# The 2nd Kilimanjaro International PhD Symposium KCMC, Moshi, Tanzania

**27th – 29th November 2013**

# FOREWORD

The Kilimanjaro International PhD Symposium, has four major components; workshop on research development, PhD manuscripts presentations, workshop on Personal Development Planning, and PhD platform. The symposium brings together young scientists from different Northern and Southern Universities to share their research work, and also get opinion or guidance from international experts in the field. It also allows the students to disseminate their scientific research findings. In a way the students use this forum as a mock PhD defence, given the range of experts who attend and comment on their work. The symposium also through the two workshops that are conducted provides to the students the opportunity to develop more skills on research proposal development, and to plan better their career path. The research development workshop also allows selected undergraduate students to present their research proposals which later can be further developed in into full proposal under close supervision of the senior faculty members. In this way the symposium motivates the undergraduate students to consider research as their potential future career. The PhD platform strengthens the network of the PhD students, and opens avenues for future research collaboration among them.

The 2nd Kilimanjaro International PhD Symposium has seen more and more PhD students from Tanzania and Europe coming together to share this experience, and more seniors joining to support and guide the milestone of these young professionals.

We are grateful for the commitment of the Executive Director of Kilimanjaro Christian Medical Centre (KCMC), Prof Dr Moshi Ntabaye, and the Provost of Kilimanjaro Christian Medical University College (KCMUCo), Prof Egbert Kessi that this year again the symposium it taking place.

The joint efforts by the following research and capacity building programmes at KCMC (i.e. Building Stronger Universities (BSU) funded by DANIDA, Denmark; Medical Education Partnership Initiative (MEPI) funded by NIH, USA; Malaria Capacity Development Consortium (MCDC) funded by Wellcome Trust, UK; and the Collaboration between KCMC, Tanzania and Radboud University, The Netherlands) have made this Symposium a success for the second year consecutively.

Welcome to Tanzania, The Land of Kilimanjaro, Zanzibar, and Serengeti
Gibson Kibiki
*Chair – Organizing Committee*

# Table of Contents

# MY CAREER PATH - PDP

**Name:** Reginald Adolph Kavishe, BSc, MSc, PhD

**University of PhD registration:** Radboud University, The Netherlands

**PhD Title:** Human glutathione s-transferase polymorphisms and Plasmodium falciparum ATP – binding cassette transport proteins in malaria

**Funding Agency:**

NWO and NACCAP (The Netherlands) through PRIOR and APRIORI

**Principal Supervisor:**

Prof Dr. Frans G.M. Russell, Radboud University, The Netherlands

**Co-supervisors:**

Dr. Jan B. Koenderink, Radboud University, The Netherlands

Prof Dr. Andre J.A.M van der Ven, Radboud University, The Netherlands

## SUMMARY:

**Background:** Malaria infection induces oxidative stress in the host and the pathophysiology of malaria is attributed to the increase in oxidative stress. The host cell antioxidant defence systems are important in containing the infection and may determine the clinical outcome of the infection. Furthermore, the *P. falciparum* genome encodes several ABC transporters. ABC transporters may play a central role in the drug resistance mechanisms either by direct efflux of antimalarial drugs or GSH-conjugated drugs. ABC transporters of the MRP subfamily are responsible for efflux of oxidized GSH (GSSG) out of the parasite into the host cell possibly for reduction and recycling. Drug resistant parasites have increased GSH metabolism, therefore ABC transporters can be good targets for new antimalarial drugs. In addition, targeting these transporters may reverse drug resistance. However, only few falciparum ABC transporters have been characterized. The aim of this thesis is to investigate the role of human glutathione transferase polymorphisms in malaria pathology and to clone and characterize putative ABC transporters of the *P. falciparum* parasite.

**Methodology:** Using polymerase chain reaction techniques we investigated the role of glutathione transferase (GST) mu, pi, and theta plymorphisms in susceptibility to severe malaria in Cameroonian and Tanzanian children. Furthermore, we used bioinformatics and phylogenetic analyses to describe the *P. falciparum* ABC transporter family. We further employed immunocytochemistry to determine the sub-cellular localization in the asexual stages of the parasite of three ABC transporters, two belonging to the ABCC subfamily and one to the ABCB subfamily. For functional characterization and in order to predict the substrates and substrate analogues for the ABC transporters, heterologous expression is necessary. We cloned seven PfABC transporters using the gateway cloning system. Attempts

to express four of the PfABCs in different heterologous systems (in baculovirus infected insect cells (Sf9), injected *Xenopus laevis* oocytes, *E. coli* and transduced mammalian HeLa cells) were done.

**Results:** We observed a significantly higher prevalence of the *GSTP1*-I105V genotype in severe than in mild malaria. The *GSTM1-null* genotype was also higher in the severe malaria group, but the difference was not significant. We also show that 11 out of 16 genome encoded ABC proteins are membrane transporters and that *PfMRP1* (*Plasmodium falciparum* Mutidrug Resistance-associated Protein 1), *PfMRP2*, and *PfMDR5* (*Plasmodium falciparum* Multi Drug Resistance protein 5) are localized on the parasite's plasma membrane in all asexual stages. There were no localization signals on the erythrocyte membrane, parasite food vacuole membrane or in cytoplasmic compartments such as endoplasmic reticulum. We observed a decrease in expression level with increasing size of native EYFP-fusion *PfMDR5* fragments and codon-harmonized full-length *PfMDR5* in both Sf9 and HeLa cells. No expression of full-length transporters was observed in any of the systems used. The codon harmonized EYFP-PfMDR5 fusion fragment was expressed in slightly higher amounts compared to the native form.

**Conclusions**: We concluded that *GSTM1-null* and *GSTP1-I105V* genotypic variants are associated with severe malaria in children. *PfMRP1*, *PfMRP2*, and *PfMDR5* are localized on the parasite's plasma membrane in asexual stages. Gene size, richness in adenylate (A) and thymidylate (T) nucleotides in genes or codon bias may affect expression of falciparum membrane proteins in HeLa and Sf9 cells. Codon harmonization may improve expression of falciparum proteins in Sf9 cells. There is an urgent need for an optimal standard expression system for *P. falciparum* proteins that may be achieved through continued optimization.

## Publications

**Kavishe,R.A**., Bousema,T., Shekalaghe,S.A., Sauerwein,R.W., Mosha,F.W., van,d., V, Russel,F.G., Koenderink,J.B. 2009. Common genotypic polymorphisms in glutathione S-transferases in mild and severe falciparum malaria in Tanzanian children. American Journal of Tropical Medicine and Hygiene 81 (2), 363-365.

**Kavishe,R.A.**, Koenderink,J.B., McCall,M.B., Peters,W.H., Mulder,B., Hermsen,C.C., Sauerwein,R.W., Russel,F.G., van,d., V. 2006. Short report: Severe Plasmodium falciparum malaria in Cameroon: associated with the glutathione S-transferase M1 null genotype. American Journal of Tropical Medicine and Hygiene 75 (5), 827-829.

**Kavishe,R.A**., van den Heuvel,J.J., van,d., V, Luty,A.J., Russel,F.G., Koenderink,J.B. 2009. Localization of the ATP-binding cassette (ABC) transport proteins PfMRP1, PfMRP2, and PfMDR5 at the Plasmodium falciparum plasma membrane. Malaria Journal. 8 (1), 205.

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Koenderink,J.B., **Kavishe,R.A.,** Rijpma,S.R., Russel,F.G. 2010. The ABC of Multidrug Resistance in Malaria. Trends in Parasitology. 26 (9), 440-446.

Shekalaghe SA, ter Braak R, Daou M, **Kavishe R**, van den Bijllaardt W, van den Bosch S, Koenderink JB, Luty AJ, Whitty CJ, Drakeley C, Sauerwein RW, Bousema T. 2010: In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. Antimicrobial Agents and Chemotherapy, 54 (5),1762-1768.

Saguti F., Sakurani B., Manjurano A., Max R., Filemon Tenu, Francis, F., Shekalaghe, SA., **Kavishe, RA.** 2013. Relationship between alpha+-thalassaemia and glutathione-*S*-transferases polymorphisms in children with severe malaria in Tanzania. Tanzania Journal of Health Research, 15, Doi: *http://dx.doi.org/10.4314/thrb.v15i2.2*

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Sawa, P., Shekalaghe, SA., Drakeley, CJ., Sutherland, CJ., Mweresa, CK., Baidjoe, AY., Manjurano, A., **Kavishe, RA**., Beshir, KB., Yussuf, RU., Omar, SA., Hermsen, CC., Okell, L., Schallig, HD., Sauerwein, RW., Hallett, RL., Bousema ,T. 2013. Malaria transmission after artemether-lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. Journal of Infectious Diseases. 207 (11), 1637-45.

Mohamed, AK., Ndaro, A., Kalinga, A., Manurano, A., Mosha, JF., Mosha, DF., van Zwetselaar, M., Koenderink, JB., Mosha, FW., Alifrangis, M,., Reyburn, H., Roper, C., **Kavishe, RA**. 2013. Trends in chloroquine resistance marker, *Pfcrt* K76T mutation 10 years after chloroquine withdrawal in Tanzania. Malaria Journal (submitted).

**Kavishe, RA**., Matondo, SI., Temba, G., Mosha, FW., Koenderink ,JB., Reyburn, H., Roper, C., Alifrangis, MA. 2013. High prevalence of *Plasmodium falciparum* dihydropteroate synthetase gene K540E and A581G mutations in Tanzania, implication for continued SP-IPTp programes. Malaria Journal (submitted)

Petro Paulo, Arnold Ndaro; Frank Mosha; Michael Alifrangis; Hugh Reyburn; Cally Roper; **Reginald Kavishe.** 2013. Surveillance of artemether-lumefantrine associated *Plasmodium falciparum* multi-drug resistance gene-1, mutations in Tanzania (in preparation).

**Name:** Hadija Hamisi Semvua, BPharm, MPH, PhD candidate

**University of PhD registration:** Radboud University, The Netherlands

**PhD Title:** Clinical Pharmacological Studies in Tuberculosis

**Funding Agency:**

NACCAP (The Netherlands) through APRIORI and

European and developing Countries Clinical Trials Partnership (EDCTP) through PanACEA Consortium

**Principal Supervisor:**

Prof Dr. David M. Burger, MSc. Pharm D, PhD, Radboud University, The Netherlands

**Co-supervisors:**

Prof Dr. Gibson S. Kibiki, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

Dr. Rob .E. Aarnoutse, MSc. Pharm D, PhD, Radboud University, The Netherlands

Prof Dr. Martin J. Boeree, MD, PhD, Radboud University, The Netherlands

**Abstract for the PhD Symposium**

**Title 1: Associations between salivary, protein-unbound and total plasma concentrations of rifampicin**

**Introduction:** Plasma is the traditional biological sample for PK studies. However saliva may be an attractive alternative matrix. The objectives of this study were (1) to compare the PK of rifampicin in saliva and plasma and (2) to assess whether saliva could be an alternative matrix for PK studies and TDM with this drug.

