Genomic Resources

Daniel E. Neafsey, PhD, Broad Institute, Cambridge, Massachusetts

For more than 10 years, the only publically available, high quality, annotated reference genome assembly for a malaria vector mosquito has been that of Anopheles gambiae. Given the effectively disjoint geographic ranges of this vector species and Plasmodium vivax, investigators in the P. vivax research community have had limited opportunity to take advantage of vector genomic resources to better understand transmission biology.

This situation is poised to change. The Broad Institute, in collaboration with VectorBase, the MR4, and many members of the Anopheles research community, has begun releasing draft de novo assemblies of 16 species of Anopheles, including several important vectors of P. vivax. Several years of research and development were invested in devising an optimal strategy for sequencing and assembling these repetitive and highly heterozygous genomes using short-read Illumina next generation sequencing, and the end result will be a collection of assemblies that will rival the original Sanger sequencing-based An. gambiae assembly in quality. It is hoped that these new vector genomic resources will enable a better understanding of vectorial capacity, and facilitate investigations into the transmission biology of all malaria parasites in a range of geographic contexts outside of sub-Saharan Africa.

Multi-OMICS Approaches for Dissecting P. vivax-Vector Interactions

Rhoel R. Dinglasan, PhD, MPH, Johns Hopkins University, Baltimore, Maryland

Although the molecular (genomics) information for Plasmodium vivax is growing steadily, the same cannot be said for the mosquito vectors of P. vivax malaria. Despite the inclusion of a few relevant anopheline vectors from P. vivax-endemic regions into the genome sequencing pipeline, the information for these vectors remains remarkably deficient. While the genome of the "model" African P. falciparum vector, Anopheles gambiae, has been sequenced, substantial evolutionary divergence limits its utility as a reference across anophelines, especially with respect to several non-sequenced P. vivax vectors. This critical gap in knowledge was highlighted in papers resulting from the malaria eradication research agenda (malERA) effort that were published in PLoS Medicine in 2011. To help fill this gap, we recently reported on the development and application of a hybrid bioinformatic approach that
provided not only whole midgut transcriptomic data for the non-sequenced \textit{P. vivax} vector (\textit{Anopheles albimanus}), but also mosquito midgut microvilli (MMV) proteomic information. This allowed us to perform a direct comparison of \textit{A. gambiae} and \textit{A. albimanus} MMV proteomes with the aim of identifying conserved, mosquito-based transmission-blocking vaccine targets. We envision that this analytical platform can be applied to other \textit{P. vivax}-vectors across the globe, even for those species which are difficult to colonize. Applying this approach to \textit{Anopheles dirus} in particular, can hopefully provide comparative insight into the complement of MMV proteins involved in \textit{P. vivax} vs. \textit{P. falciparum} invasion of the \textit{A. dirus} midgut; especially having observed that antibodies against a novel secreted MMV molecule found in both \textit{A. gambiae} and \textit{A. dirus}, only blocks \textit{P. falciparum} oocyst development.

**Systems Biology Approaches to \textit{P. vivax} Research**

\textbf{Mary R. Galinski}, PhD, Emory University, Atlanta, Georgia

We are at an unimaginable point at the cutting edge of science, where breakthroughs may be possible that were unthinkable even a few short years ago. New ways forward are important to study \textit{Plasmodium vivax}; New World Monkey infections and \textit{P. cynomolgi-rhesus} monkey model systems hold much promise. The lack of robust \textit{in vitro} culture systems for blood and liver-stage forms has been a hindrance, but much can be done regardless — and with the benefit of \textit{in vivo} and \textit{ex vivo} samples. The \textit{in vivo} environment matters. Studying the parasite in the context of its host is important and arguably the only true way to research and understand “the disease.” Basic ongoing research to understand the liver-stage forms including hypnozoites and relapses, merozoites and invasion of reticulocytes, the infected erythrocyte’s caveolae vesicle complexes, and virulence resulting from \textit{P. vivax} infections will be presented. The Malaria Host-Pathogen Interaction Center (MaHPIC) will also be introduced. The MaHPIC was created to take a systems biology approach to studying malaria infections caused by \textit{P. vivax}, \textit{P. cynomolgi} and other species (http://mahpic.emory.edu or www.systemsbiology.emory.edu). The central unifying hypothesis of this project is: “Non-Human Primate host interactions with \textit{Plasmodium} pathogens as model systems will provide insights into mechanisms as well as indicators for human malarial disease conditions.”
From Host-Parasite Biology to Antigen Discovery

