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POSTER NUMBER: 1001

Drug repurposing and screening of libraries of chemical compounds to identify new anti-parasitic agents

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Toxoplasma gondii is an intracellular parasite and the etiological agent of toxoplasmosis, a common parasitic disease capable of infecting a range of hosts, including nearly one-third of the human population. Current treatment options for toxoplasmosis patients are limited. They include the use of anti-malarial drugs or antibiotics, which often cause significant side effects. Currently, toxoplasmosis constitutes a large global burden that is further enhanced by the shortcomings of available therapeutic options, thus underscoring the urgent need to identify and develop new therapies. Non-biased screening of libraries of chemical compounds including the repurposing of well-characterized compounds is emerging as viable approach in identifying lead candidates for early drug development against parasitic diseases. As a proof-of-principle screen to identify effective anti-parasitic agents, we evaluated libraries of natural product (503), FDA-approved (640) compounds as well as imidazole derivatives (26) for potential to inhibit *T. gondii* growth. We identified 39 new compounds that potently and selectively restrict the growth of *T. gondii*. The findings are new and promising, and do not only strengthens the prospects of drug repurposing and the screening of wide range of chemical compounds as a viable approach in drug discovery toward effective anti-parasite therapy but also support imidazole-based compounds as alternative source of effective anti-parasitic agents.

Keywords: Drug discovery; Library screening; Medicinal Chemistry; Toxoplasmosis

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POSTER NUMBER: 1004

In Vivo Evaluation of The Anti-Giardia Activity of Some Hypolipidemic Compounds

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Introduction
Giardia lamblia is an intestinal parasite that causes acute and chronic giardiasis and is associated with many post infectious and chronic diseases. The parasite is known to be highly dependent on external (host) sources for lipids, cholesterol and bile salts. This is a follow up study of a previous *in vitro* one in which the action of some lipid lowering drugs have been tested for their potential anti-*Giardia* activity.

Methods
Balb/c mice were treated with antibiotic water ad libitum for three days before the infection and throughout the experiment. To establish *Giardia* infection, bioluminescent *Giardia lamblia* (WB strain) trophozoites were administered to Balb/c mice by gavage. Four compounds (Lomitapide, fenofibric acid, cholestyramine and compound X) were tested in this model for their potential anti-*Giardia* activity. The first three of them are FDA approved and clinically used as hypolipidemic compounds in man. Metronidazole and PBS were used as positive and negative control respectively. For each compound four mice were used to test its action. The response of five doses of the drugs was assessed by measuring the bioluminescence before and during treatments. Counting of intestinal parasites was also performed on the last day. GraphPad prism was used to analyse the results and the means of the responses.

Results and discussion

None of the three hypolipidemic compounds showed a significant mean difference when compared to the negative control in both the bioluminescence assay and the counting-based evaluation. Nevertheless, in a previous in vitro study, lomitapide has shown strong activity against *Giardia lamblia*. Some host factors are perceived to have contributed to its *in vivo* inactivity of the tested concentration. Compound X, a non-hypolipidemic compound, exhibited some anti-parasitic activity with a significant difference in the counting-based assessment, but not in the bioluminescence-based assay. Further studies on lomitapide and Compound X with different doses and/or chemical modification in their molecular formula are advised.

POSTER NUMBER: 1002

Development of novel antibiotics targeting the DNA double-strand break repair pathways

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An unrepaired DNA double-strand break (DSB) is lethal to cells. In bacteria, DSBs are usually repaired either via an error-prone pathway which essentially ligates the two ends of the DNA break or a pathway that utilises recombination to drive accurate repair of the DSB. Due to the lethality of an unrepaired DSB, drugs which exhibit antibacterial activity by inducing persistent DSBs have been successful in the treatment of bacterial infections. However, recurrent usage of these drugs as monotherapy has led to emergence of resistant bacterial strains which have undergone modification of the primary cellular targets of these drugs. In the present study, several diverse organic extracts from fungal sources were screened to identify candidates that exhibit antibacterial activity by either inducing persistent DSBs or inhibiting repair of a site-specific DSB that was generated in *E. coli*. The synergistic effect of the active compounds in these two categories of extracts is anticipated to exhibit robust antibacterial activity against multidrug resistant strains of bacteria. The study has also identified antibiotic-compound interactions that increase the sensitivity of *E. coli* to DSBs. These antibiotic-compound combinations would be vital for rescuing the current obsolete DSB-inducing drugs. The preliminary data from this study highlights specific cellular and molecular mechanisms that could be exploited to develop novel chemotherapy against multidrug resistant strains of bacteria. The data also indicate possible strategies for resuscitating obsolete DSB-inducing drugs.

POSTER NUMBER: 1003

A second chance for your favorite protozoan target: Repositioning opportunities for treatment of cryptosporidosis

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Cryptosporidiosis is a significant contributor to global diarrheal morbidity and mortality. The burden of the disease is especially severe in children < 2 years old in resource-limited environments where infection is associated with moderate-to-severe diarrhea, stunting, and death. In immunocompromised patients, cryptosporidiosis diarrhea maybe life threatening. The only FDA approved therapy for cryptosporidiosis, nitazoxanide, has limited efficacy in immunocompromised individuals and malnourished children. As a protozoan pathogen, there is an opportunity to reposition compounds from the extensive work in drug development for other parasites. ~ 2200 bioactive (phenotypic assays) compounds from a GSK collection were assayed for efficacy against *Cryptosporidium parvum* at the University of Washington (UW) resulting in an expected high hit rate (11%, 2 copies, > 80% inhibition @ 10 μM). Based on the initial screen, hit expansion and ADMET/potency triage have delivered 9 clusters with different ADME profiles. Following the in vitro screen, 6 GSK compounds were characterized with an IFN-gamma KO mouse model at the UW. Of the 6 compounds, a *Plasmodium falciparum* protein kinase G (PfPKG) inhibitor demonstrated excellent in vivo efficacy with a ~ 5 log reduction in oocyst shedding compared to the peak infection level of the untreated group. With CpPKG as the putative target, genetic target validation studies are currently underway. In addition, given the gastrointestinal localization of the parasite, there are questions regarding the calculation of the human dose prediction for anti-cryptosporidosis drug candidates. A human dose prediction for the PKG inhibitor was generated with novel PBPK-PD modeling supported by dose escalation studies and MALDI imaging of drug distribution in gastrointestinal tissue.

