CLINICAL TRIALS PLATFROM IN SUB-SAHARAN AFRICA

Bernhards Ogutu, Kenya Medical Research Institute (KEMRI)

Kisumu, Kenya

The last 2 decades has seen unprecedented increase in the number of clinical trials in Africa in a number of infectious diseases with malaria, HIV and TB taking the lead. This arose from the realization that there is need to evaluate new products and interventions in the areas where diseases are endemic. This led to the development clinical trials infrastructure which was championed by groups like AMANET, MCTA of INDEPTH-Network, EDCTP, NIH, UK MRC, Wellcome Trust and host of Universities and research institutions from Europe and America. The infrastructure was linked to capacity building which was initially focused on short term training to enable execution of specific projects. Most trials were mainly phase II and III. There was less focus on the long term training in clinical trials to develop a critical mass of in clinical trialists in the various centres that had the capacity. The current clinical infrastructure is mainly geared towards phase II and III field trials not early phases of product development. In the last 5 years there has been a drive to build both infrastructure and human capacity for phase I clinical trials in the sub-Saharan region. This has seen set of phase I facilities in Tanzania, Kenya, Ghana, Mali, Burkina Faso and Gabon. The development of these phase I facilities have been linked to the development of capacity for human malaria challenge studies under the aupeicies of the African controlled human malaria infections (CHMI). The development of products that largely target deployment in Africa calls for a functional pharmacovigilance platform. Pharmcovigilance in the region is week thus the need to develop a phase IV platform to ensure these new products as they get deployed are appropriately evaluated for safety and effectiveness. The last 6 years has seen the establishment of a phase IV platform through the INDEPTH-Network for antimalarials (INESS). The INESS platform has evaluated the current first line treatments of malaria in Tanzania, Ghana, Burkina Faso and Mozambique for safety and effectiveness. The formidable development of clinical trials platform in the region has not been without hurdles like provision of standard care to study participants during the studies, an evolving ethical review platform, weak but developing regulatory framework and weak health service delivery system. These circumstances add a lot of responsibilities to the African clinical trials investigator who has to work through. The investigators at times support these various stakeholders in order to execute clinical trials to meet GCP standards. At times the clinical trials investigator is seen as a villain or supporter by the health system depending on circumstances. There is need for continued investment in the clinical trials capacity in the region to sustain the current capacity and move it forward to entrench product development in the region from concept to deployment of the products.

Management of Malaria in the era of drug resistance and Elimination drive in Africa

Bernhards Ogutu, Kenya Medical Research Institute (KEMRI)

Kisumu, Kenya

Malaria remains endemic in the sub-Saharan Africa despite the reduction in disease burden experienced in the last decade. The management of malaria has heavily relied on reduction of vector human contact by use of ITNs and IRS and prompt diagnosis and treatment of the symptomatic patients. These strategies have led to the decline in disease burden but despite scale up, most countries are not seeing further significant decline disease burden. This plateau effect is further compounded by the emergence of resistance to every new antimalarial drug with latest being artemisinin derivatives in South East Asia with reports starting to emerge from Africa. In the addition to the drug resistance, insecticide resistance is spreading very fast and likely to retard gains already made on vector control and requires more attention. Despite advances made on disease modelling and epidemiology techniques malaria diagnosis relies on Microscopy as the gold standard despite largely depending on the proficiency of personnel and it also has a limit of detection. Microscopy is currently supplemented by RDTs for diagnosis but RDTs too has limit of detection and has batch sensitivity and specificity variability. This makes disease burden determination with these techniques even in the best settings estimates with wide margins of error. However, the extent of the deployment of these tools is variable across the region and areas with highest burden are the least accessed. In regions that have good accessibility the health system dynamics reduce the effectiveness of these tools to sub-optimal levels. There is need to review the malaria management in view of the elimination agenda and prevailing geopolitical landscape in Africa. A concerted regionally coordinated and led appropriate intervention development and deployment, health system alignment and long term strategy remains a key pillar.

Development of a TSOL18 vaccine for licensure

Kapil Maithal[1]*; Marshall Lightowlers*[2]*; Tim Rowan*[3]*; M. Elaiyaraja*[1]*; Meritxell Donadeu*[3]

[1]Indian Immunologicals Limited, Rakshapuram, Gachibowli Post, Hyderabad-500032, INDIA; [2]Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, AUSTRALIA; [3]Global Alliance for Livestock Veterinary Medicines (GALVmed), UNITED KINGDOM

Cysticercosis is a common disease in pig raising areas globally. The disease is caused by *Taenia solium* parasite. The life cycle of the parasite includes pigs as the normal intermediate host, harboring the larval vesicles or cysticerci and humans as the definitive host, where it causes neurocysticercosis. The use of an effective vaccine in pigs would in fact eliminate the tapeworm infection indirectly in humans by breaking the parasite life cycle. Earlier studies with a recombinant oncosphere antigen- TSOL 18 protein expressed in *E. coli* has been shown to be highly effective in preventing infection of pigs in controlled experimental trials. In recent times, the protein has been re-cloned and expressed in a *Pichia pastoris* expression system. The manufacturing process for the production of the protein at pilot fermentation scale has been optimized. A suitable formulation of the antigen has been developed with Montanide ISA 206 as adjuvant and has also been found to be safe, immunogenic and efficacious in pivotal regulatory studies conducted in compliance with veterinary Good Clinical Practice (VICH) in target animals. Currently, the vaccine candidate is entering into field trials in India for registration as a vaccine for porcine cysticercosis.

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Quantitative Analysis of Confounding Variables in IBS-Blastocystis Association Studies with Re-usable Examples in Excel

Boorom, Kenneth F[1]

[1]Blastocystis Research Foundation, 5060 SW Philomath Blvd, Corvallis, OR 97333

BACKGROUND: To most professionals, it would appear to "make sense" that population studies examining the association of an infection like Blastocystis with symptoms like IBS should consistently show either positive or negative results. However, in practice, such studies are among the least reproducible ones, and the source of the greatest contention. In this discussion, methods are illustrated for calculating the impact of confounding variables on the statistical significance value "p" in relating Blastocystis and IBS.
METHODS: Existing studies are reviewed which relate Blastocystis and IBS, with a view toward findings and study construction, and statistical significance. Prior studies relating similar infections to similar studies are reviewed, with a view toward identifying which ones did or did not demonstrate statistical significance. The studies are also reviewed for confounding factors, their value is estimated, and impact investigated through analysis in a commonly available spreadsheet program (MS Excel)
RESULTS: The analysis predicts which studies are most likely to show now association between Blastocystis infection and IBS. Lack of association will be most common in populations with a high prevalence of Blastocystis infection, or similar infections, especially where acquired or innate immunity is prevalent. Participant misclassification would also have a significant impact on study results. Study size does not improve results in these cases. Assay sensitivity has less of an effect.
CONCLUSIONS: To investigate the potential association of an infectious agent with IBS with confidence, it is necessary to exclude patients with acquired or innate immunity to the agent and reliably classify patients as IBS or non-IBS, and also to exclude patients with other potential causes for IBS.

Distinct immune profiles in amicrofilaridermic, hyperreactive and generalized onchocerciasis patients

Hübner, Marc P.[1]; Gnatoulma, Katawa[1]; Layland, Laura E.[1]; Debrah, Alexander Y.[2,3]; Arndts, Kathrin[1]; Batsa, Linda[2]; Tamarozzi, Francesca[4]; Klarmann Schulz, Ute[1]; Taylor, Mark[4]; Adjei, Ohene[2,3]; Martin, Coralie[5]; Kwarteng, Alexander[1]; Taylor, David[6]; Specht, Sabine[1]; Adjobimey, Tomabu[1]; Hoerauf, Achim[1]

[1]Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, 53105 Bonn, Germany; [2] Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana; [3]Faculty of Allied Health Sciences and School of Medical Sciences of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [4]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom, [5]UMR 7245 MCAM MNHN CNRS, Museum National d'Histoire Naturelle, Paris, France; [6]Institute of Immunology and Infection Research, University of Edinburgh, EH9 3JT, Scotland

BACKGROUND: Clinical manifestations in onchocerciasis range from generalized onchocerciasis with low pathology to severe hyperreactive sowda. Repeated ivermectin MDA further led to the occurrence of amicrofilaridermic patients. In order to investigate differences in the immune profile of those patient groups, the presented studies 1) investigated regulatory and Th17 immune responses in generalized and hyperreactive patients; and 2) compared immune responses in amicrofilaridermic and microfilaridermic patients.
METHODS: Using flow cytometry, cytokine ELISAs and PCR arrays, the Th subsets in PBMCs from generalized and hyperreactive onchocerciasis patients were elucidated. A second study combined immune profiles with parasitological and etiological data to characterize the immunoepidemiology of amicrofilaridermic and microfilaridermic onchocerciasis patients.
RESULTS: Th17 and Th2 responses were accentuated in hyperreactive onchocerciasis patients, as frequencies of IL-17A$^+$, IL-4$^+$, GATA3$^+$ and RORC2$^+$ CD4$^+$T-cell populations were increased and Th2/Th17 related genes were upregulated, while regulatory T-cells were reduced. Amicrofilaridermic patients had received more rounds of ivermectin and presented fewer nodules. Multivariable regression analysis revealed that IL-5 responses, ECP concentrations, as well as eosinophil and neutrophil frequencies were highly associated with microfilaridermia. The number of ivermectin treatments that each individual received was negatively associated with IL-10 production. Importantly for disease etiology, regions that had received more than eight rounds of ivermectin had lower IL-17 responses than regions that had received only one round of ivermectin.
CONCLUSIONS: Our data indicate that Th17 and Th2 cells contribute to the development of severe onchocerciasis. Furthermore, both the number of ivermectin treatments an individual received and the number of rounds of ivermectin MDA within the community influence immune responses, suggesting that immune responses and the risk to develop onchocerciasis is also altered in community members who did not regularly participate in MDA program.

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Role of Asymptomatic Infections in Sustained Malaria Transmission

Sócrates Herrera[1,2], Jhon Garcia[1], Andrés Amado[1], Andrés Vallejo[1], Myriam Arévalo-Herrera[2]

[1]*Malaria Vaccine and Drug Development Center (MVDC), Cali, Colombia,* [2]*Latin American Center for Malaria Research (CLAIM), Cali, Colombia*

Malaria remains a global public health problem. During the last few years, 200-300 million clinical cases and ~600 thousands fatal cases have been reported worldwide. Additionally, an unknown number of asymptomatic malaria cases are present in endemic regions. These asymptomatic cases potentially play a pivotal role in the silent transmission of the infection from the human host to mosquito vector and the subsequent spreading of the infection.

In order to assess the role of asymptomatic malaria infection in malaria transmission, we determined the following in three groups of human volunteers: the infectivity of parasite samples to mosquitoes and the maturation rate and the sex ratio of gametocytes. The groups were composed of: A) malaria symptomatic (n=16); B) asymptomatic (n=14); and C) experimentally infected volunteers (n=16). Molecular markers (Pvs16, Pvs25, PvNek-4 and PvMap-2) were used to quantify the number, sex, and sex ratio of gametocytes in parasites circulating in nature.

A total of 46 *P. vivax* blood samples were analyzed through this molecular typing and functional assay. 16 samples were collected from acutely infected patients; 14 samples were collected from asymptomatic donors recruited in a malaria endemic region in Buenaventura, Colombia; and 16 samples were collected from volunteers subjected to a *P. vivax* experimental infection trial. Seven asymptomatic individuals were able to infect mosquitoes whereas none of the experimentally infected volunteers successfully infected mosquitoes. Asymptomatic subjects had significantly greater expression of Pvs-16 (~$1x10^3$ molecules/µl) compared to experimentally infected volunteers (~$2x10^2$ molecules/µl) ($p= 0.005$). Additionally, asymptomatic volunteers displayed greater numbers of macrogametocytes (Pv-Nek4) compared to microgametocytes (Pv-MAPK2) whereas in experimentally infected volunteers, the proportion was the inverse.

These results indicate that asymptomatic patients are as expected a reservoir for mosquito infection under natural conditions and although circulating gametocytes could be found as early as day 7 post-infection, the maturation/proportion of macro and micro-gametocytes was suboptimal for inducing mosquito infection. Furthermore, the number of asymptomatic infections detected here were by cross-sectional study. It is likely that a greater number of cases would be detected if a closer follow-up of this population was conducted.