Hernando del Portillo, PhD, ICREA at Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain

Our goal is to discover new antigens to advance vaccine development against Plasmodium vivax. We have used a systems approach to identify coding-genes whose expression is dependent on an intact spleen as we hypothesize that such genes will encode proteins that are the targets of protective immune responses in natural infections. To look for associations with clinical protections we have used the sera from children 1-5 years of age from a prospective longitudinal study from Papua New Guinea. In addition, to look for mechanistics insights of protection, we have developed functional cytoadherence assays on human spleen and lung fibroblasts and tested whether P. vivax-infected reticulocyte cytoadhere to such fibroblasts. This is the first report on the discovery of novel antigens involved in acquisition of natural protective immunity in P. vivax and in challenging the dogma that human Plasmodium-infected cells avoid passage through the spleen.

SCIENCE ALLIANCE WORKSHOP

Editor’s Guide to Writing and Publishing Your Paper

Brooke Grindlinger, PhD, The New York Academy of Sciences, New York, New York

Publishing is critical to the scientific and medical professions, yet training and guidance on the topic is very limited. Brooke Grindlinger, PhD, Executive Director of Scientific Programs at the New York Academy of Sciences and former Science Editor and interim Executive Editor at the Journal of Clinical Investigation, will discuss the elements of a well written scientific paper, highlight strategies for selecting the appropriate journal, and provide an overview of the review process, including how to navigate resubmissions and rebuttals.

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Mechanisms of Naturally Acquired Immunity to P. vivax Malaria

Simón Méndez-Ferrer, Christopher L. King, MD, PhD, MPH, Case Western Reserve University, Cleveland, Ohio

Naturally acquired immunity to Plasmodium vivax malaria differs in fundamental respects compared to that of the much better studied P. falciparum. For example, individuals acquire immunity to P. vivax more quickly than P. falciparum irrespective of overall transmission intensity resulting in the peak burden of P. vivax malaria in younger age groups. Similarly, actively induced P. vivax infections in malaria therapy patients resulted in faster and generally more strain transcending acquisition of immunity than P. falciparum infections. Potential mechanisms behind the more rapid acquisition of immunity to P. vivax are poorly understood. Natural acquired immune responses to P. vivax target both pre-erythrocytic and blood-stage antigens and include humoral and cellular components. To date, only a few studies have investigated the association of these immune responses with protection, with most studies focusing on a few merozoite antigens [such as the Duffy binding protein (PvDBP), the reticulocyte binding proteins (PvRBPs), or merozoite surface proteins (PvMSP1, 3, and 9)] or the circumsporozoite protein (PvCSP). Naturally acquired transmission blocking immunity was also found in several populations. Though limited, these data support the development of a multi-stage P. vivax vaccine may be feasible.

Strategic Approaches to Developing a P. vivax Vaccine

David C. Kaslow, MD, PATH Malaria Vaccine Initiative, Seattle, Washington

“Malaria due to P. falciparum is the most deadly form and it predominates in Africa; P. vivax is less dangerous but more widespread (WHO Global Malaria Programme World Malaria Report 2012).” This epidemiologic difference — along with biological differences between these two species of the genus Plasmodium — calls for different strategic approaches and target product profiles for vaccines against P. falciparum and P. vivax. The epidemiologic differences highlight the need for P. vivax vaccines that can contribute to control and elimination efforts. The biologic differences present different challenges and opportunities at each of the three target stages of the lifecycle, particularly the liver stage hypnozoites, the Duffy blood group antigen invasion pathway, and the biology of the gametocyte. The latter may provide a unique opportunity in the controlled human malaria infection (CHMI) model to study the effect of immunization on parasite transmission. The strategic goals, the approaches to translational vaccine research and development, and some suggested priorities for a P. vivax malaria vaccine technology roadmap will be presented for discussion.
The Epidemiology of *P. vivax*