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POSTER NUMBER: 1036

In vitro antimycobacteria, immune modulatory and apoptosis inducing effects on macrophage of _Psychotria capensis_ and _Psychotria zombamontana_ species (Rubiaceae)

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The causative agent of tuberculosis (TB) *Mycobacterium tuberculosis* (Mtb), has succeeded in infecting one-third of the human race through inhibition or evasion of innate and adaptive immunity. It has been suggested that apoptosis of infected macrophages is one way in which the host deals with intracellular pathogens. To effectively combat circumvent limitations of the present TB drugs, new drugs with novel modes of action different from the presently administered drugs are urgently needed. The acetone extracts of plants belonging to the Rubiaceae family namely; *Psychotria capensis* and *Psychotria zombamontana* has been reported to be active against non-pathogenic mycobacteria. This study aims to assess the antimycobacterial activity of *P. capensis* and *P. zombamontana* against pathogenic strains of mycobacteria and also to shed some light on elucidating the anti-tuberculosis mechanism of the selected plant extracts.The antimycobacterial activities of the crude extracts of these 2 species belonging to the rubiaceae family were screened against *Mycobacterium tuberculosis* ATCC, *Mycobacterium bovis* ATCC and H37Rv strains. THP-1 cells were treated with varying concentrations of each extract for 24 and 48 h. Cell apoptosis was measured by Annexin V/Propidium Iodide apoptosis kits while pro inflammatory cytokines in the supernatants was also measured using Flow cytometry. The acetone crude extracts of studied plant species had an excellent antimycobactrial activity against the tested mycobacteria strains with a Minimum Inhibitory concentration values ranging from 78 -156 µg/mL. *P. capensis* and *P. zombamontana* extracts induced apoptotic effects on THP-1 cells following treatment in a time and dose-dependent manner. Interestingly, the acetone extracts of *P. capensis* revealed a profound macrophage apoptosis induction and also enhanced the expression of pro inflammatory cytokines in a dose dependent manner. Results obtained from this study suggest that the acetone extracts of *P. capensis* may contain compounds having potential as a candidate for anti-TB therapy.

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POSTER NUMBER: 1005

Novel Tetracyclic Iridoid Compounds Isolated from _Morinda lucida_ Induce Apoptotic Cell Death and Phenotypic Changes in _Leishmania spp_

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Leishmaniasis remains a health concern in developing nations. However current drugs are limited by high toxicity, and resistance issues. This necessitates continuous efforts in identifying alternative therapy. Previously, we identified novel tetracyclic iridoids; Molucidin, ML-2-3 and ML-F52 isolated from the leaves of *Morinda lucida*, to have anti-trypanosomal activity. This study hypothesizes that iridoids would have anti-*leishmania* properties against kinetoplastida as they share some common target sites of drug action. We investigated the efficacy of iridoids and mechanism of action against *Leishmania spp*. A 50% inhibitory concentration of compounds was determined against promastigotes and intracellular amastigotes after 48 hours of incubation. Molucidin and ML-F52 showed significant activity against *L. donovani*; IC50 = 1.44, and 0.85 µM, respectively and *L. major*; IC50 = 1.08, and 0.79 µM, respectively. Nexin assay showed Molucidin and ML-F52 to induce significant apoptotic effect, 36.1 & 64.7 %, respectively. Although no inhibition of kinetoplastid membrane protein was observed with treatment, iridoids inhibited cytokinesis and induced phenotypic changes in cells. Molucidin induced significantly higher "nectomonad-like" forms (50%), non-replicating forms, and loss of kDNA. ML-F52 induced 'cell-rounding' with loss of

flagellum. An enhanced peak at G2-M phase in Molucidin-treated cells was observed with the accumulation of mid-mitotic forms. Iridoids induced an enhanced peak at sub-G1, supporting apoptotic data of compounds. Variations in cell cycle arrest, phenotypes and cytokinesis in *Leishmania spp*. was triggered by compounds suggesting differences in their effect on parasites and therefore further investigation could present potential lead against Leishmaniasis.

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POSTER NUMBER: 1047

Design of new trypanocides targeting the ATP production pathway of African trypanosomiasis

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Neglected Tropical Diseases (NTDs) are a group of chronic infectious diseases that disproportionately cause devastating illnesses among the socially-bottom one billion people, hence, the big Pharmas consider them unprofitable for investments. NTDs are caused by diverse pathogens of which the African trypanosomes are a leading group. The presently available chemotherapies have become unsatisfactory due to their narrow spectrum of activity, reduced efficacy, and reported toxicities, prompting the need for the development new treatment options. To achieve this, we aim to design novel drug candidate compounds against previously unexplored molecular targets in the parasites. The unique energy metabolism pathway of the parasites is considered a validated rational strategy towards the development of new drugs. Our previous results show that simultaneous inhibition of the trypanosomes' glycerol kinase (TGK) and alternative oxidase (TAO), two key enzymes for ATP synthesis in the parasites' resulted in trypanosomes death. While ascofuranone (AF) is an established TAO inhibitor, there is no known inhibitor for any TGK. The present study was aimed at the discovery of novel TGK inhibitors for co-administration with AF. Here, we utilized the combination of protein X-ray crystallography, computational medicinal chemistry and Enzyme assay approach to conduct large-scale screening of a combinatorial library. The resulting hits were of compounds possessing different structural scaffolds, which potently inhibited TGK up to submicromolar-level IC_{50} values. Interestingly, a number of the inhibitors caused the expected improvement in the potency of AF against trypanosome cells, causing a shift in trypanocidal activity of AF (IC_{50}) from nanomolar to picomolar concentrations.