Because of the important contribution of asymptomatic infections to the continued malaria transmission in endemic areas, further studies using molecular markers and functional assays are required, particularly in the context of the currently ongoing or presently envisaged malaria elimination programs.

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Perspectives for Malaria Elimination in Mesoamerica and the Hispaniola Island

Sócrates Herrera[1,2] Sergio Ochoa-Orozco [1,2], Iveth J. González[3], Martha L. Quiñones [2, 5] and Myriam Arévalo-Herrera [1, 6]

[1]Caucaseco Scientific Research Center. Cali, Colombia.[2]Centro Latino Americano de Investigación en Malaria (CLAIM).[3]Foundation for Innovative New Diagnostics (FIND). Geneva, Switzerland.[4]Consultant, former Fund Portfolio Manager for Latin-America and the Caribbean/GFATM.[5] Universidad Nacional de Colombia. Bogotá, Colombia. [6] Facultad de Salud, Universidad del Valle, Cali, Colombia

Malaria remains endemic in 22 countries of the American continent with an estimate 469,000 cases/year, with ~10% of them occurring in the Mesoamerican and Caribbean regions (WHO 2013). During the last decade, malaria transmission in Mesoamerica presented a mean decrease of ~85%, whereas in the Caribbean region, the Hispaniola Island comprising Dominican Republic (DR) and Haiti presented an overall malaria transmission rise due to a steady increase in Haiti, although DR experienced a significant transmission decrease in this period (WHO 2013).Whereas *P.vivax* is highly predominant in Mesoamerica, *P.falciparum* is highly predominant in The Hispaniola. Both parasite species appear to remain susceptible to Choloroquine what makes its control more plausible.

Taking the opportunity of this significant malaria reduction in Mesoamerica, an initiative for Malaria Elimination in Mesoamerica and The Hispaniola Island (EMMIE by its Spanish acronym) has been recently launched with the active involvement of the National Malaria Control Programs of nine countries with the goal of bringing malaria transmission to zero in the region by year 2020. The EMMIE initiative is led by the Global Fund for Aids, Tuberculosis and Malaria (GMATM) with an active participation of multiple partners including Ministries of Health, all countries involved grouped in the Council of Health Ministers of Central America and Dominican Republic (COMISCA); multinational agencies and research centers.

Whereas in countries like El Salvador and Costa Rica, malaria incidence has recently reached <10 cases/year, other areas like Mosquitia in the Honduras-Nicaragua border remains ~ 6,500 cases/year, and as mentioned Haiti has presented ~20,000 cases/year. In a series of meetings facilitated by the EMMIE partners, a number of knowledge gaps have been identified by the countries and it has been proposed that academic institutions could provide scientific support for malaria elimination.

Here we will discuss the process followed by the different countries, the GMATM and the different partners involved in the initiative so far, as well as the specific aims and mechanisms proposed to reach the goal of malaria elimination by 2020 as well as the new funding mechanisms. EMMIE would represent a paradigm for other regions of the world including the rest of countries of the continent.

Plasmodium vivax malaria vaccine development

Sócrates Herrera[1,2], Nora Céspedes[1,3], Giampietro Corradin[4] and Myriam Arevalo-Herrera[1,3]

[1]Malaria Vaccine and Drug Development Center, Cali Colombia; [2]Caucaseco Research Center, Cali Colombia; [3]Universidad del Valle, Cali Colombia, [4]University of Lausanne, Lausanne Switzerland;

Because both *Plasmodium falciparum* and *P. vivax* coexist in numerous malaria endemic areas, effective anti-malarial vaccines would require protective subunits from both parasite species. Unfortunately, there is a significant gap between *P. falciparum* and *P. vivax* vaccine development. In order to address this issue, during the last two decades we have been working on establishing a Platform for *P. vivax* vaccine development in Colombia and multiple vaccine candidates have been tested in preclinical and clinical trials.

First we are currently conducting a clinical vaccine trial to reproduce the model for vaccination with *P. vivax* radiation attenuated parasites (irrad-spz). This model that was developed both for human and rodent malaria parasites induces complete protection from infectious challenge with fully infectious sporozoites, representing a formidable model to study malaria protective immunity. Ongoing studies to reproduce this model for *P. vivax* as mean to develop reagents for vaccine discovery.

Second, one of the proteins recognized by individuals protected using is the malaria circumsporozoite (CS) protein which has been the subject of extensive studies. *P. falciparum* CS protein is contained in the RTS,S vaccine candidate that has reached phase III of clinical development. A vaccine clinical development program of *P. vivax* CS protein (*Pv*CS) has been ongoing for several years in Colombia and will be presented.

Third, a *P. vivax* vaccine discovery program is addressed to identify new vaccine candidates. We are currently following two different approaches genome/proteome approaches: 1) identifying alpha helical coiled coil structures in *P. vivax* proteins which are identified *in silico*, synthesized and used to assess both antigenicity and immunogenicity and, 2) *P. vivax* genomic and proteomic analyses of reagents produced in the *P. vivax* irrad-spz trials described above.

Finally how *P. vivax* endemic countries could contribute to vaccine development will be discussed.

Host-parasite interaction: methabolic biomarkers and prothrombotic factors in Chagas disease.

Joaquim Gascon[1]*; Maria J. Pinazo*[1]*; Momar Ndao*[2]
[1] Barcelona Centre for International Health Research (CRESIB)- ISGLOBAL, Barcelona, Spain. NHEPACHA network.
[2] National Reference Centre for Parasitology, Research Institute of the McGill University Health Center, Montreal, Canada

BACKGROUND: A lack of appropriate biomarker tools limits the measurement of treatment impact for Chagas disease (CD). CD is a chronic infection, which stimulates a continuous inflammatory immune response. Molecules that are inflammatory mediators of metabolic processes are altered in chronic states of inflammation and/or infection such as chronic CD. Even though many biochemical biomarkers are easily accessible and easy to use at a reasonable cost, few of them have been considered as potential tools to measure the therapeutic efficacy in CD.

METHODS: This is a review of current evidence regarding biochemical and metabolic molecules which are potential biomarkers for therapeutic response.

RESULTS: Hemostatic biomarkers such as endogenous thrombin potential (ETP) and 1+2 prothrombin fragments (F 1+2) have altered levels in *T. cruzi*-infected patients compared with controls (50% and 77% of patients, respectively), with a significant sustained decrease during 24 months of follow-up. Other studies have demonstrated promising results focusing on key biomarkers; (mature human apolipoprotein A-I (ApoA1) (28·1 kDa); fragments of human ApoA1 (24·7, 13·6 and 9·3 kDa) and a fragment of human fibronectin (28·9 kDa). These markers were used to predict cure in Chagas disease patients treated with Nifurtimox and followed up for 3 years.

CONCLUSIONS: Some biochemical and metabolic molecules could be useful surrogates in response to treatment of *T. cruzi* infection due to their easy analysis and low cost. However, only few among them (ETP, F 1+2, potentially ApoA1 and fibronectin) have been evaluated in treated patients with chronic CD. Further studies are needed to assess their usefulness and validate their use in diagnosis or prognosis.

Detection of anti-SAPA antibodies for routine confirmation of congenital *Trypanosoma cruzi* infection

Russomando, Graciela[1] *; Sánchez, Zunilda*[1] *; de Guillen, Yvalena*[2]*.*
Dpto. Biología Molecular y Genética[1], Dpto. de Producción Bioquímica[2]. Instituto de Investigaciones en Ciencias de la Salud. Universidad Nacional de Asuncion. Asunción, Paraguay.

BACKGROUND: Congenital transmission of Chagas disease is estimated in 15,000 cases per year, and it is seen up to date as a continuous source of infection for newborns in endemic countries and in non-endemic countries with infected women immigrations. The most sensitive technique (higher than 90%) for early detection is PCR, but it is not available at primary healthcare settings in developing countries. In this study we used the shed acute-phase antigen (SAPA) protein in an ELISA system in a large-scale study on serial screening of infants born to *T. cruzi*-infected mothers.
METHODS: An ELISA system with the recombinant protein SAPA was used for the detection of IgG, IgA and IgM antibodies anti-SAPA in serum samples of 2283 infants born to *T. cruzi*-infected mothers. The cut-off optical density of the ELISA-SAPA was determined at 0.300.
RESULTS: A total of 209 babies were confirmed infected in the follow-up. In non-infected infants the conventional serology at 6, 7 and 8 months of age, were still positive due to maternal IgG (false positives) in 8%, 4,3% and 1,6% of the cases, respectively. There was a good discrimination between positive and negative infected infants aged 3–12 months. A 2% of infants infected by *T. cruzi* where not detected by conventional serology. The IgM and IgA antibodies anti-SAPA detected in infected children were in correlation with the conventional anti-*T. cruzi* IgM and IgA detection, with a loss of 70–80% infected babies.
CONCLUSIONS: The IgG anti-SAPA in the ELISA system allowed the identification of congenital cases among those that gave false positive due to maternal IgG antibodies, or false negative by conventional serology due to parasite immunosuppression. Maternal IgG antibodies may suppress the expression of fetal specific IgM antibodies in most of the cases. The assessment of congenital transmission can be done at 3 months of age with the detection of IgG antibodies against SAPA. Early detection and treatment become a relevant issue of public health, considering that early drug treatment is curative.

Mechanisms of *Trypanosoma cruzi* invasion and persistence in the host

Nisha Jain Garg[1,2,3] Fnu Nagajyothi[4], Carlos Robello[6], Herbert B. Tanowitz[4,5]

Departments of [1]Microbiology and Immunology and [2]Pathology, Faculty of the [3] Institute of Human Infections and Immunity and the Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX 77555 USA; Departments of [4]Pathology and [5]Medicine, Albert Einstein College of Medicine, Bronx, NY; [6]Department of Biochemistry, School of Medicine and [7]Unit of Molecular Biology, Institut Pasteur de Montevideo, Uruguay.

In this presentation, we will provide an update on the current knowledge of the mechanisms employed by the parasite to gain entry into the host cells and establish persistent infection despite activation of a potent immune response by the host. Recent studies point to a number of*T. cruzi* molecules that interact with a variety of host cell receptors (e.g. bradykinin receptor (B_2R), endothelin receptors ET_AR and ET_BR, low-density lipoprotein receptor) to promote parasite invasion of the diverse host cells. Likewise, T. cruzi has developed mechanisms to establish persistent infection. For example, *T. cruzi* expresses an elaborated antioxidant system and thromboxane A_2 to evade phagosomal oxidative assault and suppress the host's ability to clear parasites. Additional studies suggest that besides cardiac and smooth muscle cells that are the major target of *T. cruzi* infection, adipocytes and adipose tissue serve as reservoirs from where *T. cruzi* can recrudesce and cause disease decades later. Further, *T. cruzi* employs at least four strategies to maintain a symbiotic-like relationship with the host, and ensure consistent supply of nutrients for its own survival and long-term persistence. Ongoing and future research will continue to help refining the models of *T. cruzi* invasion and persistence in diverse tissues and organs in the host.

Adjuvanting virus-like particle and vectored malaria vaccines

Adrian V. S. Hill

Thirty years of progress in clinical assessment of novel malaria vaccines have identified some general findings. At all four stages of the parasite's life-cycle – sporozoite, liver-stage, blood-stage and the transmission-stage – exceptionally potent immune responses are required to induce any detectable protective efficacy in humans trials. At the liver-stage potent T cell responses are required and at all three other stages antibodies are of most importance.

Efforts to define the most protective antigens for subunit vaccines are ongoing in parallel with trials aiming to identify the most promising vaccine technologies for inducing very potent immune responses. Both efforts have been greatly facilitated by the availability of "challenge" models which now offer measures of efficacy at all stages of the *P. falciparum* life-cycle.

For liver-stage immunity T cell responses of thousands of antigen-specific T cells per ml (measured by interferon-gamma *ex vivo* ELISpot assays) have been required to achieve even moderate levels of protective efficacy and these T cell responses have only been attained by the use of vectored vaccines in heterologous prime-boost regimes. Recent pre-clinical data indicate that enhanced, more protective, T cell responses might be achievable by expressing a CD74 fusion protein or adding a small number of more traditional adfjuvants to vectors.