**Methods:** A descriptive PK study was performed among 15 adult Tanzanian TB patients. Time-matched samples of stimulated saliva (obtained with a Salivette® device containing citric acid) and plasma were collected at pre-dose and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after intake of rifampicin. Salivary, total (protein-unbound plus bound) and unbound plasma concentrations of rifampicin were measured with validated HPLC methods. Salivary and plasma PK parameters were also assessed.

**Results:** The geometric mean $AUC_{0\text{-}24h}$ of rifampicin in saliva (3.1 h*mg/L) was slightly but significantly lower than the protein-unbound $AUC_{0\text{-}24h}$ in plasma (5.3 h*mg/L) and these were much lower than the plasma $AUC_{0\text{-}24h}$ based on total concentrations (32.7 h*mg/L). Corresponding geometric mean $C_{max}$ values were 0.64, 1.0 and 6.8 mg/L.

**Discussions:** Salivary $AUC_{0\text{-}24h}$ and $C_{max}$ of rifampicin were much lower than those in plasma. Again prediction of total and unbound plasma concentrations based on salivary concentrations of rifampicin resulted in median percentage prediction errors (MPPE) of 13.4% and 6.0%.

**Conclusion:** It is not possible to predict total or protein-unbound plasma concentrations from salivary concentrations, due to inadequate precision associated with this prediction.

## Publications

**Hadija H Semvua**, Charles M Mtabho, Quirine Fillekes, Jossy van den Boogaard, Riziki M Kisonga, Liberate Mleoh, Arnold Ndaro, Elton R Kisanga, Andre van der Ven, Rob E Aarnoutse, Gibson S Kibiki[1], Martin J Boeree, David M Burger. Efavirenz, tenofovir and emtricitabine combined with first-line tuberculosis treatment in TB-HIV-co-infected Tanzania patients: a pharmacokinetic and safety study. *Antiviral Therapy: 2013; 18(1):105-13.*

Alma Tostmann, Charles M. Mtabho, **Hadija H. Semvua** , Jossy van den Boogaard , Martin J. Boeree, Gibson S. Kibiki , Rob E. Aarnoutse. Pharmacokinetics of first line tuberculosis drugs in Tanzanian tuberculosis patients. Journal of Antimicrobial Agents Chemotherapy. 2013; 57(7):3208.

**Hadija H. Semvua**, Gibson S. Kibiki. Atripla[R]/anti-TB combination in TB/HIV patients. Drug in focus. BMC Research Notes 2011, 4: 511.

J. van den Boogard**, H.H. Semvua**, M.J. Boeree, R.E. Aarnoutse, G. S. Kibiki. Assessment of antibacterial sale by using the Anatomic Therapeutic Chemical classification and Defined Daily Dose methodology in Moshi Municipality, northern Tanzania. Tanzania J Health Research 2010; 12(3):208-212.

Jossy van den Boogaard, **Hadija H. Semvua**, Martin J. Boeree, Rob E. Aarnoutse and Gibson S. Kibiki. Fluoroquinolone sale in northern Tanzania: a potential threat for fluoroquinolone use in tuberculosis treatment. Journal of Antimicrobial Chemotherapy. 2010; 65:145-147.

Jossy van den Boogaard, **Hadija H. Semvua**, Jakko van Ingen,[3] Solomon Mwaigwisya,Tridia van der Laan,[4] Dick van Soolingen, Gibson S. Kibiki, Martin J. Boeree, Rob E. Aarnoutse. Low rate of fluoroquinolone resistance in *Mycobacterium tuberculosis* isolates from northern Tanzania. *Journal of Antimicrobial Chemotherapy. 2011 Aug; 66(8): 1810-4.*

Mgogwe J1, **Semvua H**, Msangi R, Mataro C, Kajeguka D, Chilongola J. The evolution of haematological and biochemical indices in HIV patients during a six-month treatment period. *African Health Sciences* 2012; 12(1): 2 – 7

Alma Tostmann, Jossy van den Boogaard, **Hadija Semvua**, Riziki Kisonga, Gibson S. Kibiki4,Rob E. Aarnoutse and Martin J. Boeree. Antituberculosis drug-induced hepatotoxicity is uncommon in Tanzanian hospitalized pulmonary TB patients. Trop med Int health 2010; 15(2):268-272.

**Name:** Bilali I. Kabula, BVM, MPhil, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCo) Moshi, Tanzania

**PhD Title:** Diversity of Malaria Vectors and Their Insecticide Resistance Profile in Tanzania

**Funding Agency:**

Wellcome Trust (UK) through Malaria Capacity Development Consortium (MCDC)

**Principal Supervisors:**

Prof Franklin Mosha, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

Prof Martin Donnelly, Liverpool School of Tropical Medicine, University of Liverpool (UK)

**Co-supervisor:** Dr. William Kisinza, National Institute for Medical Research (NIMR), Tanzania

**Abstracts for the PhD Symposium**

**TITLE 1: Distribution of Anopheles gambiaes.l. in Different Ecological Zones of Tanzania: Implications for Malaria Vector Control**

**Background:** Members of the *Anopheles gambiae* complex are important vectors of malaria in Tanzania. The species within the complex exhibit an enormous diversity in their biology which impacts greatly on their importance as vectors of malaria. Describing the diversity of these vectors is crucial to developing sound and cost effective interventions for malaria control. This study investigated the distribution of *Anopheles gambiae* complex in different ecological zones of Tanzania.

**Methods:** The study was carried out in 13 districts located across three ecological zones of Tanzania namely; coastal savannah, grassland/forest savannah and highlands. Wild anopheles mosquitoes were collected using indoor resting catches. Mosquitoes were identified using a combination of morphological characters andPCR-assays.

**Results:** A total of 7,596 collected mosquitoes were morphologically identified as *An. gambiaes.l.* of which, 2,536 (33%) were subjected for PCR analysis. Out of 2,536 mosquitoes, 1,660 (65%) and 876 (35%) were identified as *An. arabiensis* and *An. gambiaes.s* respectively. Both species occurred in sympatry in 31% of the districts sampled; while An*. arabiensis* occurred alone in 69% of the study districts. *Anopheles gambiaes.s.* predominated in the highland districts while the *An. arabiensis* were found in almost all ecological zones.We also documented the replacement of *An. gambiaes.s.* by *An. arabiensis.*

**Conclusion:** The distribution of these two most anthropophilicmembers of the *An. gambiaes.l.* and malaria in Tanzania appear to be distinct, driven by different ecological factors.The predominance of *An. arabiensis* and replacement of *An. gambiaes.s.* by *An. arabiensis* have important implications for the epidemiology and control of malaria due the differences in biology and vectorial capacity of these two members of *An. gambiae* complex.

**TITLE 2: Relationship between the presence of *Vgsc-L1014S* point mutation and infection with *Plasmodium falciparum* in *Anopheles gambiaes.l.***

**Background:** The control of malaria vectors is threatened by widespread insecticide resistance. The extent to which insecticide resistance mechanisms impact upon the development of the pernicious malaria parasite in its vector remains unknown. This study compared the *P. falciparum*sporozoite rate between insecticide resistance and susceptible wild *An. gambiae*s.l.; and investigated thefeeding preferences of this mosquito population.

**Methods:** WHO standard methods were used to detect resistance in the wild female anopheles mosquitoes when exposed to 0.05% deltamethrin. PCR based molecular diagnostics were used to identify mosquitoes to species and to detect *kdr* alleles. The presence of *P. falciparum*sporozoites was detected using CSP-ELISA and blood meal source was analysed by the standard ELISA method.

**Results:** The anopheles mosquitoes were resistance to deltamethrin with mortality rates ranging from 78.6% [95% CI: 74.9-81.9%]) in the dry season to 81.2% [95% CI: 76.8-84.9%]) in the rainy season. Of 545 mosquitoes genotyped 96.5% were *An. gambiae*s.s. and 3.5% were *An. arabiensis*. The L1014S point mutation was detected in both *An. gambiae*s.s. and *An. arabiensis* at the allelic frequency of 0.45 and 0.32 respectively. The overall infection rate was 4.11% while the anthropophilic rate was 92.5% (95% CI: 89.9-94.5%). There was a significant associated between the presence of sporozoites and the kdr L1014S mutation ($X^2$ =6.89; P=0.009. Mosquitoes fed with human blood were more likely to have L1014S point mutation allele (X2=9.84; p=0.00171).

**Conclusion:** The observed association between sporozoiteand L1014S mutation allele are of great importance for the epidemiology of malaria considering the widespread nature of this resistance mutation in Africa. Further work to elucidate the physiological mechanisms which influence this association is needed.

## Publications

**Kabula, B**., Tungu, P., Matowo, J., Kitau, J., Mweya, C., Emidi, B., Masue, D., Sindato, C., Malima, R., Minja, J., Msangi, S., Njau, R., Mosha, F., Magesa, S. and Kisinza, W (2012). Susceptibilitystatus of malaria vectors to insecticides commonlyused for malaria control in Tanzania.*Tropical Medicine and International Health,*17 (6): 742-750.doi:10.1111/j.1365-3156.2012.02986.x

**Kabula, B.**, Derua, Y. A., Tungu, P., Massue, D. J., Sambu, E., Stanley, G., Mosha, F. W. and Kisinza, W. N (2011). Malaria entomological profile in Tanzania from 1950 to 2010: a review of mosquito distribution, vectorialcapacity and insecticide resistance. *Tanzania Journal of Health Research, 13 (1).* DOI: http://dx.doi.org/10.4314/thrb.v13i5.2

**Bilali Kabula,** Patrick Tungu, Robert Malima, Mark Rowland, Jubilate Minja, Ritha Wililo, Mahdi Ramsan, Peter McElroy,Jessica Kafuko, Natacha Protopopoff, Stephen Magesa,

Franklin Moshaand William Kisinza(2013). Distribution and spread of pyrethroid and DDT resistance among *Anopheles gambiae* complex in Tanzania. ***Accepted for publication to Medical and Veterinary Entomology Journal***

**Bilali Kabula**, William Kisinza, Patrick Tungu, ChachaNdege, BenardBatengana, Douglas Kollo, Robert Malima, Jessica Kafuko, Mahdi Mohamed, Franklin Mosha and Stephen MagesaCo-occurrence and Distribution of East (L1014S) and West (L1014F) African Knockdown Resistance in *Anopheles gambiae*sensulato population of Tanzania". ***Submitted***

**Bilali Kabula**, Patrick Tungu, Robert Malima, Bernard Batengana, William Kisinza, Stephen Magesa, Martin Donnelly and Franklin Mosha. Distribution of *Anopheles gambiae*s.l. in Different Ecological Zones of Tanzania: Implications for Malaria Vector Control.***To be submitted***

**Bilali Kabula,** Patrick Tungu, Bernard Batengana, Emily J. Rippon, William Kisinza, Martin Donnelly and Franklin Mosha**.**Relationship between the presence of Vgsc-L1014S point mutation and infection with*Plasmodium falciparum* in *Anophelesgambiaes.l.****To be submitted***

**Name:** Sisse Bolm Ditlev, BSc, MSc, PhD candidate
**University of PhD registration:** University of Copenhagen
**PhD Title:** Assessment of the *in vitro* efficacy of a VAR2CSA based vaccine to prevent malaria in pregnant women
**Funding Agency:**
Danish Research Council for Development Research (RUF)
**Principal Supervisor:**
Prof Ali Salanti, Centre for medical parasitology, University of Copenhagen, Denmark
**Co-supervisor:**
Prof Thor G. Theander, Centre for medical parasitology, University of Copenhagen, Denmark
Prof Madeleine Dahlbäck, Centre for medical parasitology, University of Copenhagen, Denmark

**Abstracts for the PhD Symposium**

**Title 1: Utilizing nanobody technology to target non-immuno dominant domains of VAR2CSA**

**Introduction:** Placental malaria is caused byaccumulation*of Plasmodium falciparum* infected erythrocytes (IE) in the placenta by binding of the parasite-expressed protein VAR2CSA to placental chondroitin sulfate A (CSA). The minimum binding region has been identified to be located within the ID1-ID2a domain of VAR2CSA. Defining specific epitopes responsible for CSA-binding is essential for the development of a vaccine aimed at blocking IE adhesion. However, production of monoclonal reagents targeting the minimum binding region has not been successful.