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POSTER NUMBER: 1006

Mitochondria and Mycobacteria: Small molecule aided restoration of mitochondrial function enhances anti-mycobacterial activity of human macrophages in cholesterol induced asymptomatic dyslipidemia

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Recognizing the factors fortifying susceptibility and persistence of tuberculosis (TB) is primary to the success of TB control programs. Dyslipidemia, a pathological state of metabolic imbalance, is an emerging risk factor for deregulation of host immune responses. We studied asymptomatic dyslipidemia, characteristic of borderline high cholesterol profiles, as a potential risk factor for TB susceptibility. We observed that exposure to sub-pathological concentrations of cholesterol impaired intracellular *Mycobacterium tuberculosis* (*M.tb*) clearance by human macrophages, besides increasing intracellular lipid bodies (as observed by Oil-Red-O staining), giving them a foamy phenotype prior to *M.tb* infection. This impairment correlated with perturbations in mitochondrial morphological (studied by fission and fusion gene expressions, confocal and TEM microscopy) and functional parameters (mitochondrial membrane potential and ATP/ADP). Treatment with small molecule M1 that restored mitochondrial structural and functional integrity increased the efficiency of *M.tb* clearance more effectively in cholesterol exposed macrophages. Mechanistically, M1 molecule enhanced clearance of mycobacteria by decreasing the total cellular lipid content, restoring mitochondrial morphology and function to its steady state. These observations were substantiated by infection assays in PBMC-derived macrophages from clinically healthy volunteers with borderline risk cholesterol profiles. To further elucidate the molecular events, we predicted possible mitochondrial targeting *M.tb* proteins. One of the proteins, Rv0547c, a probable oxidoreductase, targeted host mitochondria and impaired its function. Using various truncated mutants, we observed involvement of C-terminal in mitochondrial dysfunction, suggesting that C-terminal of Rv0547c can be a good anti-TB target. With these observations, we propose that

restoration of mitochondrial signalling through molecules like M1 can be explored further as host-directed therapy in tuberculosis, while proteins, like Rv0547c, can be new targets againt *M.tb*.

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POSTER NUMBER: 1007

Lysyl tRNA synthetase as a drug target in apicomplexan parasites

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In this poster we present a hit to lead project supported by the Structure-guided Drug Discovery Coalition (SDDC). This Consortium primarily supports structure-guided medicinal chemistry, with a focus on diseases of the developing world: tuberculosis, malaria and cryptosporidiosis. Our aim is to develop compounds to “early lead” status, with Proof-of-Concept in animal models of infection. We achieve this using structure-guided drug discovery on molecular targets in these diseases. The molecular targets selected have to be thoroughly validated; typically this is through association of a phenotypically (whole cell) active compound with a molecular target. These targets are then paired with crystal structures from a number of Structural Genomic Centers, giving rise to structure-guided hit to lead projects. At the DDU (Drug Discovery Unit) of the University of Dundee we focus on the hit discovery and hit to lead optimization of SDDC projects. Taking advantage of structural information we optimized a series of selective inhibitors *Plasmodium falciparum* (*Pf*) and *Cryptosporidum parvum* (*Cp*) lysyl t-RNA synthethase (KRS). We have identified a drug-like selective inhibitor of both *Pf* and *Cp* lysyl-tRNA synthetase capable of clearing parasites from mouse models of malaria and cryptosporidiosis infection. This provides very strong validation of lysyl-tRNA synthetase as a drug target in these organisms.

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POSTER NUMBER: 1008

MenA inhibitors are bactericidal against *Mycobacterium tuberculosis* alone and in combination with electron-transport chain inhibitors

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Mycobacterium tuberculosis is the leading cause of death by infectious disease worldwide. With the increasing incidence of antimicrobial resistance among *Mycobacterium tuberculosis* isolates, there is a need for both novel drugs and drug targets to treat this deadly pathogen. Recent work has produced several promising clinical candidates targeting components of the electron-transport chain (ETC) of *M. tuberculosis*, highlighting this pathway's potential as a viable drug target. Menaquinone is an essential component of the *M. tuberculosis* ETC, as it functions to shuttle electrons through the ETC to produce the electrochemical gradient required for ATP production for the cell. We show inhibitors of MenA, a component of the menaquinone biosynthetic pathway, are highly active against *M. tuberculosis*. MenA inhibitors are bactericidal in a concentration-dependent manner against *M. tuberculosis* under aerobic conditions. Furthermore, the inhibitors are even more potent against nutrient-starved bacteria, with bactericidal activity present at concentrations 10-fold lower than required to inhibit growth under aerobic conditions. When tested in combination, MenA inhibitors have enhanced activity in combination with inhibitors of other components of the electron-transport chain, including bedaquiline, clofazimine, and inhibitors of QcrB, a component of the cytochrome bc_1 oxidase. Together these data support MenA as a viable target for drug treatment against *M. tuberculosis*. MenA inhibitors not only kill *M. tuberculosis* under a variety of physiological states, but also have enhanced activity in combination with ETC inhibitors in various stages of the clinical trial pipeline.

POSTER NUMBER: 1009

Antimalarial pharmacology of primaquine: Attempting to solve a 70 year-old puzzle

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Primaquine (PQ) is an 8-aminoquinline class FDA-approved drug on the World Health Organization's (WHO) List of Essential Medicines and is currently the only registered drug available for radical cure of relapse malaria showing activity against *Plasmodium vivax* and *P. ovale* dormant liver stages. PQ is also active against *P. falciparum* liver stages and against the sexual gametocyte stages of *Plasmodium* species, making this drug available for prophylaxis and in transmission blocking strategies such as in elimination programmes. However, its widespread use in mass drug administration intervention is limited due to severe side effects occurring in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Despite being in use for some 70 years, PQ mechanism of action is still poorly understood. Metabolic activation has long been known to be required for PQ to gain activity, and recently CYP2D6 enzyme has been shown to be required to achieve *P. vivax* radical cure in humans. It is hypothesised CYP2D6 mediates the generation of hydroxylated metabolites, which in turn could exert the anti-parasitic effect via a redox cycling mechanism.