**J. Kevin Baird**, PhD, Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia

A single infectious mosquito bite does not result in a single attack of *Plasmodium vivax* malaria within two weeks — that bite typically provokes a primary attack followed by as many as 20 others for about two years. These secondary attacks are called relapses and stem from activated hypnozoites placed in the liver at the single infectious bite. Hypnozoites greatly amplify the complexity of the epidemiology of *Plasmodium vivax*, and, in turn, the prevention, control, and treatment of the infection as a public health problem. The risk, rate, and timing of relapse varies tremendously among strains and seems to hinge upon geographically defined seasonal abundance of anopheline vectors, along with the absolute numbers of sporozoites introduced (as a single bite or multiple bites). Consideration is also given to the relatively very low and self-limiting parasitemias of *P. vivax* malaria with respect to diagnostics limited to peripheral blood examination. These patterns and considerations are reviewed in the context of their impact upon fundamental epidemiological measures of force of infection, burdens of morbidity, and as critically important confounding factors in trials of chemotherapy against both asexual blood stages and hypnozoites. Strategies for coping with these confounders and deriving estimates of force of infection from both sporozoites and hypnozoites will be reviewed. The “hypnozoite reservoir” is explained as an important and useful concept in defining the epidemiology of *P. vivax*.

Relapse in vitro/ex vivo: Where Are We?

**Dominique Mazier**, PhD, National Institute of National Health and Medical Research (INSERM) and University of Pierre & Marie Curie, Paris, France

*Plasmodium vivax* hypnozoites, dormant liver forms that activate months to years after the initial infection to produce relapse episodes, pose a serious hurdle to the control and elimination of malaria in many of the endemic regions of the world. Toxicity concerns restrict the deployment of the only available drug (primaquine) that can eliminate hypnozoites. Thus, novel safe anti-hypnozoite drugs are urgently needed. However, screening for such drugs or indeed investigations on hypnozoites that might guide drug discovery has been hampered by the lack of models, molecular, biochemical, or cellular investigations being hitherto difficult to carry out in their natural hosts (humans infected with *P. vivax* or macaques infected with *P. cynomolgi*). We have recently developed a protocol for the *in vitro* cultivation of hypnozoites in their natural target cells, the primary hepatocyte, thereby making it possible for the first time to investigate hypnozoite biology (Dembele, Gego and al, PLoS ONE, 2011). This model has been improved since as follows: a) we have optimized the cultivation protocols such that the infected cultures can now be routinely maintained for forty (40) days, b) we have obtained...
The Rise of Drug Resistant P. vivax: The Role of ACTs

Ric Price, MD, Oxford University and Menzies School of Health Research, Casuarina, Northern Territory, Australia

The first-line treatment of Plasmodium vivax malaria has changed little within the last 60 years, with blood-stage P. vivax infections mostly still treated with chloroquine for the blood stage plus the primaquine for eradication of the hypnozoites. Chloroquine resistant (CQR) P. vivax was first reported 20 years ago on the Island of New Guinea, however subsequent surveillance and precise mapping of its spread has been undermined by the inherent complexity of distinguishing resistant from relapsing infections and the variation in relapsing patterns across the P. vivax endemic world. A comprehensive review of literature between 1960 and 2012, has identified 119 clinical trials and 31 case reports. These reports highlight a number of key elements of the in vivo response that are needed to detect early signs of resistance, including the measurement of in vivo drug concentrations and parasite clearance times. The literature reveals evidence for declining chloroquine efficacy in P. vivax from all but a few P. vivax endemic locations. The degree of chloroquine resistance remains modest in most areas (apart from Indonesia and Papua New Guinea), however is likely to continue to evolve, emphasising the need for alternative treatment strategies. A number of schizontocidal agents have been tested against drug-resistant P. vivax and shown to have good efficacy; many of these are available as Artemisinin Combination Therapies (ACTs) raising calls for a unified ACT-based strategy for both P. falciparum and P. vivax malaria in co-endemic areas. This presentation will review the evidence for the emergence and spread of drug-resistant P. vivax and give an overview for the role of ACTs in the treatment of P. vivax.

ABSTRACTS

compelling evidence that the small non-dividing forms are activated to pursue their development leading to mature hepatic schizonts (sumitted). This allowed the identification of a molecule which induces the activation of hypnozoite to resume maturation. In parallel, we have established new in vivo models of humanized mice engrafted with primary hepatocytes were both schizonts and hypnozoites develop. These tools will be of significant value not only for biological investigations on, but also for pre-clinical validation of compounds targeting the hypnozoite. The ultimate goal is to apply this data to rationalize the search for novel drugs that will lead to eliminate hypnozoites and their associated relapse episodes.