**Methods:** In this study we analyzed the induced immune-response from aVAR2CSA immunized Alpaca and produced VAR2CSA-specific nanobodies (Nbs) which is recombinant produced heavy-chain antibodies naturally occurring in Camelids. These were characterized for specific recognition of VAR2CSA by ELISA and tested for functionality against native VAR2CSA expressed on IE by flow cytometry and static binding-inhibition assay.

Results: Seventeen VAR2CSA-specific Nbs were identified and mapped: four to the DBL4ε, four to the DBL5ε, four to the DBL6ε domain and five to the minimum binding region. All Nbsstained the surface of IE and fourNbs reduced the IE adhesion to CSA.

**Discussion:** We demonstrate that using the nanobody technology we can produce versatile monoclonal reagentsas Nbs has capacityto recognize epitopes that are not recognized by classical IgG.

**Conclusion:** The Nbs canbe used for epitope mapping and quality control of the VAR2CSA vaccine construct, potential provides novel insights into structure and function of this complex antigen which is essential to the design of a multivalent VAR2CSA vaccine.

## Publications:

**Ditlev SB**, Nielsen MA, Resende M, Agerbæk MØ, Pinto VV, Andersen PH, Magistrado P, Lusingu J, Dahlbäck M, Theander TG, Salanti A.PLoS One. 2012;7(9):e43663. Epub 2012 Sep 6. Identification and Characterization of B-Cell Epitopes in the DBL4ε Domain of VAR2CSA.

Dahlbäck M, Jørgensen LM, Nielsen MA, Clausen TM, **Ditlev SB**, Resende M, Pinto VV, Arnot DE, Theander TG, Salanti A. J Biol Chem. 2011 May 6;286(18):15908-17. Epub 2011 Mar 11. The chondroitin sulfate A-binding site of the VAR2CSA protein involves multiple N-terminal domains.

Magistrado PA, Minja D, Doritchamou J, Ndam NT, John D, Schmiegelow C, Massougbodji A, Dahlbäck M, **Ditlev SB**, Pinto VV, Resende M, Lusingu J, Theander TG, Salanti A, Nielsen MA. Vaccine. 2011 Jan 10;29(3):437-43. Epub 2010 Nov 12. High efficacy of anti DBL4□-VAR2CSA antibodies in inhibition of CSA-binding Plasmodium falciparum-infected erythrocytes from pregnant women.

**Sisse B Ditlev**, Raluca Florea, Morten A. Nielsen, Stefan Magez, Philippe Boeuf and Ali Salanti. Accepted in PLoS One. Utilizing nanobody technology to target non-immunodominant domains of VAR2CSA

**Name:** Eva Prosper Muro, BPharm, Msc, PhD candidate

**University of PhD registration:** Radboud University, The Netherlands

**PhD Title:** Pharmacological Studies on Prevention of Mother to Child Transmission (PMTCT) of HIV infection

**Funding Agency:**

NWO (The Netherlands) through PRIOR, and European and Developing Countries Clinical Trials Partnership (EDCTP) through VITA Study

**Principal Supervisors:**

Prof Dr. David M. Burger, Radboud University, The Netherlands

Prof Dr. W.M.V. Dolmans, Radboud University, The Netherlands

**Co-supervisor:** Dr. Elton Richard Kisanga, Kilimanjaro Christian Medical University College (KCMUCO) Moshi, Tanzania.

**Abstracts for the PhD Symposium**

**Objectives:** To assess the effectiveness of different drug interventions on nevirapine resistance development after use of single-dose nevirapine as part of antiretroviral prophylaxis for prevention of HIV mother-to-child transmission (pMTCT).

**Design:** A systematic review including meta-analyses

**Methods**: Systematic search of electronic databases (MEDLINE, EMBASE and Cochrane) was performed. Studies included HIV-infected, pregnant women, who were administered single-dose nevirapine for pMTCT and who were receiving an intervention to reduce nevirapine resistance.Primary outcome was the proportion of nevirapine resistance detected in plasma samples collected ≤3 months postpartum. The reducing effect of drug interventions on nevirapine resistance was assessed in meta-analyses using random effects models and the GRADE approach for quality of evidence.

**Results**: The estimated pooled proportion of nevirapine resistance using single-dose nevirapine at labor was 31% (95%CI 7.6-54); this was reduced to 21% (95%CI 8.6-33) with addition of antepartum zidovudine. A combination of antepartum zidovudine, single-dose nevirapine and a short (<8 days) postpartum regimen resulted in a major reduction in nevirapine resistance to 0.011% (95%CI -0.11-0.13). The summary effect of 20-30 days of postpartum drug regimens combined with antepartum zidovudine and single-dose nevirapine was associated with a slightly lower incidence of nevirapine resistance, namely 0.003% (95%CI -0.054-0.060).

**Conclusions:** Antepartum zidovudine plus antiretroviral drugs postpartum have shown to nearly eliminate nevirapine resistance. Although 20-30 days post partum regimens might be slightly more effective compared to a short (<8 days) postpartum regimen, longer term antiretroviral therapy is more complex and more challenging to implement in daily practice. The WHO guideline option A (antepartum zidovudine, single-dose nevirapine and one week of lamivudine/zidovudine postpartum) should be followed to achieve a feasible minimum risk of nevirapine resistance in regions where single-dose nevirapine is still being used.

## Publications

**Muro E**, Droste JA, Hofstede HT, Bosch M, Dolmans W, Burger DM.,Nevirapine plasma concentrations are still detectable after more than 2 weeks in the majority of women receiving single-dose nevirapine: implications for intervention studies. J Acquir Immune Defic Syndr2005; 39:419–21.

**Eva P. Muro**, Quirine Fillekes, Elton R. Kisanga, Rafaëlla L□homme, Susan C. Aitken, Godfrey Mariki, Andre J. A. M. Van der Ven, Wil Dolmans, Rob Schuurman, A. Sarah Walker, Diana M. Gibb, MD, David M. Burger .Intrapartum Single-Dose Carbamazepine Reduces Nevirapine Levels Faster and May Decrease Resistance After a Single Dose of Nevirapine for Perinatal HIV Prevention., J Acquir Immune Defic Syndr 2012;59:266□273)

Quirine Fillekes, **Eva P. Muro,** Catherine Chunda, Susan Aitken, Elton R. Kisanga, Chipepo Kankasa, Margaret J. Thomason, Diana M. Gibb, A. Sarah Walker, David M. Burger. Effect of 7 days of phenytoin on the pharmacokinetics of and the development of resistance to single-dose nevirapine for perinatal HIV prevention: a randomized pilot trial Journal of Antimicrobial Chemotherapy, 2013 (accepted July 2013)

**Name:** Andrea Caroline Pehrson, MD, PhD candidate

**University of PhD registration:** University of Copenhagen, Denmark
**PhD Title:** Placental malaria, free fetal haemoglobin and preeclampsia
**Funding Agency:**

Faculty of Health and Medical Sciences, University of Copenhagen, and Novo Nordisk Foundation
**Principal Supervisor:**

Associate Professor Morten A Nielsen, Centre of Medical Parasitology, University of Copenhagen, Denmark
Prof Stefan R Hansson, University of Lund, Sweden
Prof Peter Damm, University of Copenhagen, Denmark

**Co-supervisor:** Professor Lisbeth E Knudsen, University of Copenhagen, Denmark
**Abstracts for the PhD Symposium**

**Title 1: Adhesion of malaria parasites in the *ex vivo* dual placental perfusion model**

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**Introduction:** In placental malaria (PM) *P. falciparum* infected erythrocytes (IE) sequester in the placenta through specific binding of the *P. falciparum*VAR2CSA antigen to Chondroitinsulfate A (CSA). Anti-PM vaccine development is focused on hindering theplacental parasite accumulation by identification of sub-units of VAR2CSA that induce antibodies inhibiting the binding of VAR2CSA expressing IE to CSA. In this study the *ex vivo* dual placental perfusion model is implemented to study adhesion of IE in the placenta and the inhibitory capacity of anti-VAR2CSA antibodies.

**Methods:** Placentas are obtained from healthy pregnant women undergoing elective caesarean section. The fetal and maternal circulation of a cotyledon is cannulated and the cotyledon is placed in a heated chamber. IE are added to the maternal circulation and perfusate is collected at regular time intervals to measure the parasitemia. At the end of the experiment perfused tissue is collected for histological examination. The binding characteristics of parasites expressing VAR2CSA versus other PfEMP1s, and the specificity of the binding, are investigated.

**Results:** Preliminary results show that erythrocytes infected with parasites expressing VAR2CSA accumulate in the *ex vivo* perfused placenta.

**Discussion:** *Ex vivo* dual placental perfusion can be used as a model to study adhesion of malaria parasites in the placenta. Experiments to study binding inhibition by anti-VAR2CSA antibodies are ongoing.

**Conclusion:** The *ex vivo* dual placental perfusion model is currently implemented to investigate the biology of IE adherence in the placenta and the inhibitory capacity of antibodies from immunized rodents and humans in clinical phase I trials.

## Publications

Minja DT, Schmiegelow C, Mmbando B, Boström S, Oesterholt M, Magistrado P, **Pehrson C**, John D, Salanti A, Luty AJ, Lemnge M, Theander T, Lusingu J, Alifrangis M. Plasmodium falciparum mutant haplotype infection during pregnancy associated with reduced birthweight, Tanzania. Emerg Infect Dis. 2013;19(9). doi: 10.3201/eid1909.130133.