Here, by using a chemical biology approach we provide the first direct definitive evidence of the role of hydroxylated and quinoneimine primaquine metabolites in anti-gametocyte and liver stage inhibitory activity. Furthermore, we present biochemical evidence consistent with a mechanism of action whereby redox cycling of catalytic quantities of PQ metabolites can generate pharmacologically-relevant levels of hydrogen peroxide (H_2O_2) which lead to parasite killing. Finally, we demonstrate how redox cycling of PQ metabolites by host enzymes leads to the selective killing of malaria parasite gametocytes and liver stages.

The identification of the biochemical events responsible for the anti-parasite activity of primaquine not only answers to a long-asked question, but also opens the way to the possibility of designing new 8AQs with improved therapeutic profiles.

POSTER NUMBER: 1037

Potent *Plasmodium falciparum* gametocytocidal compounds identified by exploring the kinase inhibitor chemical space for dual active antimalarials

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Novel chemical tools to eliminate malaria should ideally target both the asexual parasites and transmissible gametocytes. Several imidazopyridazines (IMPs) and 2-aminopyridines (2-APs) have been described as potent antimalarial candidates targeting lipid kinases. However, these have not been extensively explored for stage-specific inhibition of gametocytes in *Plasmodium falciparum* parasites. Here, we provide an in-depth evaluation of the gametocytocidal activity of compounds from these chemotypes and identify novel starting points for dual acting antimalarials. We evaluated compounds against *P. falciparum* gametocytes using several assay platforms for cross-validation and stringently identified hits that were further profiled for stage-specificity, speed-of-action and *ex vivo* efficacy. Physicochemical feature extraction and chemo-genomic fingerprinting were applied to explore the kinase inhibition susceptibility profile. We identified 34 compounds with sub-micromolar activity against late stage gametocytes, validated across several assay platforms. Of these, 12 were potent at <100

nM (8 were IMP and 4 were 2-APs) and were also active against early stage gametocytes and asexual parasites, with >1000-fold selectivity towards the parasite over mammalian cells. Frontrunner compounds targeted mature gametocytes within 48 h and blocked transmission to mosquitoes. The resultant chemo-genomic fingerprint of parasites treated with the lead compounds revealed the importance of targeting kinases in asexual parasites and gametocytes. This study encompasses an in-depth evaluation of the kinase inhibitor space for gametocytocidal activity. Potent lead compounds have enticing dual activities and highlights the importance of targeting the kinase superfamily in malaria elimination strategies.

POSTER NUMBER: 1048

Integration of bioinformatics and molecular modeling approaches for the discovery of novel inhibitors against falcipains as attractive malarial drug targets

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Falcipains (FPs) of *Plasmodium falciparum* are a subgroup of papain-like Clan CA cysteine proteases. Falcipain-2 (FP-2) and falcipain-3 (FP-3) are attractive malarial drug targets as they have an essential role in hemoglobin digestion pathway, the main source of amino acids for the growth and proliferation of the parasites. To date, several inhibitors have been identified, yet none of them has been approved for malaria treatment. One of the main issues is human homologs. For selective targeting of these proteases as well as their homologs from other human infecting plasmodium species, identification of sequence and structure differences with homologous human cathepsins (Cat) is necessary. This study gathers comprehensive *in silico* approaches to tackle the problem. First, large scale sequence, motif and physicochemical properties analyses are performed to identify major differences between plasmodium and human proteins. Despite the conserved structural and catalytic mechanism of human and plasmodial proteases, significant differences between the two groups are observed. This knowledge, that may provide valuable information for the development of novel antimalarial inhibitors, is then used for different purposes:
(1) A diverse set of compounds from the literature, which have been tested for activity mainly against FP-2 and/or FP-3 via wet laboratory assays, are used to determine their mode of interaction. To our knowledge this was the first attempt to report the broad spectrum inhibitory activity of these compounds using *in silico* approaches against FPs and their plasmodial and human homologs *(Musyoka et al., J Biomol Struct Dyn. 2016)*.
(2) Considering the importance of natural products in drug discovery, this part of the study aims to identify potential hits from South African natural compounds (SANCDB; https://sancdb.rubi.ru.ac.za/) with inhibitory potency against FP-2, FP-3 and homologs from other Plasmodium species; with the selectivity towards the host homologs *(Musyoka et al., Sci Rep. 2016)*.
(3) The protein substrate processing activity of these proteases is tightly controlled via a prodomain segment covering the active site making it inaccessible. Here, the information of important residues mediating the prodomain regulatory function is used for the design of peptide based antimalarial inhibitors *(Musyoka et al., BioRxiv. 2018)*.

In all cases, selective inhibitors are identified as potential hits. These hits are currently under further study for lead design purposes.

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POSTER NUMBER: 1038

The neglected vivax malaria case: coupling target-based yeast system and *in silico* modelling for drug discovery

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Plasmodium vivax is a neglected but chief parasite causing human malaria, whose biology remains poorly understood. Currently, therapeutic options are limited and resistance emergence to all antimalarials has been reported. Therefore, exploration of *Plasmodium* spp. molecular targets for new drug discovery and development is a priority and our main goal. Thirteen *P. vivax* genes have been *in silico* selected following 3 criteria: gene orthology between *P. vivax*, *S. cerevisiae*, which act as a surrogate for expressing antiparasitic targets, *H. sapiens* for drug selectivity determination, *P. falciparum* and *P. berghei* for downstream *in vitro* and *in vivo* validation; gene essentiality in yeast for protein functional complementation on constructed strains; and gene paralogy within all 3 *Plasmodium* spp., yeast and human to avoid functional redundancy. We genetically engineered *S. cerevisiae* strains for surrogate expression of 6 chosen *P. vivax* and human molecular targets. Using protein modeling and virtual ligand screening, prospective compounds against 3 different *Plasmodium* spp. targets have been identified from synthetic libraries. Chemical screens to find antiplasmodial agents are ongoing and already allowed the identification of 5 out of 132 compounds selectively inhibiting the yeast strain expressing the *P. vivax* molecular targets. *In vitro P. falciparum* drug tests for one of these compounds showed moderate parasite inhibition. Drugs showing *in vitro*, *in vivo* and *ex vivo* promising antiparasitic properties will be chemically redesigned for improved selectivity. This Swedish-Brazilian joint research funded projected using high-throughput target-based method coupled with *in silico* target modeling and drug screening is a powerful approach to find high efficacy antiparasitic lead molecules.