In contrast the generation of high level antibody responses of the order of hundreds of micrograms per ml of antibody has generally required virus-like particles. This approach includes the use of HBsAg fusion protein (as in RTS,S), hepatitis B core particles, bacteriophage VLPs expressed in E coli with conjugated antigen, the IMX313 heptamerisation approach and a wide range of other VLP technologies. All of these VLPs required potent adjuvants to achieve maximal immunogenicity and a range of potentialy acessible adjuvants will be discussed.

Acta Tropica

Felipe Guhl

Acta Tropica is an international journal that covers biomedical and health sciences with particular emphasis on topics relevant to human and animal health in the tropics and the subtropics. Its scope includes the biology of parasites and vectors, welcoming contributions concerning either basic or applied research in disciplines such as taxonomy, morphology, biochemistry, physiology and immunology; the development of tools for diagnosis and disease control; clinical and community medicine; and the epidemiology of communicable disease and health systems. Contributions may be in the form of original papers, review articles or short communications. I hope to promote questions, doubts and discussion regarding the benefits and needs for publishing in Acta Tropica.

Malaria elimination in the Americas: achievements and challenges

Keith H. Carter, PAHO, Washington D.C.

The negative effects of malaria in the Region of the Americas resulted in efforts to reduce the disease ever since the early twentieth century. As burden of the disease declined in some regions of the world, the vision of its possible eradication gave rise to the development of the global malaria eradication strategy which was launched in Mexico in 1955. As a result of success achieved, a number of countries, including some in the Americas, were certified malaria-free by WHO. With limited global success, the goal was abandoned and the strategy replaced by the Global Malaria Control Strategy in 1992. Since that time, the Roll Back Malaria initiative, the establishment of the Millennium Development goals and financing by the Global Fund have catalyzed and supported efforts to combat malaria at the global level. In spite of challenges in sustaining efforts and fluctuations in disease burden, most of the 21 remaining endemic countries in the Americas have made progress in reducing their malaria burdens since 2000; among them, Argentina, Belize, Costa Rica, Ecuador, El Salvador, Mexico and Paraguay are categorized by the World Health Organization as progressing to the goal of elimination. The Global Fund has recently approved funding a proposal aimed at eliminating malaria from the seven countries in Central America and two on the island of Hispaniola (the Dominican Republic and Haiti). In recent years, Guyana, Haiti and Venezuela have faced challenges and reported increases in disease burden; while reintroduction of malaria occurred in Bahamas and Jamaica, certified free of the disease during the eradication era.

National Survey on Schistosomiasis and Geohelminths in Brazil (2011-2014)

Naftale Katz [1], Roberto S. Rocha [2], Constança Simões Barbosa [3], Fernando S.M. Bezerra [4], Paulo Marcos Z. Coelho [1], Omar dos Santos Carvalho [1], Carlos Graeff Teixeira [5], Dilermando Fazito [6] & Jeann Marie Marcelino [7]

[1]Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil
[2]Centro de Pesquisas Leonidas e Maria Deane, Manaus, AM, Brasil
[3]Centro de Pesquisas Aggeu Magalhães, Recife, PE, Brasil
[4]Faculdade de Medicina, Universidade Federal do Ceará, CE, Brasil
[5]Faculdade de Biociências, Pontifícia Universidade do Rio Grande do Sul, Porto Alegre, RS, Brasil
[6]Faculdade de Medicina de Barbacena, Barbacena, MG, Brasil
[7]Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, DF, Brasil

There are only two nationwide Schistosomiasis surveys carried out in Brazil: the first dated 1949 (Pellon & Teixeira), and the second one named PECE, performed in 1977. In the first survey, geohelminths were also examined. The present survey (INPEG) was planned aiming to exam 220,000 school-children, 7 to 14 years old, using random samples, in 27 states of the country and in the Federal District, comprising 541 municipalities. This project was supported by the Service Health Surveillance, of the Health Ministry ("Serviço de Vigilância da Saúde, do Ministério da Saúde"), and coordenated by the Oswaldo Cruz Foundation. The school-children has been submitted to parasitological stool examination by the Kato-Katz method, the same method used in the second survey. Until now, 90% of the planned exams have been made. The results obtained show a significant decrease of schistosomiasis positivity and of geohelminths. In fact, in some states in northeastern Brazil the positivity rate of geohelminths was higher than 98% in 1949, and currently is around 20%. On the other hand, schistosomiasis also shows a significant decrease in all endemic states, as for instance in the State of Sergipe the positivity rate was 30.14% in 1949, 31.65% in 1965, and currently is 5.49%; in the State of Alagoas the positivity rate varied from 20.48% to 2.39%, in 1949 and 2012, respectively. Comparative data between the three surveys in other states will be also presented. It is possible that this marked decrease in the number of infected individuals by these parasitoses is due to increased sanitation measures in Brazil (supply of household water and waste disposal facilities), as well as treatment facility. From 1977 up to now, it is estimated that more than 15 million treatments for Schistosomiasis were performed in Brazil. In conclusion, the prevalence estimated in Brazil (2011-2014), according to data obtained in our country, is below 1% for schistosomiasis, and around 3% for *Ascaris* hookworm, and *Trichiuris*.

Regulation of human intestinal inflammation by helminths and the microbiota

P'ng Loke
Department of Microbiology, New York University School of Medicine, United States.

We have co-evolved with helminths in order to tolerate these parasites and minimize their virulence. The incidence of Inflammatory Bowel Diseases (IBD) in developing countries, where helminth colonization is endemic, is significantly lower than developed countries. Some versions of the "hygiene hypothesis" link the elimination of helminth infections in developed countries with the rise of IBD. Indeed, helminth treatment for inflammatory bowel diseases is under active investigation in clinical studies but the mechanism of action is still unclear. Based on a detailed longitudinal analyses of an individual who self-infected with *Trichuris trichiura* to treat his symptoms of ulcerative colitis, we hypothesize that enhancement of mucosal barrier function may improve conditions of ulcerative colitis. In this patient, as well as additional other ulcerative colitis patients, T_H22 cells (IL-22+, IL-17-) were reduced in tissues with active inflammation and induced in tissues colonized by worms. When macaques suffering from idiopathic chronic diarrhea were treated with *Trichuris trichiura,* there was reduced bacterial attachment to the intestinal mucosa and dramatic changes to the composition of microbial communities post treatment, which coincided with improved symptoms. These findings suggest that helminth treatment may restore mucosal barrier functions, reducing overall bacterial attachment to the epithelium, restoring the diversity and reversing the dysbiosis of attached bacteria to a normal healthy state. In normal healthy individuals from endemic regions with high helminth prevalence, we found that helminth infection was associated with increased microbial diversity of the intestinal microbiota, suggesting that helminths promote bacterial diversity. Deworming treatment reduced microbial diversity and altered the composition of the microbiota. Individuals with *Trichuris trichiura* infection resistant to deworming treatment had different bacterial communities. We are currently enrolling patients in a double-blinded placebo controlled trial to further investigate these mechanisms in human subjects, treated with *Trichuris suis* ova (TSO). The trial is designed to characterize mucosal responses to TSO treatment, alterations to the mucosal and luminal microbiota and to distinguish between responders and non-responders to TSO treatment.

The integrin-like Fibronectin (FN) receptor (β1*Eh*FNR) associates with Rab7 and Rab11 during the Host-Parasite interface.

Javier-Reyna, Rosario[1]; Hernández-Ramírez, Verónica Ivonne[1]; Garibay-Cerdenares, Olga[1]; Villegas-Pineda, Julio César[1]; Cisneros-Pano, Jonathan[1,2]; Constantino-Jonapa, Alejandro; Talamás-Rohana, Patricia[1].

[1]Departmento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN, Mexico D.F., Mexico. [2]Unidad Académica de Ciencias Químico Biológicas Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, Mexico.

BACKGROUND: In *Entamoeba histolytica*, vesicular trafficking pathways play an important role in the pathogenic mechanism during the invasion and spread of the parasite, as well as in the uptake and digestion of nutrients. The interaction of *E. histolytica* with components of the extracellular matrix (ECM) such as fibronetin (FN) causes a redistribution of the receptor of 140 kDa (β1*Eh*FNR) from internal membranes to the plasma membrane. The aim of this project was to study the involvement of Rab7 and Rab11 proteins during the mobilization of β1*Eh*FNR in trophozoites interacted with FN and recovered trophozoites from amoebic liver abscess (RTLA).

METHODS: Male hamsters were infected intrahepatically and 7 days post-infection, animals were anesthetized and killed by exsanguination. Livers were dissected and small portions were incubated in supplemented TYI-S-33 medium. After 48 h incubation, liver sections were removed and the culture medium was changed. The number of parasites as well as the attainment of the logarithmic phase was variable according to post-infection time of recovery. Then trophozoites were processed for confocal, multiphotonic, and transmission electron microscopy. The antibodies employed were: commercial anti-Rab7 and anti-Rab11 Abs and 3C10 MAb directed against β1*Eh*RFN. To visualize the actin cytoskeleton rhodamin-phalloidin was used.

RESULTS: Using confocal microscopy we demonstrated co-localization of the three elements involved (Rab proteins, the β1*Eh*FNR, and the actin cytoskeleton). Electron microscopy and FRET techniques corroborated the association between proteins and β1*Eh*FNR in trophozoites interacted with FN and RTLA. Finally, multiphoton microscopy allowed the visualization of these events *in vivo* in *E. histolytica*.

CONCLUSIONS: The above results suggest that at least Rab7 and Rab11 proteins, and the actin cytoskeleton play a key role during the β1*Eh*FNR mobilization through different cellular compartments in the exocytic pathway.

Rational Design of Vaccines for Canine and Human Visceral Leishmaniasis

Steven G. Reed, Rhea N Coler, Malcolm Duthie, Yasu Goto, and Jeff Guderian
Infectious Disease Research Institute, Seattle, USA and University of Tokyo, Japan

The quest for safe and effective vaccines for human and canine visceral leishmaniasis (VL) has proven to be more of a challenge than initially believed. Much effort has been placed in antigen selection, with many showing promise in animal models and in early human clinical trials. However, turning antigens into effective immunogens requires an understanding of the nature of the desired immune response(s) and selection of delivery platforms capable of inducing such response (s). A major breakthrough in the development of vaccine candidates against leishmaniasis, as well as other diseases requiring potent and directed T-cell responses, occurred with the identification of adjuvants capable of inducing Th1 responses. The discovery that properly formulated Toll-like receptor (TLR) agonists can stimulate Th1 immune responses has profoundly impacted vaccine development against intracellular pathogens like *Leishmania*.

In particular, the extensive experience with monophosphoryl lipid A (MPL), a TLR4 agonist obtained from the cell wall of *Salmonella,* and MPL's success in approved vaccines for hepatitis B and human papilloma virus have demonstrated the safety and efficacy of engaging TLR4. MPL is the only TLR agonist in approved vaccines, and thus has an extensive history of safety and efficacy. The first defined vaccine against leishmaniasis consisted of the recombinant fusion protein Leish-111f (L111f) together with MPL formulated in an oil-in-water emulsion, (stable emulsion, or SE). This vaccine candidate was shown to protect mice, hamsters, and rhesus macaques and was the first defined *Leishmania* vaccine to enter clinical trials. Leish 111f was shown to be safe and immunogenic, as well as to have efficacy as a therapeutic vaccine in humans and in dogs.

The experience and success with MPL-based adjuvant formulations led us to evolve the next generation of TLR 4 ligands, leading to the development of synthetic derivatives. There are several reasons for this. For one thing, continued cost-effective access to MPL, currently controlled by industry, cannot be guaranteed. In addition, we developed structures with increased potency over MPL, allowing the use of comparatively smaller doses. Using information from the crystal structure of the human TLR4, we selected one molecule for further development, based on the ability of this molecule to fit in the human MD2 structure. We have developed formulations of this novel synthetic TLR4 agonist, Glucopyranosyl Lipid A (GLA), a single molecular entity, and highly purified, which is more potent in humans than MPL, and have shown that GLA is an effective adjuvant in both prophylactic and therapeutic models of cutaneous leishmaniasis (CL). Furthermore, GLA can be synthesized in large amounts (we currently have nearly one million human doses in inventory) and, with IDRI holding the patent, is independent from control by pharmaceutical companies. In addition to being an effective adjuvant molecule, GLA can synergize with ligands of other TLRs., GLA based adjuvants have been safely tested in both human (>1000 subjects) and canine clinical trials.