Schmiegelow C, Minja D, Oesterholt M, **Pehrson C**, Suhrs HE, Boström S, Lemnge M, Magistrado P, Rasch V, Nielsen BB, Lusingu J, Theander TG. Malaria and fetal growth alterations in the 3(rd) trimester of pregnancy: a longitudinal ultrasound study.. PLoS One. 2013;8(1):e53794. doi: 10.1371/journal.pone.0053794. Epub 2013 Jan 11

Schmiegelow C, Scheike T, Oesterholt M, Minja D, **Pehrson C**, Magistrado P, Lemnge M *et al*. Development of a fetal weight chart using serial trans-abdominal ultrasound in an East african population: a longitudinal observational study.PLoS One. 2012;7(9):e44773. doi: 10.1371/journal.pone.0044773. Epub 2012 Sep 21.

**Pehrson C** & Kock K. Hydrops føtalis forårsaget af et mediastinalt teratom. Ugeskr Læger. Juli 2012 (epub ahead of print).

Minja DT, Schmiegelow C, Oesterholt M, Magistrado PA, Boström S, John D, **Pehrson C** *et al*. Reliability of rapid diagnostic tests in diagnosing pregnancy-associated malaria in north-eastern Tanzania. Malar J. 2012 Jun 21;11:211.

Schmiegelow C, Minja D, Oesterholt M, **Pehrson C**, Suhrs HE, Boström S, Lemnge M *et al*. Factors associated with and causes of perinatal mortality in northeastern Tanzania. Acta Obstet Gynecol Scand. 2012 Sep;91(9):1061-8.

**Pehrson C**, Sorensen JL, Amer-Wåhlin I. Evaluation and impact of cardiotocography training programmes: a systematic review. BJOG. 2011;118(8):926-35.

Hjortø SP, **Pehrson C**, Gernow A. Nodulær hyperplasi af Bartholins kirtel. Ugeskr Læger. 2010;172(12):969-70.

**Name:** Stellah George Mpagama MD, MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**PhD Title:** Multidrug resistant tuberculosis (MDR-TB) in Tanzania: Approaches to improve management.

**Funding Agency:**

European and Developing Countries Clinical Trial Partnership (EDCTP) through Pan African Consortium for Evaluation of Antituberculous Antibiotics (PanACEA), and National Institutes for Health (NIH) Fogarty International Center 5D43 TW008270

**Principal Supervisor:**

Prof Dr. Gibson S. Kibiki, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

**Co-supervisors:**

Prof Dr. Eric Houpt, University of Virginia, USA

Prof Dr. Martin J. Boeree, Radboud University, The Netherlands

**Abstracts for the PhD Symposium**

**Title 1: Plasma drug activity in multidrug-resistant tuberculosis**

**Background:** Little is known about plasma drug concentrations or quantitative susceptibility in patients with multidrug-resistant (MDR)-TB.

**Methods:** In adults on a MDR-TB regimen in Tanzania, patients' plasma and *M. tuberculosis* isolate were assessed for drug concentration ($C_{2hr}$), minimum inhibitory concentrations (MICs) and in the TB drug activity assay (TDA) which reports the ratio of the time to detection of plasma-cocultured *M. tuberculosis* versus control growth in the Bactec MGIT system.

**Results:** In healthy control plasma spiked with individual MDR-TB drugs at expected $C_{2hr}$ concentrations, moxifloxacin yielded superior TDA versus ofloxacin, and only moxifloxacin and amikacin yielded TDA ratios equivalent to a -2log killing. TDA <1.0 (indicative of no killing) was observed across all concentrations of kanamycin and moxifloxacin against an extensively drug-resistant (XDR) isolate. In 25 patients enrolled on a regimen of kanamycin, levofloxacin, ethionamide, pyrazinamide and cycloserine, $C_{2hr}$ concentrations were below the expected range for levofloxacin in 13 (52%) and kanamycin in 10 (40%). Three subjects with the lowest TDA (<1.5) had significantly lower kanamycin $C_{2hr}$/MIC than subjects with TDA ≥1.5, (9.8 ±8.7 v. 27.0 ±19.1) (p=0.04). In 19 subjects with sputum culture conversion, the mean TDA was 2.52 ±0.76 in subjects converting to negative in ≤2 months (N=14), and was 1.88 ±0.57in those with conversion >2 months (N=5) (p=0.08).

**Conclusions:** In Tanzania, MDR-TB drug concentrations were frequently low and a wide range of concentration/MIC was observed that ex vivo affected plasma drug activity. Opportunity exists for pharmacokinetic optimization in current MDR-TB regimens, which may improve treatment response.

Title 2: Multidrug-resistant Tuberculosis: Challenges in Diagnosis and Treatment in sub-Saharan Africa

**Background:** TB is an old infectious disease. HIV re-emerged TB to form the third epidemic M/XDR-B. This review presents an overview of MDR-TB in sub-Saharan Africa, a region where there is TB and HIV syndemic.

**Method:** Literature search using PubMed and Google with the key words drug resistant tuberculosis, HIV, MDR-TB, XDR-TB, M/XDR-TB, second-line TB, DR-TB diagnostics, DR-TB treatment, sub Saharan Africa.

**Results:** Information on the evolution of DR-TB, epidemiology of MDR-TB and pathophysiological mechanisms for development of MDR-TB was obtained. Challenges and opportunities for improving MDR-TB diagnosis and therapy are discussed. We found that MDR-TB remains a significant problem in sub-Saharan Africa, integral to the HIV epidemic, and threatens to dissolve previous successes of TB control. Current opportunities exist in diagnosis, management and follow-up of MDR-TB cases, such as molecular diagnostics and new drugs. Novel diagnostics including rapid molecular tests can decrease the time to initiation of appropriate MDR-TB therapy, and new drugs or drug regimens may ultimately shorten treatment duration while limiting default and treatment related morbidity.

**Conclusion:** Current diagnostics can decrease turnaround time from DR-TB suspicion to initiation of treatment. New drugs or regimen may shorten treatment duration. These opportunities have the potential to combat DR-TB in the region but only with proper implementation strategies, and formidable public/private partnerships to sustain these advances.

## Publications

**Mpagama SG**, Heysell SK, Ndusilo ND, Kumburu HH, Lekule IA, Kisonga RM, Gratz J, Boeree MJ, Houpt ER, Kibiki GS: Diagnosis and interim treatment outcomes from the first cohort of multidrug-resistant tuberculosis patients in Tanzania. ***PLOS One*** **2013**, 8(5):e62034

**Mpagama SG**, Mtabho C, Mwaigwisya S, Mleoh LJ, Boer IM, Heysell SK, Houpt ER, Kibiki GS: Comparison of overnight pooled and standard sputum collection method for patients with suspected pulmonary tuberculosis in northern Tanzania. ***Tuberculosis research and treatment*** **2012**, 2012:1280

**Mpagama SG,** Houpt ER, Stroupt S, Kumburu HH, Gratz J, Kibiki GS, Heysell SK: Application of quantitative second-line drug susceptibility testing at a multidrug-resistant tuberculosis hospital in Tanzania. ***BMC infectious diseases*** **2013,** 13:432.

Heysell SK, Mtabho C, **Mpagama S**, Mwaigwisya S, Pholwat S, Ndusilo N, Gratz J, Aarnoutse RE, Kibiki GS, Houpt ER: Plasma drug activity assay for treatment optimization in tuberculosis patients. ***Antimicrob Agents Chemother*** **2011**, 55(12):5819-5825.

**Mpagama SG**, Ndusilo ND, Stroupt S, Kumburu H, Peloquin CA, Gratz J, Houpt ER, Kibiki SG, Heysell SK. Plasma drug activity in patients on treatment for multidrug-resistant tuberculosis. **Provisionally accepted in the Antimicrobial Agents Chemotherapy Journal.**

**Mpagama SG**, Heysell SK, Boeree MJ, Houpt ER, Kibiki SG.Multidrug-resistant tuberculosis;Challenges in diagnosis and treatment in sub-Saharan Africa **(Submitted)**

**Name:** Florida Joseph Muro, MD, MPH, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**PhD Title:** Accuracy of the non-severe pneumonia diagnosis in Tanzanian children: the validity of the respiratory rate

**Funding Agency:**

Good Samaritan foundation (GSF) of Tanzania, and

Wellcome Trust through THRiVE

**Principal Supervisor:**

Prof Raimos Olomi, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**Co-supervisors:**

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**Abstracts for the PhD Symposium**

**Title 1: Accuracy of the non-severe pneumonia diagnosis in Tanzanian children: the validity of the respiratory rate**

**Introduction:** Pneumonia is the single commonest cause of death in young children and current IMCI WHO/UNICEF guidelines recommend antibiotic treatment to all children presenting with cough or difficult breathing and an increased respiratory rate for their age. However, respiratory rate may be transiently raised by a number of contextual factors in a busy clinic leading to overuse of antibiotics.

**Methods:** Initial respiratory rate measurements made in 2 outpatient clinics busy clinic among children 2 - 59 months of age presenting with cough or difficulty breathing were compared with the repeat respiratory rate measurements in quiet settings over 1 hour.

**Results:** A total of 167 children were enrolled. The mean (median) age was 7.1 and 27.6 months in younger and older age group respectively. The mean respiratory rate declined from 37.9 breaths per minute (bpm) at clinic to 35.1bpm final reading in quiet room. Using paired test, there was a significant evidence to support white-coat pneumonia phenomena. If the cut-offs of 'raised respiratory rate' was increased by 5 breaths/minute the corresponding proportions is not systematic however the older age group shows a good trend of decline compared to younger group.

In a logistic model age group was the only risk factor associated with over-diagnosis of pneumonia.

**Conclusion:** Noise and other contextual factors may cause a transient increase in respiratory rate and consequent over-diagnosis of non-severe pneumonia. However, this only applied to older children and not infants. Changing the respiratory rates cut-offs to higher threshold reduced the proportion of non-severe pneumonia diagnosis. More studies are needed to identify if such a policy would result in more cases of childhood illness progressing to severe pneumonia.

## Publications

**Florida J. Muro,** Suzanne P. Fiorillo, Philoteus Sakasaka, Christopher Odhiambo, Elizabeth A. Reddy, Coleen K. Cunningham, Ann M. Buchanan. "Seroprevalence of Hepatitis B and C Viruses among Children in the Kilimanjaro Region, Tanzania.". JPIDS, 2013.

George Mtove, Ilse C.E. Hendriksen, Ben Amos, Hedwiga Mrema, Victor Mandia, Alphaxard Manjurano, **Florida Muro**, Alma Sykes, Helena Hildenwall, Christopher J.M. Whitty, Hugh Reyburn, "Treatment guided by rapid diagnostic tests for malaria in Tanzanian children; safety and treatable alternative diagnoses to malaria"**. P**ublication to the Malaria Journal.