POSTER NUMBER: 1010

New developments in anti-malarial target candidate and product profiles

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A decade of discovery and development of new anti-malarial medicines has led to a renewed focus on malaria elimination and eradication. Changes in the way new anti-malarial drugs are discovered and developed have led to a dramatic increase in the number and diversity of new molecules presently in pre-clinical and early clinical development. The twin challenges faced can be summarized by multi-drug resistant malaria from the Greater Mekong Sub-region, and the need to provide simplified medicines. This review lists changes in anti-malarial target candidate and target product profiles over the last 4 years. As well as new medicines to treat disease and prevent transmission, there has been increased focus on the longer term goal of finding new medicines for chemoprotection, potentially with long-acting molecules, or parenteral formulations. Other gaps in the malaria armamentarium, such as drugs to treat severe malaria and endectocides (that kill mosquitoes which feed on people who have taken the drug), are defined here. Ultimately the elimination of malaria requires medicines that are safe and well-tolerated to be used in vulnerable populations: in pregnancy, especially the first trimester, and in those suffering from malnutrition or co-infection with other pathogens. These updates reflect the maturing of an understanding of the key challenges in producing the next generation of medicines to control, eliminate and ultimately eradicate malaria.

POSTER NUMBER: 1043

Solubility driven optimization of aminoquinolines for human African trypanosomiasis

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Target class repurposing for neglected tropical diseases (NTDs) involves identification of approved drugs or clinical candidates as inhibitors of cell proliferation and their subsequent optimization as anti-parasitic agents. Such repurposing methods could reduce the overall timeline and resources required to develop clinical candidates. Lapatinib, a human EGFR inhibitor that is FDA-approved for the treatment of breast cancer, was found to inhibit

proliferation of *Trypanosoma brucei* (etiological agent of human African trypanosomiasis). A series of lapatinib-derived analogs were produced which had potent activity against *T. brucei* and were selective against HepG2 cells. However, most members of this series displayed undesirable characteristics such as poor aqueous solubility, high plasma protein binding, and high metabolic clearance. Various medicinal chemistry strategies were employed to modulate these properties and improve the physicochemical and pharmacokinetic profile of the compounds while maintaining their high anti-trypanosomal potency. This approach resulted in an advanced, drug-like lead candidate that is orally bioavailable and arrests parasite proliferation *in vivo* in a mouse model of acute human African trypanosomiasis.

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POSTER NUMBER: 1044

Tres Cantos Open Lab: Filling the Translational Gap in Neglected Diseases Drug Discovery

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The drug discovery process for the diseases of the developing world (DDW) in the pharmaceutical industry is challenging due to the large investment and low financial return. Therefore, innovative strategies are required to discover and develop new pharmaceuticals against Diseases of the Developing World.

Since 2010, GlaxoSmithKline has adopted an open innovation strategy promoting collaboration and transfer of knowledge among the scientific community, with the final aim to deliver new effective medicines for the DDW. Within our open innovation approach, the Open Lab program has been created to facilitate the translation of basic research in the DDW field into industrial scale. The Open Lab offers the GSK Tres Cantos site (Spain) to scientists from around the world to work on their own DDW projects, providing them the drug discovery expertise and state-of-the-art facilities of the pharmaceutical industry. Results are shared with the broader community to accelerate and motivate research in DDW.

Over the last seven years, the Open Lab has funded more than 72 projects and has hosted more than 85 visiting scientists from world class institutions. Focused on developing tools, exploiting targets and discovering novel molecules to tackle malaria, tuberculosis, kinetoplastid and enteric diseases, we expect the Open Lab portfolio to expand and provide a range of exciting drug discovery opportunities that could deliver new effective medicines for the least developed countries.

Opportunities in the discovery and pre-clinical space will be discussed in this communication.

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POSTER NUMBER: 1011

Oral administration of heat-killed *Mycobacterium manresensis* as a promising coadjuvant therapy for Tuberculosis treatment

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The induction of memory specific regulatory T cells (Tregs) by orally administrating heat-killed *M. manresensis* (hkMm) daily, during 14 days, might be a useful coadjuvant therapy for standard tuberculosis (TB) chemotherapy. hkMm therapy delays the progression of TB in C3HeB/FeJ mice. There is an increase in survival and an improvement in lung pathology of *M. tuberculosis* (Mtb) infected mice that received hkMm together with a combination of rifampicin, isoniazid, ethambutol and pyrazinamide (Cardona et al., 2016).
Oral administration of hkMm increases the percentage of Tregs in mesenteric lymph nodes and spleen. The *in vitro* characterization of these cells has shown they have an anti-inflammatory profile. The adoptive transfer of spleen hkMm-induced Tregs to Mtb infected C3HeB/FeJ mice, improved the infection's outcome. There was a reduction in the bacillary load, lung pathology and inflammatory cytokines. We have also seen that by blocking IL-17, IFNaRI or TNFa, there is a reduction in bacillary load and lung pathology.

hkMm therapy increases the microbiota diversity of Mtb infected C3HeB/FeJ mice, which would be a result of the protective, anti-inflammatory effect of the treatment.
NYADATREG pilot study (NCT02076139) has demonstrated an excellent safety record and detected a significant increase in Tregs. An efficacy study (NYADAGEORG, NCT02897180) is currently running. It is a randomized, double-blinded, placebo-controlled, where 3300 close contacts of active TB cases are expected to be recruited. The primary endpoint is to assess the incidence of active TB.
The administration of hkMm as a coadjuvant therapy to the standard chemotherapy TB treatment might be a safe and useful approach to reduce the length of treatment.