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**Improving the Use of Primaquine**

George Dennis Shanks, MD, MPH, Australian Army Malaria Institute, Enoggera, Queensland, Australia

Primaquine is an 8-aminoquinoline antimalarial drug that has been in use to cure relapsing malaria for more than 60 years. Primaquine remains the only registered compound that kills the residual hepatic parasites (hypnozoites) of *Plasmodium vivax* and *P. ovale*, as well as blocks transmission by killed sexual forms ingested by the Anopheline vector. Primaquine is a problematic drug due to its propensity to cause hemolysis in persons with one of the many the genetic polymorphisms that produces glucose-6-phosphate dehydrogenase deficiency.

Despite being an old medication, many issues remain to be clarified about primaquine. These include the optimum regimen to kill hypnozoites while minimizing hemolysis and gaining maximal medication adherence from a population that does not feel ill. Drug combinations with quinine or chloroquine show synergy for uncertain reasons and the actual presumed active metabolite of primaquine is unknown.

*P. vivax* malaria is usually the last parasite remaining after highly effective control programs are instituted and malaria mortality vanishes. Killing the clinically invisible *P. vivax* hypnozoites remains an enormous challenge. Mass drug campaigns in which literally tens of millions of persons were treated have been conducted in China, Nicaragua and parts of the former Soviet Union. These massive programs result in an un-quantitated risk of death due to G6PD hemolysis and renal failure. Although field tests for G6PD deficiency are under development, it is difficult to see how these could be used in a large mass drug administration program. Further field research is required to determine how to appropriately use primaquine in populations.

**Discovery Pipeline for Relapse Prevention**

Brice Campo, PhD, Medicines for Malaria, Geneva, Switzerland

Malaria remains a disease of devastating global impact, killing more than 660,000 people every year — the vast majority being children under the age of five. *Plasmodium vivax* puts as many people at risk as *P. falciparum* and is more prevalent outside of sub-Saharan Africa. In 2007, a call for malaria eradication was made to researchers in the antimalarial community. To meet this objective, medicines that block the relapse of *P. vivax* and eliminate the asymptomatic and hepatic dormant forms (hypnozoites) of *P. vivax* need to be developed. Over the last decade there has been an increased investment in antimalarial research and development through the work of organizations such as Medicines for Malaria Venture (MMV), their partners, and others; new molecules with new modes of action are entering into preclinical development and beyond. Despite the relative abundance of projects at certain stages targeting asexual stages, particularly, very few drugs are directly targeting
Plasmodium vivax hypnozoites. This is because of the lack of robust and reliable in vitro liver stage assays to, first, allow testing of large compound libraries (to find starting points — “hits”) and, second, to support medicinal chemistry (to optimize “hits” to candidate drugs). This presentation will review the current Global Malaria Portfolio focusing on drugs in development that target P. vivax and on the MMV strategy to increase the number of hypnozoiticidal drug candidates. As part of this, the strategy to deliver accessible in vitro decision making assays for P. vivax liver and blood stages will be discussed.

An Overview on Severe P. vivax and its Pathogenesis

Nicholas Anstey, PhD, Global and Tropical Health Division, Menzies School of Health Research and Department of Infectious Diseases, Royal Darwin Hospital, Darwin, Australia

Plasmodium vivax is responsible for major morbidity and significant mortality in P. vivax-endemic areas. Mechanisms underlying the pathogenesis of severe manifestations, including severe anemia, lung injury, acute kidney injury, shock, multiorgan failure and coma remain incompletely understood. While it is uncommon for a single infection causing febrile illness in otherwise healthy individuals to progress to severe disease, particularly with early treatment and anti-relapse therapy, it has long been recognised that P. vivax can cause severe illness and fatal outcome in the presence of acute and chronic comorbidities. Such comorbidities, including malnutrition, anemia, concurrent bacterial infections and chronic liver, renal and lung disease are common in P. vivax-endemic regions. While some of these comorbidities may be alternative causes of severe and fatal disease in coincidentally parasitized patients, others are likely to be major cofactors in the genesis of severe pathology following P. vivax infections.