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**Muro F**, Mgonja A, Ndosi E and Thielman N "An investigation of HIV risk behavior among clients presenting to voluntary counseling and testing (VCT) in Moshi Tanzania"Abstract 063 selected for Poster presentation during the 6th PHASA Conference (November 29-1 Dec, 2010) in East London, South Africa.

Prabhu M, Mchome B, Mtalo A, Itemba D, Mgonja A, **Muro F J,** Wilkinson J, Ostermann J and Thielman NM "Prevalence and Correlates of Intimate Partner Violence among HIV Voluntary Counseling and Testing Clients in Northern Tanzania": Abstract 224560, selected for Poster presentation during the 138th APHA Annual Meeting (November 6-10, 2010) in Denver, CO.

Reddy E, Mtalo A, Moon A, Itemba DK, Ostermann J, **Muro FJ**, Mayhood MK, Shao JF, Bartlett JA, Crump JA, Thielman NM. Barriers to accessing HIV services in Northern Tanzania: Factors associated with awareness of ART availability among VCT clients. Abstract 6599X. VII International AIDS Conference, Mexico City, Mexico. August 3-8 2008.

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**Name:** Anne-Marie Andersson, BSc, MSc, PhD candidate

**University of PhD registration:** University of Copenhagen

**PhD Title:** Immunology and Virology

**Funding Agency:**

Lundbeckfonden

**Principal Supervisor:**

Associate Professor Peter Holst, Centre of Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen

**Co-supervisors:**

Prof Ali Salanti, Centre of Medical Parasitology, Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen

**Abstracts for the PhD Symposium**

**Title 1: Designing and Optimizing an Adenovirus Encoded VLP Vaccine against HIV**

**Introduction:** The only available target on the HIV virus particle for neutralizing antibodies is the glycoprotein env trimer and considerable work has been invested into the development of this antigen as an immunogen. A principle problem is the instability of the HIV envelope which easily dissociates. We have decided to bypass the complexity of making stable env immunogens by making use of an adenoviral vector to encode the HIV envelope and have it secreted on the surface of virus-like particles in situ.

**Methods:** The vaccine design has been validated with electro microscopy (EM) and also with Western Blot (WB). Mice have been immunized. Further validation will include the quantification of env trimers on the VLPs performed by FACS and ELISA and serum samples harvested by immunized animals will be analyzed with ELISA and in a neutralization assay.

**Results:** The EM pictures have revealed a very potent production of VLPs from Adenovirus infected cells while WB analysis shows the expression of the principal vaccine encoded proteins *gag* and *env*. Preliminary results of serum samples from mice demonstrate that virus-like particle vaccines are immunogenic.

**Discussion/Conclusions:** As to date, we have shown that we can efficiently express VLPs as encoded by an adenoviral vector, validated by EM and WB and that these constructs are immunogenic.

## Publications

Increased Immunogenicity and Protective Efficacy of Influenza M2e Fused to a Tetramerizing Protein"; Accepted: **2012-10-01;** Journal: **PLoS ONE**

**Name:** Jovin Kitau, BSc. MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**PhD Title:** Differential responses of malaria vectors to insecticides and repellents

**Funding Agency:**

Wellcome Trust through Malaria Capacity Development Consortium (MCDC)

Prin**cipal Supervisors:**

Dr. Stephen Magesa, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

Prof Mark Rowland, London School of Hygiene and Tropical Medicine (LSHTM), UK

**Co-supervisors:** Dr. Robert Malima, National Institute for Medical Research (NIMR), Tanzania

**Abstracts for the PhD Symposium**

**Title 1: Long-lasting insecticide treated blanket for protection against Anopheles arabiensis and Culex quinquefasciatus: an experimental hut evaluation in Tanzania**

**Introduction**: Insecticide treated blankets or bed sheets are a potential alternative to long lasting insecticide-treated nets (LLINs), particularly in low net usage, disaster or emergency situations and may provide additional control when used together with an LLIN. In this trial, factory treated, long-lasting insecticidal blankets (LLIB) were evaluated in laboratory bioassays and in experimental huts against wild, free-flying *Anopheles arabiensis* mosquitoes.

**Methods**: Testing was done for treated blankets based on WHOPES guidelines for evaluation of LLINs. Treatments tested were unwashed untreated blanket (negative control 1), untreated blanket washed 5 times (negative control 2), unwashed blanket factory-treated with permethrin (LLIB), LLIB washed 5 times, unwashed blanket conventionally treated with permethrin (ITB), ITB washed 5 times and unwashed, holed Olyset net (positive control). WHO cone bioassays and arm-in-cage tests were done in the laboratory to assess insecticidal efficacy and repellency against female *An. gambiae and An. arabiensis* mosquitoes. The same blankets treatments tested in the laboratory were then tested in veranda-trap experimental huts in Northern Tanzania against wild *An. arabiensis* mosquitoes. The primary outcomes were mortality, and blood-feeding inhibition.

**Results**: In laboratory tests of pyrethroid susceptible *An. gambiae* Kisumu, percentage mortality achieved with unwashed ITB and LLIB was similar at 47% and 45% ($P>0.05$) respectively but these decreased after 5 washes to 20% and 38% respectively. Protective efficacy of LLIB either unwashed or washed 5 times was superior to Olyset LLIN ($P<0.01$), which provided no protective efficacy against landing in arm-in-cage tests. Percentage *An. arabiensis* mortality in huts with factory treated LLIB unwashed (26%) may seem low, but was not statistically different to Olyset net (31%, $P=0.501$). However, mortality was significantly lower when the LLIB was washed (22%, $P=0.037$). Olyset and washed LLIB provided considerable personal protection and both reduced blood-feeding by 49% compared with the washed control ($P<0.001$).

**Conclusion**: This trial demonstrated the potential of insecticide treated blankets to provide personal protection and kill malaria vectors which makes the tool particularly useful where LLINs are not suitable and where net usage is low.

**Title 2: Experimental hut Evaluation of DEET residual spraying against *Anopheles arabiensis* and in North-eastern Tanzania.**

**Introduction**: Widespread pyrethroid resistance among Anopheles mosquitoes and changing pattern in mosquito feeding raise concerns about the continuing efficacy of this group of insecticides. In these circumstances, repellents could provide a valuable additional complement. Therefore, the efficacy of DEET residual spraying has been evaluated in experimental huts against wild, free flying populations of *An*. *arabiensis*.

**Methods:** Plywood panels treated with DEET, lambdacyhalothrin, permethrin, actellic or DDT were evaluated for 36 test nights. Primary outcomes were mortality, inhibition of blood-feeding and treatment-induced mosquito exit.

**Results:** Huts with boards sprayed with DEET recorded a mosquito mortality rate (82%) higher than that achieved with lambdacyhalothrin (77%, P=0.043) and equivalent to that achieved with actellic and DDT (86%, 81% respectively, P>0.05). Although not significant the mortality of unfed mosquitoes was higher than fed mosquitoes for all treatments. There was over 40% reduction in the proportion of *An*. *arabiensis* blood feeding in huts with DEET treated boards. There was a reduction in mosquito entry into huts in the first two weeks post spraying suggesting a spatial effect of freshly sprayed chemicals. There was a 10% increase in the proportion of mosquitoes exiting DEET treated huts, a proportion similar to that seen with lambdacyhalothirn and permethrin.

**Conclusion** DEET induced mortality in mosquitoes that is comparable to the currently used residual insecticides, highlighting its residual potential when used on substrates. DEET, coupled with micro-encapsulation technology which ensures gradual and prolonged release of the chemical, merits further investigation for management of insecticide resistance.

## Publications

Kitau, J. et al. 2012. Species shifts in the *Anopheles gambiae complex*: do LLINs successfully control *Anopheles arabiensis?* PLoS One 7: e31481.

Oxborough RM, **Kitau** J, Matowo J, Feston E, Mndeme R, Mosha FW, Rowland MW (2013) ITN mixtures of chlorfenapyr (Pyrrole) and alphacypermethrin (Pyrethroid) for control of pyrethroid resistant Anopheles arabiensis and Culex quinquefasciatus. PLoS One 8(2): e55781. doi: 10.1371/journal.pone.0055781

Kabula B, Tungu P, Matowo J, **Kitau** J, Mweya C, Emidi B, Masue D, et al (2012) Susceptibility status of malaria vectors to insecticides commonly used for malaria control in Tanzania. Tropical Medicine and International Health 17(6):742-50

**Kitau J**, Oxborough RM, Tungu PK, Matowo J, Malima RC, et al. (2012) Species Shifts in the Anopheles gambiae Complex: Do LLINs Successfully Control Anopheles arabiensis? PLoS One 7(3): e31481. doi:10.1371/journal.pone.0031481

Mwanziva CE, **Kitau J**, Tungu PK, Mweya CN, Mkali H, Ndege CM, Sanga A, Mtabho C, Lukwaro C, *Azizi S*, Myamba J, Chilongola J, Magesa SM, Shekalaghe S and Mosha FW (2011) Transmission intensity and malaria vector population structure in Magugu, Babati District in northern Tanzania. Tanzania Journal of Health Research 13(1) 68-78.

**Kitau J**, Pates H, Rwegoshora TR, Rwegoshora D, Matowo J, Kweka EJ, Mosha FW, McKenzie K & Magesa SM. (2010) The Effect of Mosquito Magnet® Liberty Plus Trap on the Human Mosquito Biting Rate Under Semi-Field Conditions. American Journal of Mosquito Control Association 26 (3) 287-94.

Oxborough RM, **Kitau J**, Matowo J, Mndeme R, Feston E, Boko P, Odjo A, Irish S, N'Guessan R, Mosha F & Rowland M. (2010) Evaluation of indoor residual spraying with the pyrrole insecticide chlorfenapyr against pyrethroid susceptible Anopheles arabiensis and Culex quinquefasciatus mosquitoes. Transactions of the Royal Society of Tropical Medicine and Hygiene 104 (10) 639-45.

Matowo J, Kulkarni MA, Mosha F, Oxborough R, **Kitau J**, Tenu F & Rowland M. (2010) Biochemical basis of permethrin resistance in Anopheles arabiensis from Lower Moshi, North-Eastern Tanzania Malaria Journal 7;9:193.

**Kitau J**, Rwegoshora RT, Rwegoshora D, Matowo J, Mosha FW & Magesa SM (2009) The effect of combined use of Mosquito Magnet Liberty Plus trap and insecticide treated net on human biting rates of *Anopheles gambiae* s.s. and *Culex quinquefasciatus*. *Tanzania Journal of Health Research* 11 (2), 84-89.

Eliningaya J Kweka, Mosha F, Lowasa A, Mahande AM, **Kitau J**, Matowo J, Mahande MJ, Masenga CP, Tenu F, Feston E, Lyatuu EE, Mboya MA, Mndeme R, Chuwa G and Temu EA (2008) Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania. *Malaria Journal* **7**:152doi:10.1186/1475-2875-7-152.