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POSTER NUMBER: 1012

Screening for Modulators of *ATM* Splicing Behavior

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Ataxia-telangiectasia is a rare human genetic disorder that is characterized by cancer predisposition, neurodegeneration, immunodeficiency, radiosensitivity, chromosomal instability and cell-cycle checkpoint defects. Most mutations in Ataxia-telangiectasia patients are truncating or splice-site mutations that give rise to shorter, unstable ATM proteins. In the present study we analyzed the c.1065+1G>T mutation in the ATM gene that creates a non-consensus TT donor site. As a result, 5 nucleotides of intron 8 are retained, leading to a premature termination codon, and classical Ataxia-telangiectasia phenotype. Using a luciferase reporter minigene, we screened a library of bioactive compounds (approximately 2500 compounds) to assess the possibility of restoring correct splicing in the donor splice site mutation. For the identification of these small-molecule modulators of *ATM*, we used HeLa cells expressing two types of *ATM* minigene. The mutant version of the minigene included the c.1065+1G>T mutation that was compared with a wilt-type minigene without the mutation. The mutant version had no expression of luciferase unless splicing was corrected, whereas the wild-type version constitutively expressed luciferase. Hits were selected on the basis of splicing correction above 10%, relative to the control (wild-type minigene). We hope that this approach will enable the discovery of novel therapeutic strategies that will ultimately allow the repurposing of some of the identified hits.

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POSTER NUMBER: 1013

Sorafenib suppresses TGF-β responses by inducing degradation of cell-surface type II TGF-β receptors: implications in development of effective adjunctive therapy for hepatocellular carcinoma

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Sorafenib is the only FDA approved drug for the treatment of advanced hepatocellular carcinoma (HCC) and other malignancies. Studies indicate that TGF-β signalling is associated with tumour progression in HCC. Autocrine and paracrine TGF-β promotes tumour growth and malignancy by inducing epithelial-mesenchymal transition (EMT). Sorafenib is believed to antagonize tumour progression by inhibiting TGF-β-induced EMT. It improves survival of patients but HCC later develops resistance and relapses. The underlying mechanism of resistance is unknown. Understanding of the molecular mechanism of sorafenib inhibition of TGF-β-induced signalling or responses in HCC may lead to development of adjunctive effective therapy for HCC. In this study, we demonstrate that sorafenib suppresses TGF-β responsiveness in hepatoma cells, hepatocytes, and animal liver, mainly by downregulating cell-surface type II TGF-β receptors (TβRII) localized in caveolae/lipid rafts and non-lipid raft microdomains via caveolae/lipid rafts-mediated internalization and degradation. Furthermore, sorafenib-induced downregulation and degradation of cell-surface TβRII is prevented by simultaneous treatment with a caveolae disruptor or lysosomal

inhibitors. On the other hand, sorafenib only downregulates cell-surface TβRII localized in caveolae/lipid rafts but not localized in non-lipid raft microdomins in hepatic stellate cells. These results suggest that sorafenib inhibits TGF-β signalling mainly by inducing caveolae/lipid raft-mediated internalization and degradation of cell-surface TβR-II in target cells. They may also imply that treatment with agents which promote formation of caveolae/lipid rafts, TGF-β receptor kinase inhibitors (e.g., LY2157299) or TGF-β peptide antagonists (by liver-targeting delivery) may be considered as effective adjunct therapy with sorafenib for HCC.

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POSTER NUMBER: 1045

Developing Therapeutics to Reduce *Cryptosporidium* Morbidity and Mortality Among Children in Low-Resource Settings

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Cryptosporidium is a leading cause of diarrheal disease among impoverished children in low-resource settings. There are no vaccines and only one drug (nitazoxanide) is approved by the United States Food and Drug Administration for cryptosporidiosis. Nitazoxanide has poor efficacy in malnourished children and is ineffective in severely immunocompromised patients. Therefore, there is an urgent need for new treatments to reduce the burden of diarrheal disease, and their appropriate integration with other interventions, such as oral rehydration, vaccines, and improvements in water, sanitation, and hygiene. In addition to the substantial burden of acute diarrhea, there is an emerging understanding of the contribution of *Cryptosporidium* to the chronic condition of environmental enteric dysfunction and its devastating sequelae of malnutrition, growth stunting, reduced oral vaccine effectiveness, and cognitive dysfunction. In many endemic countries, the burden due to chronic, asymptomatic infection exceeds that of acute diarrheal disease. This further underscores the need for new interventions to reduce this burden.

PATH is a global organization that works to accelerate health equity by bringing together public institutions, businesses, social enterprises, and investors to solve the world's most pressing health challenges. This poster will review the consensus target candidate profile and target product profile that have been established for new therapeutics targeting *Cryptosporidium* to ensure that they are suitable for use by populations for whom they will have the greatest impact. A screening cascade that has been established to characterize drug candidates will also be described. The current global pipeline of preclinical and clinical candidates against *Cryptosporidium* will be summarized and examples of PATH's contributions to the current portfolio of *Cryptosporidium* drug development projects will be discussed in detail.

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POSTER NUMBER: 1041

Application of metabolite-specific fluorescent protein biosensors for multiplexed high-throughput screening of compounds active against *Trypanosoma brucei* and other kinetoplastid parasites

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Trypanosoma brucei, the causative agent of human African Trypanosomiasis (HAT), survives in the mammalian host using glucose as its sole carbon source. Identifying small molecules that target glucose uptake or regulation in the parasite is challenging in a high-throughput assay format. We recently developed a high-throughput screening assay using an endogenously expressed fluorescent protein glucose sensor expressed in either the cytosol or glycosomes of *T. brucei* and monitored intracellular glucose depletion in the parasite via a flow-cytometric-based assay in the presence of 50,000 individual compounds (1). Identified hits reduced intracellular glucose concentration in parasites. Some hits we identified also have activity against *Leishmania donovani* with no toxicity in mammalian cells.

We have now expanded on our original assay format and developed a method for simultaneously monitoring multiple metabolites for drug screening. Briefly, expressed fluorescent protein biosensors that monitor key metabolites (e.g. glucose, ATP, pH) are targeted to different intracellular locations (cytosol, specific organelles) via appropriate targeting sequences. Biosensor expressing parasites are rapidly labeled with various concentrations of

two or three surface reactive dyes (cellular “barcoding”), then mixed into a single population to enable flow cytometric monitoring of multiple metabolites in different cellular locations in a single experiment. We have demonstrated observation of interrelated metabolite changes in four biosensor/location combinations in live parasites, with as many as 36 possible in a single experiment. We have screened a small library (Pathogen Box) of parasite active compounds using this cellular barcoding approach and have demonstrated that we can achieve Z' and other assay performance metrics that are competitive with single metabolite monitoring. Importantly, this new method allows a much higher density of analytes and interrelated relationships than can be monitored in a single screening campaign.