Fatal P. vivax and Implications for Understanding its Pathophysiology

Marcus Vinícius Guimarães Lacerda, MD, PhD, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Amazonas, Brazil

Severe disease attributable to Plasmodium vivax infection is already well described worldwide, however, autopsies in these patients are scarce. From 1996 to 2010, 19 patient deaths with a clinical diagnosis of P. vivax infection occurred in a tertiary care center in the Brazilian Amazon. Seventeen of these 19 deaths were fully autopsied. Clinical charts, macroscopic autopsy reports and stored paraffinized tissue blocks were retrieved. Nested-PCR was performed in paraffinized samples of spleen and lung to confirm P. vivax mono-infection. Immunohistofluorescence was used to detect P. vivax parasitized red blood cells (RBCs). Of 17 autopsies, 13 revealed that death could be attributed to P. vivax infection; and in the other remaining four; acute diseases other than malaria were
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A Multicenter Descriptive Study of *Plasmodium vivax*-Associated Admissions to Reference Hospitals in Brazil and India: Is Severe Disease the Same Everywhere?

Quique Bassat, MD, PhD, Barcelona Centre for International Health Research (CRESIB), Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain

Severe *Plasmodium vivax* malaria has been increasingly described in the last decade in many endemic areas. Many case reports and series of cases worldwide are consistent with increasing morbidity and mortality, however, clinical characterization in distinct settings is not homogeneous and highly depends on subjective clinical expertise, local laboratory capacity, and characteristics of the population. From 2009 to 2011, a standard clinical protocol was applied in two tertiary care hospitals in Brazil and India to fully characterize patients hospitalized with the diagnosis of *P. vivax* malaria. Brazilian Amazon (Manaus) and Rajasthan (Bikaner) share similar epidemiological characteristics, with more than 90% of *P. vivax* malaria reports and low to moderate transmission, leading to malaria in older age ranges, as compared to the more typically pediatric disease in high endemic areas. During the period of the study, 314 patients were hospitalized in Manaus and 462 in Bikaner. In Manaus, 46.4% presented one or more of the WHO severity criteria (defined for *P. falciparum*), and in Bikaner 37.4%. Severity was very similar in both sites and the major causes of hospitalization were hyperbilirubinemia, severe anemia, and acute renal failure. However, in Manaus co-morbidities and co-infections were more frequent, namely severe hemolysis due to the use of primaquine in G6PD deficient males and dengue virus co-infection. Case Fatality Rate amongst the hospitalized patients was 2.0% in both sites, which is very similar to what was already previously described in places like Indonesia. In conclusion, comparing complications attributable to *P. vivax* infection with a standard clinical protocol in Brazil and India, it was possible to detect very similar types of complications, however in Manaus, co-morbidities and co-infections were more frequent, suggesting that severe *P. vivax* disease may be different depending on the local epidemiological profile. Further similar multicentre studies are needed.

found to be the cause of death. The primary complication in patients in which malaria contributed to death was acute respiratory distress syndrome (ARDS) and pulmonary edema associated with the accumulation of neutrophils in the inter-alveolar space (six cases). Spleen rupture (three cases) and multi-organ dysfunction syndrome (MODS) (three cases) were the second most common complications. One child evolving with coma was also characterized, but no parasite was detected in the brain tissue. In one patient, which developed ARDS, and presented negative peripheral parasitemia by the time of death, scattered parasitized RBCs were seen inside pulmonary capillaries, suggesting some sequestration in the lung. In 13 out of the 17 deceased patients, *P. vivax* infection was the plausible cause of death. However, more studies are needed to understand pathogenesis related to severe disease.
Plasmodium vivax in African Apes