We have continued to optimize both antigen and adjuvant for leishmaniasis vaccines. Whereas Leish-111f was developed primarily as a therapeutic candidate, recent efforts have focused on antigens that meet more stringent criteria for prophylactic use. These criteria include sequence conservation among *Leishmania* spp., ability to protect in CL and VL models, and ability to be expressed and purified at high levels. All of these criteria are important for the optimal leishmaniasis vaccine. As a result of optimization of vaccine

development for VL, we have developed Leish F3, a fusion protein consisting of SMT (sterol methyl transferase) and NS (nucleosidase), both of which have been shown to protect in animal models. The Leish F3 antigen, formulated in GLA-SE has proven safe and immunogenic in human clinical trials. . Recently, a second VL vaccine candidate, Leish F4, has been developed and is progressing to human clinical trials as well. Vaccine candidates for canine VL are also in further development, and will be discussed.

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Macrophage Subsets in Fibrosis

Thomas A. Wynn

Program in Tissue Immunity and Repair, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Macrophages are found in close proximity with collagen-producing myofibroblasts and play key roles in the pathogenesis of fibrosis. They produce growth factors and pro-fibrotic mediators that directly activate fibroblasts, including transforming growth factor beta, insulin-like growth factor, vascular endothelial growth factor, and platelet-derived growth factor. They also regulate extracellular matrix turnover by influencing the balance of various matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases. Macrophages also regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells and by producing various inflammatory and anti-inflammatory cytokines. With their potential to act in both a pro- and anti-fibrotic capacity at distinct stages of the wound healing response, macrophages and the factors they express are integrated into all stages of the fibrotic process. These various and sometimes opposing functions are performed by distinct macrophage subpopulations. In this presentation I will describe our recent studies that have focused on elucidating the regulatory role of macrophages and specific macrophage subpopulations in the pathogenesis of type-2-dependent liver and lung fibrosis.

Leishmaniases

Leishmaniases are diseases caused by protozoan parasites and transmitted to humans by the bites of infected *Phlebotomus,* also known as sandflies. There are three main forms of the disease: cutaneous, visceral and mucocutaneous. The cutaneous form of the disease is the most common, causing ulcers on exposed areas of the body, leaving permanent scars and possibly leading to disability. Visceral Leishmaniasis is the most severe form of the disease, and could be fatal if it is not threated on time; it affects vital organs causing fever, weight loss, anemia, splenomegaly and hepatomegaly. The last of the main forms of the disease is mucocutaneous; it is the most destructive causing partial or total mutilation of mucous membranes in the nose, mouth and throat.

Worldwide distribution of Leishmaniases

Leishmaniases is prevalent in 98 countries with 1.3 million new cases per year, only 600,000 of which are reported. Out of the new cases, around 300,000 are visceral and 90% are identified in Bangladesh, Brazil, Ethiopia, India, Nepal, Sudan and South Sudan; the other million cases are cutaneous, mainly occur in Afghanistan, Argelia, Brazil, Colombia, Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia, Syria and Tunisia, and mucocutaneous more commonly in Brazil, Peru and Bolivia.

Leishmaniases in Mexico: Overview and Plans of Action

From 2000 until 2013, 11,459 new cases have been reported. The states registering the largest number of cases are: Tabasco, Quintana Roo, Campeche, Chiapas, Nayarit, Veracruz, Oaxaca, Sinaloa and Yucatán; it primarily affects men during their reproductive years. Mexico has implemented a Specific Plan of Action (Plan de Acción Específico, PAE) for Leishmaniases 2013-2018 in order to promptly diagnose and treat all the cases through the following strategies: 1) Strengthening the epidemiological surveillance system 2) Laboratory confirmation of probable cases, 3) Administering the appropriate treatment in a timely matter, 4) Promoting preventive measures for individuals, families and groups in endemic areas, and 5) Training vectors personnel to conduct integrated surveillance. Each strategy includes specific lines of action in order to decrease Leishmaniases incidence in the country. To the present day, we have managed to strengthened training and supervision of Leishmaniases through a collaboration between the Pan American Health Organization (PAHO), Institute of Diagnosis and Epidemiological Reference (InDRE) and the National Autonomous University of Mexico (UNAM), as well as supervise and give follow up of the established goals for each one of the States that are endemic to the disease such as: Chiapas, Tabasco, Campeche, Quintana Roo and Veracruz.

Scientific Publishing based on public funding and '*pro bono*' work: an alternative model of open access? The case of Memórias do Instituto Oswaldo Cruz.

Brandão, Adeilton[1]*; Lourenço-de-Oliveira, Ricardo* [2]

[1]Associated Editor for Publishing Policy; [2]Editor-in-chief;
Memórias do Instituto Oswaldo Cruz
Instituto Oswaldo Cruz - Fiocruz, Av. Brasil 4365, 21040-360 - Rio de Janeiro, Brazil

BACKGROUND: The science publishing activity has been evolving from the traditional author zero publication fee – reader subscription service model to the author publication fee – reader open access initiative. According to some scholarly research, this new model has enormous potential to increase the scientific knowledge dissemination, especially in the countries that cannot afford the high costs of subscription service of the traditional scientific journals. Due to potential for new business opportunities an exponential number of open access titles has been created in last five years. This uncontrolled proliferation has led to two serious consequences: a) an increased burden on the peer review system; b) the appearance of 'predatory' journals. Taking into account these consequences, a question emerges: is it possible to build an intermediate model for scientific publishing in which the publication costs might be the lowest possible for authors, readers, libraries and editors?
METHODS: critical review of publishing policies and editorial practices of both traditional and open access journals
RESULTS: initiatives like the Scielo network of Latin American journals is a good proxy for an intermediate model. This is a government backed initiative (mostly Brazilian) and among the supported journals is the Memórias do Instituto Oswaldo Cruz, a centenarian scientific publication that since 1909 has been continuously disseminating original research in the fields of Tropical Medicine, Parasitology and Infectious Diseases. These fields include some of most relevant challenges in public health for the developing nations. Memórias policy towards a very low cost publishing alternative for author, readers and libraries has remained unaltered since the publication of the first issue in 1909. The advent of world wide web technologies has consolidated this trend and changed the previous Memórias model to a complete "no publishing fee" journal for authors and readers. Historically, this might be viewed as the first example of a traditional scientific publication towards a full open access before this model had been conceived by publishing innovators. Memórias do Instituto Oswaldo Cruz has also made available all the issues published so far for free downloads.
CONCLUSION: While there is no 'free lunch" publication system, Memórias do Instituto Oswaldo Cruz has proved that a full open access model to both readers and authors is a feasible one thanks to the uninterrupted support from a Brazilian federal organization and the '*pro bono*' work of the editorial staff.

Immune modulation by pathogens: How dengue viruses circumvent and turn off the insect vector's innate immune responses.

Coatsworth, Heather[1], Caicedo, Paola[2], Ocampo, Clara[2], Lowenberger, Carl[1].

[1]Departament of Biological Sciences, Simon Fraser University, Burnaby BC Canada
[2]CIDEIM, Carrera 125 # 19-225, Cali, Valle del Cauca, Colombia

BACKGROUND: Dengue is the most widespread arthropod-borne virus (arbovirus) in the world, with >50 million cases annually. Dengue is transmitted by mosquitoes of the genus Aedes, especially *Ae. aegypti*, that lives in close association with humans. However in Cali Colombia, ~30% of field collected *Ae. aegypti* are refractory to all 4 serotypes of Dengue virus. To date, the mechanisms involved in this refractory phenotype have not been fully characterized. We have used several techniques to identify differentially expressed genes linked to the refractory and susceptible phenotypes.
METHODS:We established lines of susceptible (S) and refractory (R) *Ae. aegypti* in the lab. These were exposed to a blood meal containing, or not, Dengue virus. Midguts (2-120h post infection) and carcasses (48-120h post infection) were dissected, RNA was extracted for use in Suppressive Subtractive Hybridization, microarrays, and RNA-seq to identify differentially expressed genes. Some of these were knocked down using RNAi to test their impact on the R/S phenotype
RESULTS: We identified an upregulation of apoptosis-related genes. Within 24h in the S strain there was an increase in an inhibitor of apoptosis (IAP1) that stopped apoptosis, allowing the virus to develop and replicate. Many other genes associated with immunity, regulatory pathways, immune recognition molecules, and immune adaptor molecules were also identified. RNAi knockdown of specific genes altered the phenotype of the mosquitoes, implicating these genes in contributing to the phenotype. We believe that CONCLUSIONS: Dengue viruses manipulate the antiviral immune responses of mosquitoes to prevent being killed directly, or through apoptosis of Dengue-infected cells.

In vitro activity of 2-aryl-3-hydroxymethyl imidazo[1,2-*a*]pyridines and pyrimidines against _Giardia lamblia_ WB and _Boophilus microplus_ ticks

Velázquez-Olvera Stephanía[1]; Salgado-Zamora Héctor[1]; Jiménez-Cardoso Enedina[2]; Ibarra-Velarde Froylan[3]; Pérez-González Cuauhtémoc[4]

[1]Departamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Unidad Profesional Lázaro Cárdenas, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomas Zip Code 11340 Mexico City, Federal District; [2]Laboratorio de Investigación en Parasitología, Hospital Infantil de Mexico "Federico Gomez". SS, Calle Dr. Marquez #162 Col. Doctores Zip Code 06720, Mexico City, Federal District; [3]Departamento de Parasitología, Facultad de Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán, Zip Code 04510, Mexico Cith, Federal District. [4]Sistemas Biológicos, Universidad Autónoma Metropolitana Unidad Xochimilco. Calzada del Hueso 1100, Col. Villa Quietud, Delegación Coyoacán, Zip Code 04960, Mexico City, Federal District.

BACKGROUND: *Giardia duodenalis* (also known as *G. lamblia* or *G. intestinalis*), a flagellated protozoan, is the most common causative agent of persistent diarrhoea worldwide. The life cycle includes motile, flagellated trophozoites parasitizing the upper intestine, and thick-walled cysts forming in the lower intestine that are shed with the faeces. Antigiardial chemotherapy is directed against the trophozoites. In the past, various antiparasitic drugs have been developed but only a few turned out to be effective for treatment of giardiasis.
The cattle tick Riphicephalus (Boophilus) microplus is one of the most important ectoparasites because it causes economic losses of several million dollars to the world economy. The control of this tick in Mexico is based on the use of ixodicides; however, nowadays a considerable number of them produce resistance due to excesive and inadequate use.
METHODS: Two series of 2-aryl-3- hydroxymethylimidazo[1,2-*a*]azines were synthesized by a sequence of steps, starting from the corresponding 2-aminopyridine or 2-amino pyrimidine.
A density of trophozoites of *Giardia lamblia* WB in TYI-S-33 medium pH 6.8 supplemented with 10% bovine serum and 0.1% bile was incubated at 37 °C for 24-48 h. Trophozoites were harvested by chilling the tubes in ice water for 10-15 min then counted in a Neubauer cell counter chamber. For *in vitro* inhibition assays, 50000 cells /mL were used as an optimum population, which were cultured in the presence of 2-aryl-3-hydroxymethylimidazo[1,2-*a*]pyridines (6a-g), pyrimidines (7a-g) and albendazole, in a range of 7 different concentrations (0 to 200 µmol/mL). Cultures were incubated for 24 h at 37°C. Furthermore, the synergetic action of albendazole in combination with the active 2-aryl-3-hydroxymethyl imidazo[1,2-*a*]pyridines and pyrimidines (6c, 6f, 7a, 7b, 7c, 7d, 7e) was evaluated in the same way as before.
Five discriminant doses of 2-aryl-3-hydroxymethylimidazo[1,2-*a*]azines and cypermethrin (1%, 0.50%, 0.25%, 0.125%, 0.0625%) were prepared for the treatment groups and an untreated control group (using vegetal oil and trichloroetilene) was included. For the larval packets Whatman No. 1 filters (7.5 × 8.5 cm) were used, the name of the compound being identified for each dilution. With the aid of a small paintbrush taking approximately 100 fourteen-day old larvae were placed inside the packet. All packets were incubated at 28°C ± 2°C for 24 h with an 80% - 90% relative humidity. With the aid of a table counter dead and live ticks from each packet were counted to estimate the response percentages of mortality for each dilution.
RESULTS: Based on the IC_{50} values obtained, the best anti-*Giardia* activity in comparison to that of albendazole was provided by imidazo[1,2-*a*]pyrimidine

derivatives 7c and 7d, followed by 7a and 7b, which showed similar concentrations as albendazole. Compounds 6c and 7e showed concentrations slightly above albendazole and concentration of compound 6f is higher than albendazole, nevertheless it falls within the range of acceptable concentrations. The results of the synergetic action of albendazole in combination with the active 2-aryl-3-hydroxymethyl imidazo[1,2-*a*]pyridines and pyrimidines (6c, 6f, 7a, 7b, 7c, 7d, 7e) indicated that the combinations of albendazole with 7a, 7b, or 7c exhibited the best anti-*Giardia* action and increased the albendazole efficiency as demonstrated by the viability of *Giardia* left.