(1) Voyton, C.M., Morris, M.T., Ackroyd, P.C., Morris, J.C., Christensen, K.A,. “FRET Flow Cytometry-Based High Throughput Screening Assay To Identify Disrupters of Glucose Levels in Trypanosoma brucei”, *ACS Infectious Disease* (2018) DOI: 10.1021/acsinfecdis.8b00058

POSTER NUMBER: 1014

Redox homeostasis as a novel drug target in asexual and gametocyte stage *Plasmodium falciparum* parasites

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Chemotherapy, coupled with vector control, has remarkably reduced malaria disease mortality. However, current elimination strategies are threatened by emerging drug resistance towards currently used antimalarial therapies. Therefore, there is an urgent need for novel drug combination strategies, which not only target the proliferative asexual stages, but also the transmissible gametocyte stages of *P. falciparum* parasites for current drug resistant phenotypes.
Oxidant drugs, such as artemisinins, oxidise reduced flavin cofactors to maintain reduced glutathione levels for interception of reactive oxygen species (ROS), disrupting redox homeostasis in the parasite. Using novel 10-amino artemisinin derivatives, artemisone and artemiside, we showed nM *in vitro* activity against *P. falciparum* asexual proliferative and sexual transmissible gametocyte stages. Albeit with slower kill-kinetics against mature stage gametocytes compared to the asexual stages. Furthermore, co-treatment of these compounds with a pro-oxidant redox partner drug, methylene blue (MB), showed notable synergism particularly against mature gametocyte stages. Therefore, the induction of oxidative stress by artemisone and artemiside is sustained and even enhanced by the redox cycling action of MB specifically in mature gametocytes. Thus, we show that redox-homeostasis is essential for proliferating asexual parasites but also to maintain gametocyte viability prior to transmission, making it an ideal pan-reactive drug target.
Furthermore, through utilizing such dual-acting oxidant and redox-active partner drugs, we aim to develop a distinctive antimalarial combination strategy with the addition of a complementary third partner drug with a different mode-of-action, i.e. novel prophylactic active quinolone derivatives, that target multiple stages of the parasite's life-cycle for effective disease treatment, blocking of parasite transmission whilst limiting drug resistance formation.

POSTER NUMBER: 1015

Novel Antiplasmodial Compounds from Fungi

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In our pursuit to discover next generation of antimalarials from novel areas of chemical space, we are screening a large library of diverse fungi to discover secondary metabolites active against malaria parasite. Filamentous fungi are rich source of secondary metabolites possessing a wide range of novel pharmacophores. The Cichewicz fungal

collection contains tens of thousands of fungal isolates that were secured from diverse habitats and ecological niches across the United States. We hypothesize that fungal secondary metabolites, which are underexplored for antimalarial drug discovery, will provide us with a unique opportunity to explore medicinally relevant, but untapped chemical space for the discovery of essential malaria therapeutics. In this effort, we have screened a libraries of 750 pure compounds, and of 460 extracts obtained from diverse fungal sources for their ability to inhibit intraerythrocytic growth of chloroquine-resistant *P. falciparum* Dd2 using a SYBR Green I-based fluorescence assay. As a counter screen, we evaluated the cytotoxicity of the top hits in HepG2 cells using the MTS ((3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) cell proliferation assay. This screening has identified potent and selective novel antiplasmodial compounds, including a novel histone deacetylase inhibitor. These unique pharmacophores from wide areas of chemical space would provide chemical starting points to develop lead compounds against diverse cellular targets.

POSTER NUMBER: 1016

Antimalarial drug discovery targeting *Plasmodium* glutathione S-transferase

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Enzymes in the *Plasmodium* glutathione biosynthetic pathway are predicted as antimalarial drug targets. Glutathione S-transferase (GST) roles include detoxification of toxic compounds, reduction of hydroperoxides and mediate detoxification of ferriprotoporphyrin IX. Using reverse genetics, we showed that *Plasmodium* GST is essential for parasite survival, making this enzyme a prime candidate for screening of novel antimalarials. Based on structure-based *in silico* screening we analyzed a total of 4,900,000 small compounds for binding and potential inhibition of this enzyme. A 3D structural model of *P. berghei* GST (PbGST) was generated by comparative modeling and used for *in silico* screening of two libraries: the ChEMBL-NTD archive and the ChemBridge library. Virtual library hits were visually inspected for binding to the G and H sites. A total of 61 compounds were identified as potential PbGST inhibitors: 21 from ChEMBL-NTD and 40 from ChemBridge. One compound (CB27) displayed *in vitro* inhibition of *P. falciparum* and *P. berghei* asexual blood stages in the nanomolar range (0.9 and 0.5 μM) and four other compounds showed IC_{50} values at low micromolar concentrations (1-3 μM) in *P. berghei*. A shape similarity screening using compound CB27 as a query identified 24 additional compounds with similar shape and electrostatic properties, of which 6 showed antimalarial activity (0.6-4.9 μM). Initial toxicological evaluation of 5 novel compounds showed a lack of hemolytic activity and no cytotoxicity when assayed against mammalian lung fibroblasts. Analysis of GST inhibition using *P. berghei* protein extracts revealed concentration-dependent inhibition for some compounds. Eleven novel compounds that inhibit malaria parasite growth were identified as potential antimalarial candidates for further development.

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POSTER NUMBER: 1046

Synthetic natural products as a source of novel chemical matter for antimicrobial discovery

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*Funded by the Bill & Melinda Gates Foundation.