**Name:** Irene Kiwelu, BSc, MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**PhD Title:** Molecular Epidemiology of HIV-1 among Female bar and Hotel workers in Northern Zone of Tanzania

**Funding Agency:**

Good Samaritan Foundation (GSF) of Tanzania,
International Partnership for Microbicide (IPM), and
Harvard International Fogarty Fellowship, USA

**Principal Supervisor:**

Prof Max Essex -Harvard School of Public Health, University of Harvard, Boston, USA

**Co-supervisors:**

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Prof Saidi Kapiga, London School of Hygiene and Tropical Medicine (LSHTM), London, UK & Mwanza

Interventional Trials Unit (MITU), NIMR, Mwanza, Tanzania

Dr. Rachel. Manongi, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**Abstracts for the PhD Symposium**

**Title 1: HIV-1 *pol* diversity among female bar and hotel workers in Northern Tanzania**

**Background:** The National ART program was launched in Tanzania in October 2004. The success of the National ART programs partly depends on the rate of drug resistance among HIV-1 variants circulating in the local HIV/AIDS epidemics. In regions like Tanzania where multiple HIV-1 subtypes and recombinant viruses co-circulate, it is important to determine rates of drug resistance.

**Methodology:** The prevalence of HIV-1 subtypes and drug resistance mutations among 50treatment naïve HIV-1 infected female bar and hotel workers, a high-risk population of HIV-1 infection in Moshi, Tanzaniainfected with different HIV-1 subtypes was investigated. Samples collected at enrollment in 2005 were analyzed. A fragment of the HIV-1 *pol* gene of about 1,660 bp encoding the entire protease (PR) and part of reverse transcriptase(RT) was amplified using modified single genome amplification and sequencing technique.

**Results:** The prevalence of HIV-1 subtypesA1, C, D and inter-subtype recombinant viruses was; 36%, 29%, 9% and 27% respectively. Thirteen different recombination patterns were observed: D/A1/D, C/A1, A1/C/A1, A1/U/A1, C/U/A1, C/A1, U/D/U, D/A1/D, A1/C, A1/C, A2/C/A2, CRF10_CD/C/CRF10_CD and CRF35_AD/A1/CRF35_AD. The CRF35_AD is a new genetic variant in Tanzania. All recombinant viruses in this study were unique, suggesting ongoing recombination processes among circulating HIV-1 variants. The prevalence of multiple infections in this population was 15% (n=7).

Primary HIV-1 drug resistance mutations to RT inhibitors were identified in three (7%) subjects (K65R plus Y181C; N60D; and V106M). In some subjects polymorphisms were observed at the RT positions 41, 69, 75, 98, 101, 179, 190, and 215.Secondary mutations associated with NNRTIs were observed at the RT positions 90 (7%) and 138 (6%).

In the protease gene, three subjects (7%) had M46I/Lmutations. All subjects in this study had HIV-1 subtype-specific natural polymorphisms at positions 36, 69, 89 and 93 that are associated with drug resistance in HIV-1 subtype B.

**Conclusion:** These results suggested that HIV-1 drug resistance mutations and natural polymorphisms existed in this population before the antiretroviral drugs were used in the National ART program. With increasing use of ARV drugs in Tanzania, these results highlight the importance of drug resistance monitoring in ART-naïve individuals.

## Publications:

**Kiwelu IE**, Renjifo B, Chaplin B, Sam N, Nkya WM, Shao J, Kapiga S, Essex M. HIV-1 subtypes among bar and hotel workers Moshi, Tanzania. AIDS Res Hum Retroviruses. 2003 Jan 1:19(1):57-64

**Kiwelu IE**, Koulinska IN, Nkya WM, Shao J, Kapiga S and Essex M. Identification of CRF10_ CD viruses among bar and hotel workers in Moshi, northern Tanzania. AIDS Res Hum Retroviruses 2005, Oct; 21 (10) : 897 -900

**Kiwelu IE**, Novitsky V, Margolin L, Baca J, Manongi R, Sam N, Shao J, McLane MF, Kapiga SH, Essex M. HIV-1 subtypes and Recombinants in Northern Tanzania: Distribution of viral Quasispecies. 2012PLoS One 8:e47605

**Kiwelu IE**, Novitsky V, Margolin L, Baca J, Manongi R, Sam N, Shao J, McLane MF, Kapiga SH, Essex M. Frequently intra-subtype recombination among HIV-1 circulating in Tanzania. 2013,PLoS One 8:e71131

**Name:** Anine Engholm Jeppesen, BSc. MSc, PhD candidate

**University of PhD registration:** Center for Medical Parasitology, University of Copenhagen

**PhD Title:** Immune evasion strategies of *Plasmodium falciparum*

**Funding Agency:**

Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

**Principal Supervisor:**

Associate Professor Lea Barfod, Center for Medical Parasitology, University of Copenhagen, Denmark

**Co-supervisor:** Prof Lars Hviid, Center for Medical Parasitology, University of Copenhagen, Denmark

**Abstracts for the PhD Symposium**

### Title 1: Discovery of novel IgM binding PfEMP1 variants in *Plasmodium falciparum* line NF54

The antigen family *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates binding of infected erythrocytesto a variety of human receptors. The binding phenotype depends on which PfEMP1 variant is being expressed. Each parasite genome contains about 60 *var* genes encoding different PfEMP1 variants. This and the substantial interclonal*var* gene diversity together make the repertoire of PfEMP1 variants immense. PfEMP1 variants involved in placental malaria (VAR2CSA) and some variants displaying the rosetting phenotype (correlated with severe childhood malaria) are able to bind non-immuneIgM. To determine how frequent non-immune IgM binding among PfEMP1 variants is, we set out to identify the IgM-binding PfEMP1 variants in the well-characterized *P. falciparum* line NF54.

Switching among transcription of different *var*genes rarely occurs in long-term *in vitro* cultures, probably as a reflection of epigenetic memory. Therefore, the selection of IEs expressing particular PfEMP1 variants can be troublesome. We overcame this problem by establishing a novel method based on the NF54 clone G6 that had its epigenetic memory deleted. This clone was used in flow cytometry assisted single-cell sorting experiments to select for infected erythrocytes binding non-immuneIgM.

So far, we have identified three additional PfEMP1 variants in NF54 that bind non-immuneIgM. Furthermore, we have further defined the binding site for IgM to the C-terminal part of IgM-binding PfEMP1 variants. These results pave the way for a more comprehensive analysis of the functional significance of the binding of non-immune IgM that is characteristic of some, but by far all, PfEMP1 variants.

**Name:** Johnson Matowo, BSc. MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO) Moshi, Tanzania

**PhD Title:** Characterization of insecticide resistance in *Anopheles gambiaes.l,* the principal malaria vectors in northern Tanzania

**Funding Agency:**

Wellcome Trust through Malaria Capacity Development Consortium (MCDC)

**Principal Supervisor:**

Prof Franklin Mosha, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

**Co-supervisors:** Prof Mark Rowland, London School of Hygiene and Tropical Medicine

**Abstracts for the PhD Symposium**

**TITLE 1: Pyrethroid resistance and *kdr* mutation in *Anopheles arabiensis*, the primary malaria vector in rural villages of Lower Moshi, North Eastern Tanzania**

**Background:** The major foci of pyrethroid resistance in 1990-2010 were in West and Central African populations of *Anopheles gambiaes.s.* Recently, pyrethroid resistance in *Anopheles arabiensis* has been reported in several countries of East and Central Africa. Trends in susceptibility status of *Anopheles arabiensis* in Lower Moshi, North-Eastern Tanzania to different classes of insecticides commonly used in malaria control were assessed.

**Methods:** Three cross-sectional surveys of *Anopheles arabiensis* were conducted in Lower Moshi Tanzania in 2004, 2009, 2011, 2012 and 2013 to determine levels of resistance to pyrethroids, DDT, organophosphates and carbamates using WHO susceptibility tests and standard diagnostic dosages. Sub-samples of mosquitoes that were collected from 8 villages of Lower Moshi in 2013 were identified and genotyped for both L1014S and L1014F mutations byReal time PCR using TaqMan assays.

**Results:** Reduced susceptibility to permethrin was observed in all localities in 2004 (84%-87% mortality). This remained the same in 2009, except in one locality where resistance was recorded (67% mortality). The 2011 survey data showed an increase in the frequency of resistance for permethrin. The KdT50 for permethrin ranged from 19-20 minutes in 2004 to 38-45 minutes in 2011. The populations of *An. arabiensis* were fully susceptible to deltamethrin in 2004. Reduced susceptibility to deltamethrin was observed in all localities in 2009 (87%-97%). However, resistance to deltamethrin was recorded in most localities in 2011 (63%-76% mortality). The KdT50 for deltamethrin ranged from 15 minutes in 2004 to 16-55 minutes in 2011. Resistance to lambdacyhalothrin was observed in 2009, with mortality ranging from 66% to 97%. The recent 2011 survey revealed high levels of resistance to lambdacyhalothrin in all villages (mortality <60%). The KdT50 for lambdacyhalothrin ranged from 38-69 minutes in 2009 to 57-81 minutes in 2011.There was a significant decrease in percentage mortality in all pyrethroids tested in all villages from 2011 to 2013.The *An. arabiensis* remains susceptible to DDT, organophosphates and carbamates. Of 1156 mosquitoes that were genotyped for

L1014S and L1014F mutations, only 1 mosquito from Mtakuja village was found to have L1014F mutation.

**Discussion and Conclusion:** These results clearly demonstrate the presence of pyrethroid resistance in *Anopheles arabiensis* in Lower Moshi. Detection of West African *kdr* mutation in *An. arabiensis* suggests spreading of the mutation across villages, since the same mutation was detected in Msituwatembo village, about 30km from Mtakuja village. The lack of DDT resistance coupled with previous studies showing very low frequency *kdr*suggests that enzyme-based mechanisms are responsible for resistance in *An. arabiensis*. Regional monitoring of resistance should continue to provide an early warning so that alternative insecticides can be considered if resistance levels become operationally significant.

**TITLE 2: The effect of combining two interventions of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) on *Anopheles gambiaes.s* and malaria transmission in North-western Tanzania**

**Introduction:** Continuous usage of insecticides through insecticide treated nets (ITNs) and indoor spraying of insecticides (IRS) is likely to cause insecticide resistance in major malaria vector species. The study that was carried out in Muleba in 2011 revealed very high resistance of *Anopheles gambiaes.s*to pyrethroids and DDT; and reduced susceptibility to bendiocarb with *kdr* frequency nearly reaching fixation (99.8%). Muleba had been under lambdacyhalothrin spray operations since 2006. CDC bottle assays indicated metabolic mechanism involvement.A study was carried out to assess the effect of combining bendiocarb IRS with Olyset nets (LLINs) compared with LLINs alone on *An. gambiaes.s* and malaria transmission in the area.