The compounds that presented best ixodicide activity at the dose of 1% were 7f that showed 97.7% of mortality and 6f, which caused 86.2 %. But with the dose of 0.0625% the compounds that cause more mortality in ticks than cypermethrin (66.5%) were 7a, 7e, 7f, and 7g.

CONCLUSIONS: The significant anti-*Giardia* activity of some 2-aryl-3-hydroxymethyl imidazo[1,2-*a*]pyridines and pyrimidines and the synergistic effect of these in combination with albendazole represents a first approach to the use of these compounds in the treatment of giardiasis.

In the other hand, the ixodicide activity of 2-aryl-3-hydroxymethylimidazo[1,2-*a*]azines evaluated against *Boophilus microplus* ticks showed high efficacy against larvae ticks *in vitro*.

Ocular cysticercosis

Fleury, Agnès Odile Marie[1,2].

[1]Unidad periférica del Instituto de Investigaciones Biomédicas (Universidad Nacional Autónoma de México) en el Instituto Nacional de Neurología y Neurocirugía, México D.F. [2]Consulta Externa, Clínica de Neurocisticercosis, Instituto Nacional de Neurología y Neurocirugía, México D.F.

BACKGROUND: Cysticercosis corresponds to the localization in different organs of the larvae of *Taenia solium*, the cysticercus. Due to its possible fatal complications, attention is mostly put on the affection of the Central Nervous System. Although cysticercosis of the eye does not present this seriousness, it can be associated with a high morbidity, mainly due to the possible loss of vision. For this, the knowledge of this localization of the cysticercus is important.
METHODS: We made a review of the literature published in indexed Journals. Information regarding epidemiological and clinical characteristics of the patients, and regarding diagnosis and treatment of the disease were retrieved. The prognosis of the patients was evaluated.
RESULTS: Cysticercosis of the eye is mainly reported in Asian countries. Contrary to cysticercosis of the brain, parasites rarely calcified in this location. All the segments of the eye can be affected, mainly the posterior segment (subretinal or intraretinal), the vitreous and the subconjunctival area. Diagnosis is based on ophthalmoscopy, high-resolution ultrasonography, CT scan and MRI. Treatment will be individualized; surgery and anthelmintic drugs are the options.
CONCLUSIONS: Although not frequent, cysticercosis of the eye is a potential source of serious complications. Comparing with neurocysticercosis, differences in the epidemiology of the patients and the evolution of the parasite exist. Studies should be conducted to understand better the reasons of these differences.

Transcription profiling of L4 Ostertagia ostertagi; identification of an ATP-diphosphohydrolase capable of controlling the innate immune response in the gastric glands

Zarlenga, Dante S.

Agricultural Research Service, Animal Parasitic Diseases Lab, Beltsville, Maryland 20705

BACKGROUND: *Ostertagia ostertagi* is a stomach worm of cattle that causes significant morbidity, mortality in heavily infected animals by damaging the abomasum during the infection process. The adult parasites cause most of the pathology; however, the L4 are capable of controlling the innate inflammatory response amidst substantial cell damage and hyperplasia. This research identifies genes specifically associated with the L4 stage that are involved in attenuating or controlling innate immune responses.
METHODS: Subtraction libraries were constructed using photoprobe biotin to tag L3 “substractor” dscDNA hybridized with L4 cDNA synthesized with SMART “B” primers. The product was mixed with Streptavidin, phenol extracted then column purified.. The L4-enriched cDNA was PCR amplified, cloned then differentially-screened for L4 specific sequences.
RESULTS: An ATP-diphosphohydrolase (apyrase)was identified and expressed as a biologically-functional enzyme. In vitro mutagenesis studies identified the active site of the apyrase. In vitro activity of the enzyme could be attenuated with mouse polyclonal antiserum to the recombinant protein.
CONCLUSIONS: The *Ostertagia* apyrase is functionally active on ATP, ADP, UTP and UDP, coinciding with the range of nucleotides involved in purinergic signaling across the P2 receptor complex. We hypothesize that parasite uses the native protein to reduce extracellular nucleotides secreted by locally-damaged tissues in the gastric glands to control inflammation. Western blot analyses demonstrated that the native OoAP is secreted and produced or collects within the glandular bulb of the esophagus at the esophageal/intestinal junction, and is developmentally-confined to L4. We showed that both targeted drug development and immunity may be used to control the activity of the enzyme and therefore the longevity of the parasite within the host.

An approach to Identify of influencing factors for parasitoses in residents of localities of Santa Marta.

Villalba Vizcaíno, Vivian[1]*; Sanchez, Jose*[1]*; Medina, Denisse*[1] ; Castro, Lyda Raquel[2]; Simone Cacciò[3]

[1]Centro de Investigación en Medicina Tropical, CIMET, University of Magdalena, Santa Marta, Colombia
[2]Grupo Evolución, Sistemática y Ecología Molecular, University of Magdalena, Santa Marta, Colombia
[3]Istituto Superiore di Sanità, Italy

BACKGROUND: Parasitoses are important health problem and constitute one of the main worldwide causes of human morbidity and mortality. Enviromental and socio-economical factors can influence in frequency and kind of parasites are transmitted. Santa Marta is a touristic city with 9 different localities and many people with low income.
METHODS: Population for this study was selected of two different localities of Santa Marta city. Locality number 1 and 5. Data about socio-economical status, education level, gastrointestinal symptons were collected by questionnaire. Fecal samples were collected of participants and examination was done with lugol staining by optical microscopy. Statistical analysis was done by Chi-square and Regression analysis to determine influencing factors in parasitoses.
RESULTS: A total of 36 questionnaires and samples were collected. Differences between localities were in geographical localization, proximity to river and conditions of houses. Mean of age of participants was 21 years old. The majority of participants only have a high school level. Only 27.8% of participants had gastrointestinals symptoms (most frequently: abdominal pain and diarrhea). Microscopy examination was positive in 80.6%. Most frequent parasite was *G. intestinalis* (55.5%) and we found mixed parasitoses in 41.6% of participants. In cases of mixed parasites, a combination between *G. intestinalis* and *E. hystolitica* were found.
CONCLUSIONS: This study was an initial approach to found frequency of parasites and influencing factors in Santa Marta. We need more samples to extend study and obtain information to develop an strategy to prevent parasitoses in Santa Marta.

An innovative model for strengthening research and development of vaccines against neglected tropical diseases.

Betancourt-Cravioto, Miguel[1]; Hotez, Peter[2]; Bottazzi, Maria Elena[2]; Ortega, Jaime[3], Dumonteil, Eric[4]; Valenzuela, Jesus[5]; Kamhawi, Shaden[5]; Tapia-Conyer, Roberto[6]

[1]Dirección de Soluciones Globales, Instituto Carlos Slim de la Salud, Mexico City, Mexico; [2]Texas Children's Hospital and Baylor College of Medicine, Houston, Texas; [3]Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados - Instituto Politécnico Nacional, Mexico City, Mexico; [4]Laboratorio de Parasitología, Centro de Investigaciones Regionales ''Dr. Hideo Noguchi'', Universidad Autónoma de Yucatán, Merida, Mexico; [5] Vector Molecular Biology Section, Laboratory of
Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland; [6]Fundación Carlos Slim, Mexico City, Mexico.

BACKGROUND: The neglected tropical diseases (NTDs) represent a group of chronic parasitic and related infections that occur predominantly among the poor and that contribute to the persistence of poverty due to poor school performance in children because of impaired growth and development, and work absenteeism and poor productivity. These diseases affect millions of individuals in the World's poorest regions yet there are little efforts from the industry and other relevant actors to research and develop tools for their prevention and control, and to incorporate them into public health policy. In this report we describe a new model for developing and bringing innovation in the prevention of two of the most prevalent NTDs in the Americas: Chagas disease and Leishmaniasis: *The Slim Initiative for the Development of Vaccines against NTDs*.
METHODS: The model consists on a public-private international partnership that has brought together actors from academia, research, government and the private sector, in order to join efforts for the development of two new vaccines: a preventative vaccine against cutaneous Leishmaniasis and a therapeutic vaccine against Chagas disease. The idea behind the partnership is to carry out knowledge and technology transfer to Mexican institutions to strengthen vaccine R&D capacity.
RESULTS: In the first phase, the consortium has advanced successfully in the first stages of vaccine development with collaboration between Mexican and U.S. institutions that has included: training of technical personnel, provision of laboratory equipment, and methodological transfer. In a second stage, further pre-clinical work will be carried out.
CONCLUSIONS: New collaborative models are required to establish innovative technologies to address the needs of the populations exposed to NTDs by strengthening national capacities in order for countries to address their own public health needs.

Pentamidine inhibits the polyamine transport in *Trypanosoma cruzi*, exerts *in vitro* antichagasic activity and enhances the effect of benznidazole *in vivo*.

López-Muñoz, Rodrigo[1]; Díaz, María[1]; Seguel, Verónica[1]; Miranda, Mariana[2]; Campos-Estrada, Carolina[1]; Reigada, Chantal[2]; Maya, Juan Diego[1]; Pereira, Claudio[2].

[1]Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [2]Laboratorio de Biología Molecular de *Trypanosoma cruzi*, Instituto "Alfredo Lanari", CONICET- UBA. Buenos Aires, Argentina

BACKGROUND: Pentamidine is a drug used against leishmaniasis and African trypanosomiasis. Little evidence exists about its effects in *Trypanosoma cruzi*. Pentamidine blocks a polyamine transporter of *Leishmania major*; consequently, its might also block these transporters in *T. cruzi.* Due that *T. cruzi* is unable to synthesize putrescine; the inhibition of polyamine transport can bring a new therapeutic target against the parasite.
METHODS: We evaluated the effect of pentamidine on the transport of radiolabeled polyamines in isolated *T. cruzi* forms. We assessed viability by MTT and flow cytometry, and followed the parasite burden in infected cell cultures by DAPI stain of DNA and qPCR. Finally we studied the effect of pentamidine alone or in combination with benznidazole in *T. cruzi*-infected mice. In these mice we followed survival, parasitemia, heart histology and parasite burden.
RESULTS: Pentamidine inhibits the polyamine transport in *T. cruzi* epimastigotes and amastigotes. Pentamidine decreases, the viability of *T. cruzi* trypomastigotes, and the parasite burden of infected cells. In *T. cruzi*-infected mice pentamidine decreases heart inflammation, parasite burden and parasitemia; resulting in an increased survival rate. In addition, pentamidine increases the effect of benznidazole over the parasitemia and parasite load in chagasic hearts from infected mice.
CONCLUSIONS: Contrary to the results reported in the early 40's, pentamidine showed antichagasic activity, and enhanced the activity of benznidazole; this results could lead new antichagasic strategies based in polyamine transport inhibitors.
ACKNOWLEDGEMENTS: FONDECYT 11110182.

Simvastatin and benznidazole reduces E-selectin, ICAM-1 and VCAM-1 expression in *Trypanosoma cruzi*-infection.