Paul M. Sharp, Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

Plasmodium vivax infection in humans is absent from much of sub-Saharan Africa, due to the near fixation of a mutation that inhibits expression of the P. vivax receptor (the Duffy antigen) on the surface of red blood cells. It is widely considered that P. vivax originated as an ancient cross-species transmission from macaques in SE Asia, since those monkeys harbour the closest known relatives of P. vivax; based on the genetic diversity within P. vivax, and molecular clock assumptions, it has been estimated that this species jump occurred more than 300,000 years ago. We have tested more than 5,000 ape faecal samples, from 76 field sites across central Africa, for the presence of Plasmodium DNA sequences. We first found evidence of six distinct species related to P. falciparum, three infecting chimpanzees and three infecting gorillas. Using a single template amplification approach, we have also been able to obtain P. vivax-like sequences, of mitochondrial, apicoplast and nuclear DNA, from samples from both chimpanzees and gorillas. These ape-derived P. vivax sequences are very closely related to those from human P. vivax. Phylogenetic analyses reveal that the human P. vivax sequences form a monophyletic clade, often within the radiation of sequences from apes; for all loci examined, the P. vivax -like sequences from apes show more diversity than those from humans. These results suggest that human P. vivax had its origin in Africa. Reconsideration of the rate of mutation in Plasmodium suggests a much more recent timescale for human P. vivax infection, consistent with it having selected for the spread of the Duffy-negative mutation.

Burden of P. Vivax Infection During Pregnancy: Preliminary Results from the PregVax Multicenter Collaborative Project

Azucena Bardaji, MD, PhD, Barcelona Centre for International Health Research (CRESIB)-Hospital Clinic, University of Barcelona

It is widely recognised that pregnant women have an increased risk of P. falciparum malaria infection and disease. However, very little is known about the burden of P. vivax in pregnancy and its impact on maternal and child health.

The analysis makes part of a multicentre collaborative cohort study [PregVax, funded 7th FP-HEALTH-201588, the Spanish Government, EUROSAUD Programme, and the Malaria in Pregnancy Consortium] that aims to estimate the burden of P. vivax infection in pregnancy in Brazil, Colombia, Guatemala, Papua New Guinea (PNG) and India. A total of 9325 pregnant women were enrolled at the routine antenatal care and 52% (4888) of them followed-up until delivery. Blood samples were collected for detection of malaria parasitaemia by microscopy and anaemia determination. In a subsample of women the prevalence of P. vivax submicroscopic infections...
by real time PCR was assessed at recruitment and delivery. Preliminary data on the average prevalence of microscopic *P. vivax* infection was of 1.2% and 0.6% at recruitment and delivery, respectively. The average prevalence of submicroscopic *P. vivax* infection was of 9.6% and 6% at recruitment and delivery, respectively. Further data on burden of infection and frequency of anaemia will be presented during the symposium for all the sites.

These findings show that the prevalence of microscopic *P. vivax* infections was low across sites but that of submicroscopic infections was higher. This evidence may contribute to better understand the burden of *P. vivax* infection during pregnancy in low transmission areas and may be of help to guide development of effective malaria control and surveillance strategies.

**Successful Elimination of Malaria in P. falciparum and P. vivax Co-endemic Areas**

Speaker to be announced.

*Abstract not available at the time of printing.*

**The Need for a Specific Focus on P. vivax in the Malaria Elimination Science Agenda**

**Pedro Alonso,** PhD, Barcelona Institute for Global Health (ISGlobal) / Barcelona Centre for International Health Research (CRESIB)

The Malaria Eradication Research Agenda (malERA) initiative identified critical knowledge gaps and tools needed for the eradication of malaria. Most significantly, the outcomes of this consultative process highlighted the need to address the particularities of *Plasmodium vivax*, defining specifically targeted research questions and features of innovative tools for eradication. Interrupting *P. vivax* transmission is envisaged to be particularly challenging due to early development of gametocytes even before the appearance of the first symptoms, development of hypnozoites and therefore relapses, transmission by outdoor biting mosquitoes, and the ability of this human malaria species to complete its life cycle in a wider range of climatic and ecological conditions. Tools intended for *P. vivax* elimination should include drugs that completely clear the parasite from infected humans, including hypnozoites, with a prophylactic effect and that can be used in Mass Drug Administration (MDA) campaigns; vaccines that elicit long lasting immune responses that prevent infection or inhibit gametocyte development, or that induce clearing of infected hepatocytes by cellular immune responses; and new diagnostics techniques for detecting both hypnozoites and G6PD carriers, which would allow MDA. *P. vivax* specific lines of research include: study of the basic biology of the parasite, particularly the stages that could hit transmission; models that help understanding coinfections and, most importantly, the development of *in vitro* and *in vivo* culture systems of the entire life cycle of this crucial species for malaria elimination.
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