**Methods:** It was two arms clusters randomized control trial where the two arms received lambdacyhalothrin IRS and LLINs in the first year. In second year, IRS was withdrawn from one arm and in the second arm lambacyhalothrin IRS was replaced by bendiocarb IRS.Insecticide resistance was then monitored in one cluster in each arm with WHOsusceptibility bioassays using diagnostic concentrations of DDT, lambdacyhalothrin, deltamethrin, permethrin, and bendiocarb Circumsporozoite ELISA was used for sporozoite rate determination.

**Results:** A sudden reduction in % mortality in the bendicarb tests was observed in the IRS+LLIN arm after the second round of bendiocarb spraying with a mortality of 76% in May 2012 and only 31% in November while the mortality in the LLIN arm was constant at 88% in May and 84% in November 2012. Significant decrease in percentage mortality was observed when *An.gambiaes.s* was exposed to lambdacyhalothrin after the first round of bendiocarb IRS (from 28% to 12%). For DDT, the percentage mortality remained the same (12%) after the first round of bendiocarb. However there was a decrease in mortality in the LLINs arm (from 40% to 17%). The CSP ELISA assays are not yet completed although preliminary results indicates that there is no effect of insecticide resistance on malaria transmission.

**Conclusion:** The sudden reduction in % mortality in the IRS+LLIN arm after the second round of bendiocarb IRS indicates increased resistance. This may be due to selection of bendiocarb resistant *Anopheles gambiaes.s* that existed before the first round of bendiocarb IRS.Further increase of observed bendiocarb resistance may compromise the success of future IRS with this insecticide. Therefore, there is a need to monitor and investigate mechanisms underlying bendiocarb resistance in the area.

## Publications

High level of resistance in the mosquito *Anopheles gambiae* to pyrethroidinsecticidesand reduced susceptibility to bendiocarb in north-western Tanzania

NatachaProtopopoff, **Johnson Matowo**, Robert Malima, Reginald Kavishe, Robert Kaaya, Alexandra Wright[1],Philippa A West, ImmoKleinschmidt, William Kisinza, Franklin W Mosha and Mark Rowland *Malaria Journal* 2013, **12**:149

Biochemical basis of permethrin resistance in *Anopheles arabiensis* from Lower Moshi, north-eastern Tanzania

**Matowo J,**Kulkarni MA, Mosha FW, Oxborough R, Kitau J, Tenu F,RowlandM*Malar J* 2010, **9**:193

**Name:** George Semango BSc. MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO) Moshi, Tanzania

**PhD Title:** association of Anti-β2 glycoprotein-I antibody and ischemic stroke in Tanzania

**Funding Agency:**

Commission for Science and Technology (COSTECH), Tanzanian Ministry of Communication, Science, and Technology (MoCST), Tanzania

**Principal Supervisors:**

Prof Dr. Gibson S. Kibiki, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

Dr. Leo Joosten, Radboud University, The Netherlands

Prof Dr. Andre Van de Ven, Radboud University, The Netherlands

**Co-supervisor:** Dr. Quirijn de Mast, Radboud University, The Netherlands

**Abstracts for the PhD Symposium**

**Title 1: Anti-β2 glycoprotein-I antibody is associated with ischemic stroke in Tanzania: a case-control study**

**Background**: Ischemic stroke is an important cause of death and disability in Sub Saharan Africa (SSA). The pathogenic mechanisms for the disproportionate burden of stroke in SSA are not well known yet. Antiphospholipid antibodies, including anti-β2 glycoprotein-I (anti-β2GPI) antibodies, are associated with an increased risk for stroke. Chronic infections are highly prevalent in Tanzania and it is well known that anti-β2GPI antibodies may arise during infectious diseases. We hypothesized that anti-β2GPI antibodies are a common risk factor for stroke in SSA.

**Methods:** A case control study was performed that included consecutive patients with an ischemic stroke presenting to the Kilimanjaro Christian Medical Centre in Moshi, Tanzania, and a group of controls. Anti-β2GPI antibodies were determined in this population by ELISA.

**Results**: 157 stroke patients and 79 controls were enrolled. Anti-β2GPI antibodies were detected in 56 (35.7%) of the stroke patients compared to 18 (22.8%) in the control group (P=0.044, chi-squared test). This translated into a crude odds ratio of 1.9 (95% confidence interval 1.01 – 3.5) and an adjusted odds ratio of 2, 9 (95% CI 1.02 – 8.0) in a multivariate logistic regression model. There was no significant difference in the prevalence of β2GPI antibodies across age categories or the type of ischemic stroke.

**Conclusions**: Anti-β2GPI antibodies had a very high prevalence in this Tanzanian population and were associated with an increased risk for stroke. Their contribution to the etiology of ischemic stroke and the relation with infectious diseases is intriguing and deserves further study.

## Publications

Plantenga*et al*., A promoter polymorphism in human interleukin-32 modulates its expression and influences the risk and the outcome of epithelial cell–derived thyroid carcinoma 2013

Semango et al., Exploring IL-32 splicing in Thyroid Cancer and Kaposi Sarcoma (submitted)

**Name:** Jacklin Mosha, MD, MSc, PhD candidate

**University of PhD registration:** Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania

**PhD Title:** Investigation of malaria hotspots in order to progress towards control strategies targeted at hotspots.

**Funding Agency:**

Wellcome Trust through Malaria Capacity Development Consortium (MCDC)

**Principal Supervisor:**

Prof Dr. Gibson S. Kibiki, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

Dr. Daniel Chandramohan, London School of Hygiene and Tropical Medicine (LSHTM), UK

**Co-supervisors:** Prof Brian Greenwood, London School of Hygiene and Tropical Medicine (LSHTM), UK

**Abstracts for the PhD Symposium**

**Title 1: Epidemiology of sub-patent *Plasmodium falciparum* infection: implications for detection of hotspots with imperfect diagnostics**

**Introduction***:* At the local level, malaria transmission clusters in hotspots, which may be a group of households that experience higher than average exposure to infectious mosquitoes. Active case detection, often relying on rapid diagnostic tests for mass screen and treat campaigns, has been proposed as a method to detect and treat individuals in hotspots. Data from a cross sectional survey conducted in north-western Tanzania were used to examine the spatial distribution of *Plasmodium falciparum* and the relationship between household exposure and parasite density.

**Methods:** Dried blood spots were collected from consenting individuals from four villages during a survey conducted in 2010. These were analyzed by PCR for the presence of *P. falciparum*, with the parasite density of positive samples being estimated by quantitative PCR. Household exposure was estimated using the distance-weighted PCR prevalence of infection. Parasite density simulations were used to estimate the proportion of infections that would be treated using a screen and treat approach with RDTs compared to targeted mass drug administration and MDA.

**Results:** PCR analysis revealed that of the 3,057 blood samples analysed, 1,078 were positive. Mean distance-weighted PCR prevalence per household was 34.5%. Parasite density was negatively associated with transmission intensity with the odds of an infection being sub-patent increasing with household exposure (OR 1.09 per 1% increase in exposure). Parasite density was also related to age, being highest in children 5-10 years old and lowest in those >40 years. Simulations of different MDA strategies showed that treating all individuals in households where RDT prevalence was above 20% increased the number of infections

that would have been treated from 43% to 55%. However, even with this strategy, 45% of infections remained untreated.

**Conclusion:** The negative relationship between household exposure and parasite density suggests that DNA-based detection of parasites is needed to provide adequate sensitivity in hotspots. Targeting MDA only to households with RDT positive individuals may allow a larger fraction of infections to be treated. These results suggest that community wide MDA, instead of screen and treat strategies, may be needed to treat the asymptomatic, sub-patent parasite reservoir successfully and reduce transmission in similar settings.

**Title 2: Spatial methods to determine malaria transmission hotspots**

**Background:** At the micro-scale, malaria infections are frequently clustered into single or groups of households that consistently have significantly more infections than others throughout the year. Identifying and targeting interventions at these hotspots of malaria transmission, could lead to a rapid reduction in malaria transmission. To date there is no agreed method for the detection of malaria transmission hotspots. The most used geospatial method is the spatial scan statistic. This study seeks to determine which geospatial method best describes a malaria transmission hotspot by comparing methodologies using cross sectional data collected in 2010 to predict the distribution of malaria infections in the following year.

**Methods:** Three geospatial methods were used to explore baseline clustering of malaria infection and serological markers. The three methods explored were spatial scan statistic, kernel analysis and weighted local prevalence analysis. These geospatial methods were compared in their ability to predict malaria infection in the second year of the study. To compare the predictive performance of the different methods, we used mixed effects logistic regression models, and calculated and compared the area under the receiver operating curve (AUC) for each model. During the first year, we measured malaria infection through detection of parasite DNA using nested polymerase chain reaction (nPCR), and we measured serologic markers (AMA-$1_{19}$, MSP-19 antibodies) for malaria infection. Malaria infection in the second year was determined by nPCR.

**Results:** Guided by AUC values, the kernel method of cluster identification appeared to allow the most accurate prediction of malaria infection in the following year. The spatial scan statistic had similar AUC values but only discriminated two hotspots amongst the villages. The weighted local prevalence method showed a similar spread of high prevalence areas as the kernel method but was a poorer predictor of infection in the second year. When using the Kernel method for both nPCR prevalence and AMA-1 sero-prevalence, a 500m and 1km radii were optimum radii to predict malaria infection in the second year.

**Conclusion:** Hotspots can be defined using geospatial methods and are stable over a period of at least one year. Hotspots can be detected either by using parasite prevalence and sero-

prevalence to AMA-1 antibodies or both. It was also found that the kernel method was better in predicting subsequent malaria infection than the other two geo-spatial methods explored.

## Publications

**Mosha JF**, Sturrock HJ, Greenhouse B, Greenwood B, Sutherland CJ, Gadalla N, Atwal S, Drakeley C, Kibiki G, Bousema T, Chandramohan D, Gosling R. *Epidemiology of subpatent Plasmodium falciparum infection: implications for detection of hotspots with imperfect diagnostics.* Malar J. 2013 Jul 1;12:221. doi: 10.1186/1475-2875-12-221

**Mosha JF**, Sturrock HJ, Greenhouse B, Greenwood B, Sutherland CJ, Gadalla N, Atwal S, Hemelaar S, Brown JM,Drakeley C, Kibiki G, Bousema T, Chandramohan D, Gosling R. Spatial methods to determine malaria transmission hotspots. Submitted

Gosling RD, Okell L, **Mosha J**, Chandramohan D. *The role of antimalarial treatment in the elimination of malaria.* Clin Microbiol Infect. 2011 Nov;17(11):1617-23. doi: 10.1111/j.1469-0691.2011.03660.x. Epub 2011. Review.