Campos-Estrada, Carolina[1], López-Muñoz, Rodrigo[1], Kemmerling, Ulrike[2], Leiva, Mario[1], Maya-Arango, Juan[1]

[1]Department of Clinical and Molecular Pharmacology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile. [2]Department of Anatomy and Developmental Biology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile.
Email: ccampos@ciq.uchile.cl

BACKGROUND: Chagas disease is caused by *Trypanosoma cruzi*. This parasite triggers an inflammatory response to control host's infection. As the inflammatory response persists, the patients develop Chronic Chagas Cardiomyopathy (CCC). Important pathophysiological mechanisms involved in the CCC include microvascular alterations, due to endothelial dysfunction. This process is characterized by increased expression of vascular Cell Adhesion Molecules (ICAM-1, VCAM, and E-selectin), allowing inflammatory cell recruitment. Benznidazole is the current treatment of Chagas disease, but it has no proven efficacy in the chronic phase. However, simvastatin may improve the therapeutic efficacy of benznidazole, due to its pleiotropic roles in modulating inflammatory responses in the *T. cruzi* infected endothelium in CCC.
METHODS: The effect of both drugs on ECAMs expression in *T. cruzi* infected endothelial cells (EA.hy926) was determined by flow cytometry, immunofluorescence and western blot. Leukocyte adhesion to EA.hy926 cells was also assayed by cell adhesion assay. Finally, the effect of simvastatin and benznidazole pre-treatment upon NF-κB activation was determined by confocal microscopy.
RESULTS: After 16 hours of infection, the peak of ECAMs expression is reached in *T. cruzi* infected EA.hy926 cells. This effect was sustained for further 48 hours. Simvastatin and benznidazol treatment, during 24 hrs before infection, decreased ECAMs expression and cell adhesion, without affecting the cell viability and cytoskeleton. Thus, the effect is independent of their trypanocidal activity. Furthermore, both drugs blocked NF-κB activation. In conclusion, both drugs modulated the *T. cruzi*-induced endothelial activation. As the inflammation has a key role in the pathogenesis of Chagas disease, simvastatin and benznidazole may contribute to the treatment of inflammation in Chagas disease.

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Control of Taeniasis-cysticercosis

Garcia, Hector H.[1,2]

[1]Centro de Salud Global – Tumbes and Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru; [2]Cysticercosis Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru.

The infection of the human nervous system by the larvae of the pork tapeworm *Taenia solium* (neurocysticercosis, NCC) is a frequent event in wide regions of the world. NCC is a major contributor to the burden of seizures and epilepsy worldwide. Development leads to elimination of transmission, however most endemic regions are precisely the poorest regions and appropriate living conditions are not to be expected in the close future.

Active interventions for the control of the transmission of *Taenia solium* have been performed for over 20 years. A series of control measures including antihelmintic treatment of humans, health education, antihelminthic treatment of pigs, and porcine vaccination hav been tested alone or in combination. A large program in Peru performed along the past ten years demonstrated the feasibility of focal elimination, and is currently examining the persistence of the effect and potential re-introduction pathways.

A large pending agenda in control still exists, including making controls tools available and accessible, tailoring control programs to local scenarios, as well as defining and homogenizing monitoring strategies. So far, however, existing evidence provides the basis to consider it feasible.

Neurocysticercosis

Garcia, Hector H.[1,2]

[1]Centro de Salud Global – Tumbes and Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru; [2]Cysticercosis Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru.

The infection of the human nervous system by the larvae of the pork tapeworm *Taenia solium* (neurocysticercosis, NCC) is a frequent event in wide regions of the world. While NCC may be asymptomatic in a substantial proportion of infected individuals, a minority will develop neurological symptoms, particularly late onset seizures, headache, and intracranial hypertension. NCC is a major contributor to the burden of seizures and epilepsy worldwide.

The clinical expression of NCC is extremely variable and depends on several factors which include the number, size and evolutive stage of the parasites, as well as the inflammatory response of the immune system of the host. Understanding this variability is crucial for an appropriate diagnosis and management, and as such, patient work up should be individualized.

Management of individuals with neurocysticercosis may involve symptomatic medications including anti-inflammatory drugs, antiparasitic drugs, and surgery. The use of antiparasitic drugs has been a matter of discussion for years and only recently appropriate data from randomized controlled trials became available.

Finally, interventions for the control of *Taenia solium* transmission in endemic areas have been performed in diverse settings, raising the hopes for eventual elimination and eradication.

Global control of soil transmitted gastrointestinal helminths

Albonico Marco [1]*; Antonio Montresor* [2]*; Albis Francesco Gabrielli* [2]
[1]Ivo de Carneri Foundation, Milan, Italy; [2]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

BACKGROUND: STH affects more than 1 billion people worldwide and thrive in neglected poor populations that lack education and access to drugs, and in a neglected environment of poor sanitation and insufficient safe water supply. Preventive chemotherapy (PC) is the control strategy recommended by WHO that focuses on regular administration of broad spectrum anthelmintic drugs through community or school-based intervention, improvement of health education, sanitation, and access to safe water. STH control is integrated into a rapid-package intervention that targets several diseases like schistosomiasis, lymphatic filariasis, onchocerciasis, and trachoma.

METHODS: PC entails distribution of albendazole 400 mg or mebendazole 500 mg twice or once /year to the population at risk, depending on the endemicity of STH infections. Pyrantel pamoate and levamisole are the two other drugs recommended by WHO though less used worldwide. Different goals and thresholds are used to define different phases of 1) control of STH (reaching 75% of drug coverage in school-age children), 2) elimination of STH as public health problem (reducing heavy infection < 1%), 3) elimination of transmission of STH infections (reducing incidence to 0). In the second and in the third phase PC must be increasingly joint with reduced contamination of the soil through good coverage of sanitation and provision of safe water supply.

RESULTS: More than 800 million treatments are being delivered annually for the control/elimination of neglected tropical diseases (NTD) due to helminths and amenable to PC. In 2012, 86 M preschoolers (27.7%of those in need) and 252 M school-age children (37.2% of those in need) were treated for STH infections. GSK and J&J has donated 400 M albendazole and 200 M mebendazole tablets annually, respectively. Morbidity assessment is ongoing in several endemic countries.

CONCLUSIONS: The is a need to scale up PC intervention to reach the 2020 target of 75% national coverage in 100% STH endemic countries. Major challenges are enhancing access to drugs at country level, and sustaining drug efficacy by using drug in combination and close monitoring. Implementation and use of sanitation as well as health education are keys for interrupting transmission.

Laboratory and radiologic data of infants with congenital toxoplasmosis

Jose Gil Montoya
Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, California, USA

BACKGROUND: infants with congenital toxoplasmosis (CT) may be born apparently normal or have clinical manifestations that range from mild to severe disease. Some of the newborns may die as a result of the infection with the parasite.
METHODS: The Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL) database was searched for data on infants 0 to 180 days old, in whom CT had been confirmed and who had been tested for *Toxoplasma gondii*-specific immunoglobulin G (IgG), IgM, and IgA antibodies. We reviewed available clinical data and laboratory profiles of 164 infants with CT whose mothers had not been treated (UnRx) and 25 infants with CT whose mothers had been treated (Rx) with a drug regimen for the parasite during gestation. Rx included spiramycin, pyrimethamine + sulfadiazine +folinic acid, or spiramycin followed by pyrimethamine + sulfadiazine + folinic acid, in an attempt to prevent or treat infection in the fetus. In these women, detailed information regarding doses, duration of treatment or gestational age at which these drugs were administrated was not available.
RESULTS:

	No. of Infants	
	UnRx (n=164)	Rx (n=25)
Eye disease/ eye exam	119/129 (92.2%)	10/16 (62.5%)
Calcifications/radiologic exam	94/118 (79.7%)	13/17 (76.5%)
Hydrocephalus/radiologic exam	68/99 (68.7%)	5/13 (38.5%)
IgM(+)/IgM performed	142/164 (86.6%)	11/25 (44%)
IgA(+)/IgA performed	127/164 (77.4%)	15/25 (60)

In the treated cohort, major clinical manifestations of congenital toxoplasmosis (eye disease, brain calcifications and hydrocephalus) and positive toxoplasma antibody test (IgM and IgA) results were consistently lower in frequency than in the untreated cohort, except for brain calcifications.
CONCLUSIONS: Our study reveals that major clinical signs of congenital toxoplasmosis and positivity for IgM and IgA appear to occur at a higher frequency in the unRx than the Rx group. However, our results in both groups contrast remarkably with those of European investigators who rarely observe severe clinical signs in infants wit congenital toxoplasmosis.

New ways of working in the discovery of new medicines for Diseases of the Developing World

Fiandor, José M.

Diseases of the Developing World, GSK, Kinetoplastid DPU, Tres Cantos (Spain)

Diseases of the Developing World (DDW) like malaria, tuberculosis and kinetoplastid diseases accounted in 2010 for 10% of the disease burden globally, measured as disability adjusted life years, with around four million deaths and preventing adults from work and children going to school, not enabling the affected population to escape poverty.

The little commercial opportunity in the DDW has made that historically the DDW have received limited R&D investment. To mitigate the costs and risks of investing in R&D for DDW new ways of working in drug discovery in this field must be explored.

Since 2010 GSK has adopted an open innovation strategy as the best way of working to deliver new effective medicines for DDW. As part of this approach GSK started with a new concept, “The Open Lab”. The objective of the Open Lab is to accelerate discovery of new medicine for DDW by bringing together scientist from universities, research institutions and GSK to work in the approved projects by and independent Foundation (The Tres Cantos Open Lab Foundation; www.openlabfoundation.org) established with funding from GSK. Since its establishment the foundation has received more than 150 grant applications and approved 38 projects involving 34 different organizations. To illustrate this initiative some examples in the Kinetoplastid diseases field will be presented.

The Lead Optimization Latin America (LOLA) consortium: collaborative drug discovery for Neglected Tropical Diseases (NTDs)

Luiz Carlos Dias[1]; Marco A. Dessoy[1], Brian Slafer[1], Adriano Andricopulo[2], Dale Kempf[3], Brian Brown[3], Mira Hinman[3], Yvonne C. Martin[3], Simon F. Campbell[4], Charles E. Mowbray[4]

[1]Instituto de Química – UNICAMP, Campinas, Brazil ; [2]Laboratorio de Química Medicianl e Computational Centro de Biotecnologica Molecular Estrutual– USP, Sao Paulo, Brazil; [3]AbbVie Inc., Chicago, USA; [4]Drugs for Neglected Diseases *initiative* (DND*i*), Geneva, Switzerland

BACKGROUND: A strategic international collaboration for the expansion of drug discovery in Brazil as a pioneering precedent for early phases of R&D in endemic countries delivering open innovation. First phase based on a screening campaign by WHO TDR that identified a novel series of cyanopyridines with interesting activity against *Trypanosoma cruzi*. Initial exploration of the structure activity relationships (SAR) of this series already provided leads.

METHODS: In the collaboration, with Unicamp's expertise in chemical synthesis of compounds and DNDi's access to libraries of compounds of partners, new analogues of lead compounds are designed and prioritised in a collective and collaborative process taking into account *in vitro* activity against *T. cruzi*, selectivity against mammalian cells, stability and routes of metabolism in liver microsomes, aqueous solubility, permeability and synthetic accessibility. Synthetic routes are then designed and these novel compounds are synthesized, fully characterized and sent for parasitology and Absorption Distribution Metabolism and Elimination (ADME) testing by other partners in the consortium.

RESULTS: The on-going optimization of the cyanopyridine series will be presented with a particular focus on chemical synthesis to deliver compounds with improved *in vitro* parasitology and Absorption Distribution Metabolism and Elimination (ADME) testing profiles in the search for compounds ready for *in vivo* pharmacokinetic and efficacy studies.

CONCLUSIONS: The LOLA consortium is making exciting progress in the search for new treatments for Chagas Disease. The model brings together scientific expertise from Latin America with drug discovery experience and advice from DND*i* and its consultants and partners such as AbbVie Inc.

New mechanisms to accelerate drug discovery for Neglected Tropical Diseases (NTDs)

Charles E. Mowbray[1]

[1]Drugs for Neglected Diseases *initiative* (DND*i*), Geneva, Switzerland

BACKGROUND: The currently available treatments for Neglected Tropical Diseases such as Visceral Leishmaniasis and Chagas Disease suffer serious limitations. There is an urgent need for new medicines to deliver improved efficacy and greater safety in convenient and field-adapted formulations for the patients of these diseases.

METHODS: DNDi and its extensive network of public and private partners aim to discover and develop new drugs for kinetoplastid diseases through innovative collaborations. DNDi accesses diverse chemical collections, exploits innovative high throughput screening methodology, and applies modern drug discovery approaches in the hit to lead and lead optimisation stages. These activities are guided by a clear focus on meeting the needs of patients developed through an extensive network of clinical and public health researchers in the endemic regions.

RESULTS: Some of the lessons learned in DNDi's first 10 years will be shared and the current Drug Discovery model will be described and illustrated with examples of emerging new chemical entities (NCEs). Some new mechanisms to accelerate the drug discovery process will be presented.

CONCLUSIONS: Drug Discovery for kinetoplastid diseases has evolved from an era of reformulation and repurposing of existing agents and has moved onto fully integrated programmes to discover NCEs. We aim to accelerate the delivery of new treatments to neglected patients through the implementation of new scientific breakthroughs coupled with novel partnerships and initiatives.

Use of data-mining and chemoinformatics in the identification and optimization of high throughput screening hits for NTDs

James Mills[1]; Gibson, Karl R[1]; Whitlock, Gavin[1]; Glossop, Paul[1]; Ioset, Jean-Robert[1]; Tadoori, Leela Pavan[2]; Mowbray, Charles E.[2]

[1]Sandexis, Innovation House, Discovery Park, Sandwick, Kent, UK; [2]DNDi, 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland

BACKGROUND: A typical problem encountered by organisations such as DNDi is the extraction of the maximum possible amount of information from the huge amounts of data that are generated, whether this be from high-throughput screens,ongoing medicinal chemistry programs or published data sets. In this presentation we demonstrate how computational methodologies have been developed to extract previously unidentified series of hits from phenotypic screens, assess the chemical landscape of putative actives to inform prioritisation of hits, and identify and build on SAR additivity in the optimisation of hit series.

METHODS: Re-triage of legacy DNDi screening data was carried out by using novel clustering methodologies to identifying series of compounds enriched in weak actives that showed at least some evidence of a window over general cell toxicity. For each such series, a full chemoinformatic work-up was carried out, identifying the local chemical space in terms of (i) available chemical matter for screening (eMolecules) and (ii) evidence for bioactivity in any targets (using ChEMBL). Series optimization was approached by identifying whether or not the SAR was additive, using double mutant analysis to identify squares of compounds. In series for which additivity was demonstrated, the method was used in a predictive sense to identify holes in the current series with predicted improved potency

RESULTS: The re-triage work was summarized in automated web-based reports and provided to a group of external consultants, allowing them to make decisions on each series based on a comprehensive summary of all relevant data for all series. This led to the identification of over 10 series that are currently being followed up with a targeted *Leishmania* screening program. A number of series being prosecuted by DNDi have shown SAR additivity and a number of more potent compounds have been synthesized as a result of applying the methodology.

CONCLUSIONS: A comprehensive analysis of high-throughput screens can unearth novel series that have been missed by previous triage methods. This involves extraction of signals in the more noisy data and therefore relies on computational methods to ensure that the most appropriate criteria are used to define genuine hits that are worthy of follow-up.

How Latin America can become a R&D leader in the field of tropical neglected diseases: towards new mechanisms for innovation

[1]*Eric Stobbaerts*

[1]Drugs for Neglected Diseases *initiative Latin America*, Rua Santa Heloisa 5, Jardim Botanico, Rio de Janeiro, Brazil

BACKGROUND: Historically, research capacity for tropical neglected diseases have been mostly planned and funded by the developed world. In the last decade however the urgent need to shift this pattern and enhance commitment and adequate funding from endemic countries has been growing. In this route, these same countries have moved to a much clearer protagonist role in order to implement NTDs innovation on their own terms and needs.

METHODS: Efforts are currently made to experiment innovative mechanisms to fund and coordinate public health R&D to address unmet medical needs in developing countries, especially in Latin America. Similar trends are taking in other BRICS countries. These efforts, driven by public policies and private initiatives, look at new frameworks with the aim to demonstrate that R&D projects can be successful by optimizing guiding principles such as cross-regional collaboration of existing networks, open-innovation and knowledge sharing, equitable access to new products, and sustainable funding secured through existing and new funding mechanisms.

RESULTS: New approaches began about ten years ago, with the product development partnerships (PDPs). With eight offices around the world, DNDi has proven the possible achievements in close partnership with endemic countries. In Latin America, a cooperation agreement with the Department of Science, Technology and Strategic Inputs (SCTIE) is one example. Plus, global mechanisms such as UNITAID, Medicines Patent Pool, supported by public and private donors, ought to be mentioned. More recently, the scope of the demonstration projects perform as the 2.0 version of such enterprise, such as the Chagas R&D Accelerator Initiative, aiming to pull public and private funding into a virtual pooled fund to delivery of new tools to treat and control Chagas disease through a coordination mechanism based on open knowledge and innovation principles.

CONCLUSIONS: With the right commitment, coordination and collaboration, the public and private sectors from the endemic countries can work together to enable people suffering from NTDs to have access to advanced R&D through partnerships and provision of funding to find next-generation treatments and interventions.

Abstract – Opening Ceremony

Research & Development for neglected patients: What has changed over the past two decades and what are the future challenges to guarantee access to treatments for patients in need?

Dr. Bernard Pécoul, Executive Director, Drugs for Neglected Diseases initiative (DNDi)

Over one billion people, including 500 million children, are affected by neglected diseases, some of which are fatal without treatment. As diseases are mostly poverty-related, the patients do not represent a lucrative market for drug development, and hence little investment in research and development (R&D) for new treatments.

Twenty years ago, existing treatments had serious limitations (toxicity, cost, lack of effectiveness, difficult to administer, painful). Despite an obvious urgent medical need to develop new, better adapted treatments, R&D pipelines were virtually empty.

However, improvements over the past decade in particular have helped address neglected diseases' R&D gaps: new initiatives, including product development partnerships, have flourished and new actors have joined forces, offering innovative ways to develop new health technologies. Furthermore, emerging economies and neglected disease-endemic countries have begun to engage these issues.

In Latin America approximately 200 million people are suffering from neglected tropical diseases (NTDs), with 8-10 million cases of Chagas disease, tens of millions of cases of intestinal worm infections, and other diseases such as schistosomiasis, leishmaniasis, and onchocerciasis. Countries have demonstrated a key-role in developing regional approaches to control and eliminate endemic NTDs.

Today, the fact that elimination for some diseases is on the global agenda is a clear illustration of progress made. For instance, WHO's NTDs Roadmap has defined specific global or regional targets and milestones for the control or elimination of their 17 defined NTDs by 2020. WHO/Roll Back Malaria has similarly targeted the elimination of malaria in at least 8-10 new countries (since 2008) by end-2015, including the entire WHO European Region. Chagas disease is also a good illustration of progress made over the past decade for control and prevention, as well as for R&D of new health tools. However, more coordinated effort is needed to treat patients and to support long-term achievements.

Major R&D challenges remain in order to progress from isolated and fragmented successes to sustainable change. A global framework is certainly needed to define priorities, address gaps, and so guarantee the sustainability of the R&D landscape for NTDs. This would also further reinforce or enable international collaboration, strengthen regulatory capacities in developing countries to streamline clinical development and marketing authorization, estimate and raise adequate financial resources and establish new incentive mechanisms, so paving the way for equitable global public health for neglected patients.

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Pitfalls in the VL treatment (Regional differences and challenges)

A. Musa [1], B. Sharma [2], M. Wasunna [2,5], EAG. Khalil [1], V. Goyal [2], F. Alves [2], R. Kimutai [2,5], T. Dorlo [3], T. Edwards [4], N. Alexander [4], A. Hailu[6], J. Olobo[7], M. Balasegaram [2], S. Ellis [2], N. Strub [2], G. Bilbe [2], J. Alvar [2]

1. Institute of Endemic Diseases, University of Khartoum, Sudan

2. Drugs for Neglected Disease *initiative,* Geneva
3. Utrecht University, Holland
4. London School Hygiene and Tropical Medicine, London
5. Kenya Medical Research Institute (KEMRI), Kenya
6. Addis Ababa University, Ethiopia
7. Makerere University, Uganda

DNDi and others have conducted studies to optimize current drug regimens for VL treatment. In India and Bangladesh phase-III trials showed efficacy above 95% for 3 double-drug combinations (miltefosine/paromomycin, AmBisome/miltefosine, AmBisome/ paromomycin) and single-dose AmBisome. In Nepal, miltefosine is the first line drug with an initial cure rate of 95.8%. However, after seven years of use the relapse rate at six and twelve months has become 10.8% and 20.0%, respectively. Pharmacokinetic studies (PK) have shown that children are under-exposed to miltefosine, which is associated with higher risk of relapses. In Bangladesh AmBisome 10mg/kg single infusion has shown a cure rate of 97,7% at six months follow up in a feasibility/efficacy study in a sub-district hospital. In India, follow-up of patients treated with multiple doses of Ambisome (20mg/Kg) showed that a high proportion of relapses occur between 6 and 12 months after the end of the treatment.

SSG&PM for 17 days (91% efficacy rate) has been recommended as a first line treatment for East Africa (WHO EC, 2010). In order to find an alternative option less painful and toxic, DNDi and the LEAP have finalized a phase II trial conducted in Kenya and Sudan. This study assessed the safety, efficacy, PK/PD of Ambisome+SSG, Ambisome+miltefosine and miltefosine. Results showed an unsatisfactory efficacy to justify inclusion in a Phase III trial as compared with the standard SSG&PM regimen. PK findings showed also under-exposure to miltefosine in African children, meanwhile the parasite clearance was slower when AmBisome was administered in one single infusion as compared to multiple doses. Innovative, efficacious, safe and affordable new (oral) treatments are undertaken. A proof-of concept trial is currently ongoing in Sudan to assess efficacy of oral fexinidazole.

Part of these studies have been granted by the European Commission (FP7)

Elimination of VL in the Indian subcontinent – is it achievable?

P.Das[1] J.Alvar[2], B. Sharma[2]

1. Rajendra Memorial Research Institute, Patna
2. Drugs for Neglected Disease *initiative,* Geneva

In an effort to eliminate VL in the Indian subcontinent a joint initiative between the government of the three countries India Nepal and Bangladesh was launched in 2005 with the support of WHO and SEARO. The MOU was for mutual cooperation, to achieve the objective that was to reduce the annual VL incidence below 1/10,000 population in a district, subdistrict and upzilas by 2015 based on detection, treatment of VL cases and vector control. The elimination was possible as in this region man is the only host and P. argentipes only vector for L. donovani. The additional aspect in favour of elimination were the availability of rapid diagnostic test and oral drug miltefosine. The disease is restricted to defined area in the region now therefore intense vector could be implemented. There are positive experiences historically in the region which was endemic has successfully eliminated the disease. The plan for elimination was in four phases the preparatory phase of two years, attack phase of five years, consolidation phase of three years and then maintenance of two to three years.

The programs of the countries have implemented the tools with considerable effort but still some challenges remain in achieving the target. The lessons or the reason for reemergences of the disease inspite of achieving control of disease needs to be deliberated. The emerging issue of coinfection in VL with HIV needs to be monitored and treated as special population due high relapse rate. PKDL and asymptomatic are a subpopulation which may have sufficient Vl parasitic for transmission. In order to eliminate this group could be the carriers and lead to re-emergence of the epidemic after a decades. An important aspect that needs attention is the information systems to accurately assess the total burden of disease including Kala azar, PKDLand asymptomatics.

Challenges in Cutaneous leishmaniasis: Perspectives for treatment development and disease control

Byron Arana[1]

[1]Drugs for Neglected Diseases Initiative, 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland.

BACKGROUND: Cutaneous Leishmaniasis is caused by over 15 different species of the parasite *Leishmania*. The exact incidence of is not known. An estimated 1.2 million cases/year from approximately 90 countries worldwide suffer from different forms of CL. Most cases are reported from Syria, Afghanistan, Iran, Morocco, Brazil, Colombia and Peru. Due to the complexity of transmission cycle of leishmaniasis, early diagnosis and treatment remain as the more important and feasible control strategies for CL. Pentavalent antimonial continue to be the first-choice drug despite their toxicity, difficulty in administration and high cost.

METHODS: A review of published studies reporting the efficacy of different treatment modalities for Cl was conducted

RESULTS: A great variety of topical or systemic treatments options have been used to treat CL. However the majority of these modalities have been tested in non-controlled studies, with no well-accepted cure definition and with only few subjects. In addition, the lack of adequate animals models to evaluate the efficacy of drug candidates, coupled with the spectrum of the clinical manifestations and the variability on drug response to the different *Leishmania* species, have hampered the development of new drugs for CL.