**Name:** Charles Michael Mtabho, MD, MPH, PhD candidate

**University of PhD registration:** Radboud University, The Netherlands

**PhD Title:** Improving management of Tuberculosis in Tanzania; epidemiological and clinical studies

**Funding Agency:**

NACCAP (The Netherlands) through APRIORI and

European and developing Countries Clinical Trials Partnership (EDCTP) through PanACEA Consortium

**Principal Supervisor:**

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**Co-supervisors:**

Prof Dr. Gibson S. Kibiki, Kilimanjaro Christian Medical University College (KCMUCO), Moshi, Tanzania.

Dr. Rob .E. Aarnoutse, MSc. Pharm D, PhD, Radboud University, The Netherlands

Prof Dr. Martin J. Boeree, MD, PhD, Radboud University, The Netherlands

**Abstracts for the PhD Symposium**

**Title 1: The effect of diabetes mellitus on the pharmacokinetics of tuberculosis drugs in Tanzanian patients**

**Introduction:** Diabetes mellitus (DM) is a well-known risk factor for tuberculosis (TB). With the current global increase in type 2 DM, more attention is needed on optimum treatment of TB in TB-DM patients. Pharmacokinetic profiles of TB drugs in Asian and American TB-DM patients have been described, but such data are lacking in Africa. We performed a pharmacokinetic study in Tanzanian patients.

**Methods:** Forty Tanzanian adult TB patients (20 TB only, 20 TB and Diabetes) who were in the intensive phase of TB treatment for at least two weeks were recruited at a Tanzanian out-patient TB clinic. Plasma concentrations were determined in venous blood samples taken just before and at 1, 2, 3, 4, 6, 8, 10, 24 hours after observed drug intake to estimate pharmacokinetic parameters of isoniazid, rifampicin, pyrazinamide and ethambutol using validated HPLC methods.

**Results:** The geometric mean exposure ($AUC_{0-24}$) of rifampicin and isoniazid (in h*mg/L) were significantly lower in TBDM patients (29.3 rifampicin, 5.4 isoniazid) than TB only patients (39.9 rifampicin, 10.6 isoniazid). $C_{max}$ (mg/L) of isoniazid was also lower in TBDM patients (1.6 versus 2.8, p=0.01). 73.7% versus 55% of patients had $C_{max}$ of isoniazid below reference range in the TBDM group as compared to the TB only group, and for rifampicin, the proportions were 43.4% against 35%. In a multiple linear regression analysis, age, bodyweight, or BMI did not affect the association between DM and the PK parameters. All PK parameters for pyrazinamide and ethambutol were not significantly different between the groups

**Conclusion:** Exposure to isoniazid and rifampicin is reduced in Tanzanian diabetic patients with TB. These effects are most likely explained by the diabetes disease. Increasing the doses of these drugs in treating TBDM patients may be considered in view of accumulating

evidence that exposure to TB drugs is related to response.

## Publications

**Mtabho, C. M**., Irongo, C. F., Boeree, M. J., Aarnoutse, R. E., & Kibiki, G. S. 2010, "Childhood tuberculosis in the Kilimanjaro region: lessons from and for the TB programme", *Trop.Med. Int.Health*, vol. 15, no. 5, pp. 496-501.

*Suba,M.R, Ayana, S.M,* ***Mtabho, Charles.M****, Kibiki, G.S (2010). "*The aetiology, management and clinical outcome of upper gastrointestinal bleeding among patients admitted at the Kilimanjaro Christian Medical Centre in Moshi, Tanzania", *Tanzania Journal of Health Research,* Vol 12, No 4

*Charles E. Mwanziva, Jovin Kitau, Patrick K. Tungu, Clement N. Mweya, Humphrey Mkali, Chacha M. Ndege, Alex Sanga,* ***Charles Mtabho****, Charles Lukwaro, Joseph Myamba, Salum Abdulalazizi, Stephen M. Magesa, Jaffu Chilongola, Seif Shekalaghe, Franklin W. Mosha (2011).* "Transmission intensity and malaria vector population structure in Magugu, Babati District in northern Tanzania", *Tanzania Journal of Health Research,* Vol 13, No 1

Scott K. Heysell, **Charles Mtabho**, Stellah Mpagama, Solomon Mwaigwisya, Suporn Pholwat, Norah Ndusilo, Jean Gratz, Rob E. Aarnoutse, Gibson S. Kibiki and Eric R Houpt. Plasma Drug Activity Assay for Treatment Optimization in Tuberculosis Patients. *Antimicrob. Agents Chemother.* 2011, 55(12):5819. DOI: 10.1128/AAC.05561-11.Published Ahead of Print 3 October 2011.

Stellah G. Mpagama, **Charles Mtabho**, Solomon Mwaigwisya, Liberate J. Mleoh, I Marion Sumari-de Boer, Scott K. Heysell, Eric R. Houpt, and Gibson S. Kibiki. Comparison of Overnight Pooled and Standard Sputum Collection Method for Patients with Suspected Pulmonary Tuberculosis in Northern Tanzania," Tuberculosis Research and Treatment, vol. 2012, Article ID 128057,2012. doi:10.1155/2012/128057.

Hadija H Semvua, **Charles M Mtabho,** Quirine Fillekes, Jossy van den Boogaard, Riziki M Kisonga, Liberate Mleoh, Arnold Ndaro, Elton R Kisanga, Andre van der Ven, Rob E Aarnoutse, Gibson S Kibiki, Martin J Boeree, David M Burger. Efavirenz, tenofovir and emtricitabine combined with first line tuberculosis treatment in TB–HIV-coinfected Tanzania patients: a pharmacokinetic and safety study. Antivira therapy, 2012. **doi:** 10.3851/IMP2413

Alma Tostmann, **Charles M. Mtabho**, Hadija H. Semvua, Jossy van den Boogaard, Gibson S. Kibiki, Martin J. Boeree, Rob E. Aarnoutse. Pharmacokinetics of first line tuberculosis drugs in Tanzanian patients. Accepted *Antimicrob. Agents Chemother*

**Charles M. Mtabho**, Hadija H. Semvua, Jossy van den Boogaard, Constantine F. Irongo, Martin J. Boeree, Angela Colbers, David M Burger, van Crevel R, van der ven AJAM, Gibson S. Kibiki, Alma Tostmann, Rob E. Aarnoutse. The effect of diabetes mellitus on exposure to tuberculosis drugs in Tanzanian patients. *In preparation.*

**Name:** Sidsel Nag, BSc, MSc, PhD candidate

**University of PhD registration:** University of Copenhagen, Denmark

**PhD Title:** Validating the applicability of malaria rapid diagnostic tests and whole genome sequencing to survey molecular aspects of malaria epidemiology and epidemic risks

**Funding Agency:**

**Principal Supervisor:**

Associate Professor Michael Alifrangis, Center for Medical Parasitology, University of Copenhagen, Denmark

**Co-supervisor:** Professor Ole Lund, Center for Biological Sequence Analysis, Danish Technical University,

Prof Frank Aarestrup, Danish Technical University, Denmark Prof Poul-Erik Kofoed, Institute of Regional Health Research, University of Southern

Denmark

Dr. Johan Ursing, Department of Infectious Diseases Solna, KarolinskaInsitute, Stockholm. Sweden

**Abstracts for the PhD Symposium**

**Title 1: Validating the applicability of malaria rapid diagnostic tests and whole genome sequencing to survey molecular aspects of malaria epidemiology and epidemic risks**

Successful disease control requires successful disease surveillance. In this regard, we propose an application of malaria rapid diagnostics tests (RDTs) provided for sub-Saharan Africa in numbers larger than 70 million in 2011 alone, for surveillance of molecular epidemiology of *P. falciparum* malaria. We are attempting to setup regular RDT-collection in collaboration with local health centers in Tanzania and Guinea-Bissau. We wish to investigate whether used RDTs can be applied for PCR-based methods to detect the presence of antimalarial resistance markers represented by single-nucleotide polymorphisms in *P. falciparum* genes. Regular high-throughput analysis of the prevalence of these resistance markers would provide evidence for a basis for molecular surveillance of resistance to antimalarial drugs. Furthermore, we wish to investigate to which extent used RDTs collected at local health centers can be applied for serological analysis, and lastly whether it is feasible to acquire DNA suitable for whole genome sequencing (WGS). WGS is a major player in pathogen surveillance, applied for the purpose of keeping up to date with the spread of different genotypes and identification of evolutionary events with potential effect on pathogenesis or treatment. We wish to investigate the differences in intra-regional and inter-regional parasite diversity in Tanzania and Guinea-Bissau, as well as differences in putative selection of parasites due to differences in transmission intensity and fluctuations. Elucidating these differences is key to providing policy guidance of containment of e.g. artemisinin resistant parasites and focusing surveillance in high risk-areas.

## LIST OF FACULTY MEMBERS WHO PROVIDED EXPERTISE OPINION TO THE PhD STUDENTS DURING THE SYMPOSIUM

1. Adam Sander

   Post Doc fellow at Centre for Medical Parasitology at University of Copenhagen, Denmark

2. Ahaz Kulanga

   Deputy Provost, Administration and Finance, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

3. Augustine Mallya

   Associate Professor, department of Orthopaedics and Traumatology Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

4. Bakari Msangi

   Ag NFAST, Tanzania Commission for Science and Technology (COSTECH), Dar Es Salaam, Tanzania

5. Balthazar Nyombi

   Senior Lecturer, department of Medical Microbiology, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

6. Bernard Njau

   Lecturer, department of Community Health, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

7. Blandina Mmbaga

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11. Didi Bang

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12. Egbert Kessi

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    Lecturer, department of Orthopaedics and Traumatology, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

14. Elisante Masenga

    Associate Professor, department of Dermatology and Venereology, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    Lecturer, Faculty of Nursing, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

16. Elton Kisanga

    Senior Lecturer, department of Pharmacology Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    Professor of Medicine, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    Lecturer department of Internal Medicine, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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Professional Development and Educational Advisor on the Malaria Capacity Development Consortium (MCDC) programme, Université de Dakar, Senegal

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    Lecturer, department of Internal Medicine, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

28. Jaffu Chilongola

    Senior Lecturer, department of Biochemistry and Molecular Biology, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

29. Joackim Kessy

    Lecturer, department of development studies Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

30. John Bartlett

    Professor of Medicine, and International Health, Duke University, USA, and Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    Senior Lecturer, department of Paediatrics and Child Health, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

32. Maryselina Msuya

    Lecturer, Faculty of Nursing, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    Lecturer, department of Community Health, Kilimanjaro Christian Medical University College (KCMUCo), Moshi, Tanzania

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    PhD in Childhood TB diagnosis and performance of interferon-gamma release assays, Department of Infectious Diseases, Hvidovre University Hospital, Denmark

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    Associate professor at Centre for Medical Parasitology at University of Copenhagen, Denmark

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    Laboratory technologist, Senior Scientist, Technical University of Denmark (DTU), Copenhagen, Denmark

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**The Organising committtee of the 2nd Kilimanjaro International PhD Symposium**

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