CONCLUSIONS: In alignment with WHO recommendations, DNDi CL strategy proposes to treat CL based on the clinical presentation of the diseases: Local therapies for patients with small and few lesions located in anatomical areas amenable to be treated topically; systemic, preferable oral drugs, for subjects suffering from numerous or large lesions, subjects with lesions potentially disfiguring, disabling or located in anatomical areas which make local therapy impossible, whilst subjects with leishmaniasis recidivans, diffuse CL or PKDL and anthroponotic CL should benefit from using anti-Leishmania drugs in combination with an immune response modifier to accelerate and enhance a Th-1 type immune response.

Scaling up Diagnostics and Treatment of Chagas disease, the MSF experience

Dr. Judith Herrera, Director Medical Department, Médecins Sans Frontières Spain (MSFS) /OCBA

BACKGROUND: MSFS started to work on Chagas disease since 1999. Projects were initially vertical and focused on children; adults started to be included in 2007. The main objective was to demonstrate the feasibility of diagnosis and treatment and to advocate for more support in research and management of the disease. Since 2007, the importance of scaling up became a priority, introducing the objective of integration of the services at the PHC MoH structures in projects in Bolivia, Paraguay and more recently in Mexico.

METHODS: To scale up access, models have been developed to simplify diagnosis and treatment procedures and processes to increase the possibility for the MoH to integrate them in their own structures, often with limited resources. Different approaches have also being used to provide services within the PHC structures and at the community level with different degree of involvement and support of the MSF team. Further adaptations were made giving more emphasis in promoting from the beginning the ownership of the national authorities, health professionals and community members to increase the possibilities to achieve the scaling up objective.

RESULTS: So far, around 30,000 patients have been screened and 6500 received treatment in the different MSFS supported projects. Substantial achievements were reached in increasing the awareness and the consensus that to treat children and specially adults is useful, feasible and safe. Simplification of the model of care has increased access during the time of intervention, but the integration to the MoH services has not yet been achieved due to limitations in its adaptation to the local conditions and structural barriers, not allowing so far replicability and sustainability. Adjustments to recent projects should yield better outcomes.

CONCLUSIONS: Despite the achievements, access is still a major problem due to a number of barriers at the political, organizational, strategic and programmatic levels. MSF remains committed to improve its approach to make sure models are better adapted to local conditions to make integration more feasible. The political commitment and active involvement of national and international key actors play a central role if more access to the affected population is to be achieved.

Chagas disease drug discovery: Towards a new era

Eric Chatelain[1]

[1]Drugs for Neglected Diseases *inititative* (DND*i*) 15 Chemin Louis Dunant, 1202 Geneva, Switzerland

BACKGROUND: Chagas disease, the result of human infection by *Trypanosoma cruzi* parasite, is endemic in Latin America and its impact is reaching global dimension through migrating populations. New drugs that are safe, efficacious, low cost and adapted to the field are critically needed. Two new compounds –posaconazole and the ravuconazole prodrug E1224 - that had shown promise in *in vitro* and *in vivo* models, entered Proof of Concept clinical study. The outcome of these recent trials was however disappointing, as treatment failure in Chagas patients treated with the azoles reached 70 to 90% as opposed to 10-30% failure for benznidazole-treated patients.
The lack of translation from *in vitro* and *in vivo* models to the clinic observed for the azoles raises several questions.

METHODS: We reviewed and challenged if we are indeed using the right tools and decision-making processes to progress compounds forward for the disease.

RESULTS: Technological innovations are changing the way Chagas drug discovery is being performed: the development of phenotypic high-throughput screening assays for intracellular *T. cruzi* using imaging software allows the detection of single parasite inside cells *in vitro*; and the use of transgenic parasites with BioLuminescence Imaging enables the live tracking of the parasitic infection in mice. Additional secondary assays, such as testing against a panel of *T. cruzi* strains, time-kill and reversibility assays among others, should allow for a better decision-making during the discovery process and the profiling of compounds with the best chances of a positive clinical outcome.

CONCLUSIONS: These developments open new opportunities and will be very useful in redefining the current screening sequence for Chagas drug discovery. Overall it should lead to an improved prediction model for human studies and increase our chance of success in providing Chagas patients with new drugs, as long as sustained funding remains available.

New treatment opportunities for Chagas Disease

[1]*Isabela Ribeiro*

[1]Drugs for Neglected Diseases *initiative*, 15 Chemin Louis Dunant, 1202 Geneva, Switzerland.

BACKGROUND: Chagas Disease (CD) is an important public health issue, both in Latin America and increasingly around the world. Current treatment is limited to only two drugs for decades: nifurtimox and benznidazole (BNZ).

METHODS: Matching needs and opportunities, DNDi's portfolio is a mix of projects at different stages of the development process, balancing short- and long-term objectives. In the short and medium-term, DNDi presently evaluates new regimens of BNZ in monotherapy and combination for the treatment of adult patients with chronic indeterminate CD. DNDi also presently investigates Fexinidazole, as a potential candidate for a monotherapy, as it has been shown to be effective in experimental models of CD. To meet long term objectives, DNDi collaborates with partners to conduct drug screening, hit-to-lead and lead optimization activities targeting the identification of new chemical entities.

RESULTS: The current CD clinical portfolio leverages data from recently concluded studies. Two recent azole proof-of-concept (PoC) trials showed that BNZ had a rapid and sustained parasitological response, but with safety and tolerability limitations. Data from two BNZ Population Pharmacokinetics studies showed significantly lower exposures in children <7y, but with comparable efficacy. These data lead to the hypothesis that new BNZ treatment regimens may address the existing efficacy and tolerability gaps and two PoC studies of BZN in monotherapy and combination are planned. Regarding Fexinidazole, a Phase II, PoC trial evaluating six dosing regimens is under-way in Bolivia. The efficacy and safety results from these clinical studies will inform the decision to proceed to Phase III evaluation in different epidemiological settings.

CONCLUSIONS: DNDi is collaborating with partners to develop urgently needed new, field-relevant tools for CD. Data obtained over the past year offer a new landscape in which to advance improved treatments for this neglected disease, using old and new drugs.

"Immune participation of *Anopheles albimanus* pericardial cells against pathogens and malaria parasites"

Salvador Hernández-Martínez. Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública. Cuernavaca, Morelos, México.

BACKGROUND: Insects have highly efficient innate immune mechanisms that allow them to recognize and neutralize potential pathogens; however, the identification of organ and tissues involved in these mechanisms is essential for the development of control strategies in vector-borne diseases. The fat body and the hemocytes are the most studied tissues in terms of their immune response. On other hand, the insect heart contains accessory cells called pericardial cells (PCs) or nephrocytes, with functions of filtration and recycling of molecules from the hemolymph. Recently, several studies have demonstrated the presence of response immune markers in mosquito PCs (STAT PCs; Sp22D, TEP-I and SRPN10), suggesting their possible involvement in the defense mechanisms. Currently, we are studying the response of PCs of *Anopheles albimanus* (vector of *Plasmodium vivax* in Mexico) against different pathogens, including malaria parasites. METHODOLOGY: Female mosquitoes challenged with bacteria, yeasts, zimosan, Peptidoglycan, lipopolysaccharide or RPMI were dissected and the heart analyzed. Histochemical tests were conducted in the heart and to evaluate lysosomal enzymes and lytic activities. The synthesis of DNA in the PCs was evaluated by means of BrdU incorporation after different challenges. Interaction with sporozoites of *P. vivax* was analyzed by conventional paraffin histology and transmission electron microscopy. Finally, the heart transcriptome of challenged and non-challenged mosquitoes was obtained by pyrosequencing. RESULTS: Enzymatic and histochemical results showed lysosomal and acid phosphatase activity only in the PCs of mosquitoes injected with bacteria, yeasts or components of these microorganisms, which was confirmed by measuring their relative activities. PCs obtained from mosquitoes previously inoculated with zymosan, presented strong lytic activity on *Micrococcus lysodeikticus*. BrdU incorporation showed DNA synthesis in PCs nuclei, which was differential depending of the challenge. In *P. vivax* infected mosquitoes, sporozoites near PCs were aggregated, surrounded by an electron-dense precipitate and showing signs of damage. The distribution of parasites damaged and undamaged, allows us to suggest a new mechanism of displacement of the sporozoites to reach the salivary glands of the mosquito. Bioinformatics analysis on the transcriptome shows significant differences in the expression of genes related to immunity. CONCLUSIONS: The continuity of these studies is essential to identify and validate the expression of genes related to immunity in the PCs. The results provide new knowledge of the immune mechanisms of diseases vectors.

Molecular characterization of BRF1, a subunit of Pol III transcription factor TFIIIB, in *Trypanosoma brucei*

Vélez-Ramírez, Daniel Eduardo; Florencio-Martínez, Luis Enrique; Romero-Meza, Gabriela; Martínez-Calvillo, Santiago

Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, 54090 Tlalnepantla, State of Mexico, Mexico

BACKGROUND: BRF1 is a subunit of the RNA polymerase III (Pol III) transcription factor TFIIIB, together with TBP and BDP1. BRF1 is essential for Pol III activity and, hence, for cell survival. A putative orthologue of BRF1 in *Trypanosoma brucei* (TbBRF1) has been found, but definitive identification and characterization are still lacking.
METHODS: Sequences of several BRF1 orthologues were aligned with ClustalΩ to identify specific BRF1 domains. TbBRF1 conditional knock-down cell lines were generated using a tet-inducible RNA interference (RNAi) system. Growth curves were performed to analyze cell viability, and mRNA and protein decrease was confirmed by Northern-blot and Western-blot, respectively. The effect of TbBRF1 depletion on Pol III transcription was analyzed by nuclear run-on assays.
RESULTS: We found that TbBRF1 contains the typical conserved domains: a zinc finger motif and two TFIIB-related regions. In the knock-down cell line, RNAi induction effectively reduced the TbBRF1 mRNA and protein levels. As expected, ablation of TbBRF1 led to a growth arrest. Run-on assays showed that TbBRF1 is necessary for Pol III-mediated transcription.
CONCLUSIONS: TbBRF1 contains the conserved domains found in other BRF1 orthologues. TbBRF1 participates in Pol III transcription in *T. brucei* procyclic forms and is needed for cell viability.

Early steps in the evolution of malaria drug resistance

White, John1; Guler, Jennifer2; Staverosky, Julia1; Rathod, Pradipsinh K.1

1Department of Chemistry, University of Washington, Seattle, WA, USA;
2Department of Biology, University of Virginia, Charlotte, VA, Washington, USA

BACKGROUND: Emergence of malaria drug resistance is characterized by large number of mutations in the relevant parts of the parasite genome. Two important questions are: (1) Can malaria parasites acquire many mutations in the right places without extraordinary damage elsewhere in their genome, and (2) What are the earliest genotypic and phenotypic traits accompanying *de novo* emergence of drug resistance. Our laboratory uses *in vitro* selection for drug resistance against novel experimental antimalarials to both identify targets of potential new drugs and to understand evolutionary strategies underlying acquisition of malaria drug resistance.
METHODS: We used *in vitro* culture of *P. falciparum* to select for clones resistant to a variety of new classes of antimalarials, often with different mechanisms of action and some with unknown targets. Using careful special laboratory selection strategies to capture early events in mutagenesis, independently derived resistant parasite clines were subjected to Whole Genome Sequences to identify the nature of the earliest resistance traits.
RESULTS: In these studies, we successfully capture gene duplication, and higher order copy number variations, as an early and important step in the acquisition of targeted mutagenesis: these initial amplicons can be as large as 100 Kb. They can subsequently give changes in number and sizes of CNVs, point mutations, and even disappearance of CNVs, with little or no collateral damage elsewhere in the genome. We also show variations in the earliest genotypes and phenotypes captured by continual maintenance of antimalarial pressure during selection and cell cloning.
CONCLUSIONS: Our results demonstrate that haploid forms of *P. falciparum* are capable of targeted mutagenesis. We demonstrate a population-based strategy of malaria parasites to identify a locus for advantageous mutagenesis, and a way for parasite populations to target mutagenesis at the advantageous locus without large collateral damage elsewhere in the haploid genome.