The rapid emergence of multi-drug resistant pathogens is a burgeoning global health crisis and novel antimicrobials will be required to combat this threat. Natural products (NPs) have been and continue to be a rich source of antimicrobial compounds. However, the challenges in identifying new sources of NPs, in de novo chemical synthesis

of complex NPs and in dereplication of previously known NPs have greatly slowed NP-based drug discovery. To circumvent these limitations, we have established a platform for production of new-to-nature NP-like chemical matter, termed synthetic natural products or SynNPs, within a genetically tractable host chassis, the budding yeast *Saccharomyces cerevisiae* (see abstract by van der Sloot et al for details). We have implemented a high-throughput metabolomics strategy to identify novel chemical matter produced by these libraries, which we demonstrate can be observed between different library clones and across different libraries. To mine the chemical diversity of our libraries, we have implemented target-based positive selection assays for modulation of protein interactions, rescue of toxicity caused by over-expression of heterologous pathogen-encoded proteins and modulation of reconstituted pathogen protease (ClpP) activity. We have adapted classic Waksman co-culture overlay assays to directly screen yeast colonies for production of antimicrobial compounds. Assay sensitivity has been improved by implementation of established and novel stress-specific luminescence assays in *Mycobacterium smegmatis*. As guided by biological assays and informed by metabolomics, we have purified and determined the structure of a number of novel compounds with antimicrobial activity.

POSTER NUMBER: 1017

Biological evaluation of novel synthetic heterocyclic compounds against resistant bacteria

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Compounds with highly selective antimicrobial activity and novel targets are required to fight back bacterial resistance. The biological activities conferred by structural modifications of synthetic heterocyclic compounds have been explored for designing novel therapeutic agents.The aim of this work was to identify leading molecules in a library of novel heterocyclics and its derivatives of easy and simple synthesis at low cost.

Methods: A total of 570 compounds including series of quinolines, chalcones, pyridines, sulfonamides, pyrazolines, prolines, isobenzofuranones and indazole-derivatives were designed and synthesized. The compounds were assessed for antibacterial activity against wild type and multidrug resistant gram-negative and gram-positive bacteria including *E. coli ATCC*25922, methicillin-susceptible *S.aureus ATCC*25923 (MSSA), methicillin-resistant *S.aureus ATCC43300* (MRSA), vancomycin-intermediate *S.aureus*(VISA), carbapenemase+ *K. pneumoniae BAA1705, K. pneumoniae ATCC700603 (ESBL+), P. aeruginosa ATCC27853* and *N. gonorrhoeae ATCC 31426* (beta lactamase+). Minimal inhibitory concentrations (MIC) were determined by broth microdilution in those compounds with reproducible antimicrobial effects in the screening. For *N.gonorrhoeae* agar microdilution was performed.

Results: The pyridine and pyrazoline derivatives were the most active compounds of the library particularly against MSSA, MRSA and *N. gonorrhoeae*: the substituted fluorophenylpyridine chalcone (MICs MRSA 2µg/mL, MSSA 16µg/mL) and the phenylpyridine chalcone (MICs MRSA 16µg/mL, MSSA 31.25µg/mL). One pirazole indolin-derivative was selectively active against VISA (MIC 3.9µg/mL) other pyrazole was active against MRSA (MIC 3.9µg/mL) and *N. gonorrhoeae* 2.9µg/mL. *E. coli* was inhibited by two pyridine derivatives (MIC 7.8-15µg/mL). Three proline derivatives inhibited *N. gonorrhoeae* growth (MIC 3.9µg/mL).

Conclusions: The compounds exhibited greater activity against *S. aureus* and little or no growth inhibition of gram-negative bacteria, none of them seemed to be active against *P. aeruginosa*.The most biologically active compounds were the 4-chloro-substituted chalcones two of them having effects on VISA. We also describe two compounds substantially active against MRSA for further study.

POSTER NUMBER: 1018

Antimalarial pantothenamide metabolites target acetyl-CoA synthesis in *Plasmodium falciparum*

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Malaria eradication is critically dependent on novel drugs that target resistant *Plasmodium* parasites and block transmission of the disease. Here we report the discovery of potent pantothenamide bioisosteres that are active against blood-stage *P. falciparum* and also block onward mosquito transmission. These compounds are resistant to degradation by serum pantetheinases, show favorable pharmacokinetic properties and clear parasites in a humanized rodent infection model. Metabolomics revealed that CoA biosynthetic enzymes convert pantothenamides into drug-conjugates that interfere with parasite acetyl-CoA anabolism. *In vitro* generated resistant parasites showed mutations in acetyl-CoA synthetase and acyl-CoA synthetase 11, confirming the key roles of these enzymes in the sensitivity to pantothenamides. These new pantothenamides provide a promising class of antimalarial drugs with a unique mode of action.

POSTER NUMBER: 1019

Rational design of a multi-target antimalarial compound with *in vivo* activity

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Malaria, particularly that caused by *P. falciparum* and *P. vivax*, remains a global health concern. Artemisinin combination therapies, the gold standard of treatment, have played a major role in reducing the malaria burden. However, parasites resistant to artemisinin treatment have emerged, and are spreading rapidly. We implemented an ambitious strategy to use rational drug discovery to develop a single compound capable of inhibiting two antimalarial drug targets, the M1 and M17 aminopeptidases, both key players in the blood stage of malaria infection. This strategy was designed to improve the efficacy of a compound by taking advantage of the synergistic effect achieved by inhibiting multiple targets within the same metabolic pathway, and additionally, to reduce the capacity of parasites to generate resistance, which occurs rapidly when parasites are treated with single-target therapeutics. We discovered potent dual inhibitors of M1 and M17 that show nanomolar *in vitro* activity against both *P. vivax* and *P. falciparum* (including drug resistant strains). Further, in mouse models, our most potent compound is effective against *P. berghei* infection after oral administration (97% reduction in parasitemia). We have therefore developed a multi-target inhibitor capable of potent activity across multiple *Plasmodium* species, which represents an exciting lead for further development into a novel antimalarial therapeutic.

POSTER NUMBER: 1020

MS-CETSA based de-orphanization of antimalarial drugs reveals Purine Nucleoside Phosphorylase as a target of Quinine and Mefloquine

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Despite decades of research, surprisingly little is known about exact mechanisms of action of the vast majority of

antimalarial drugs. Identifying cellular binding partners and downstream effectors of existing and potential therapeutics is essential for better understanding of the parasite's biology, molecular mechanisms of drug action and characterization of new druggable targets.

Here we used the cellular thermal shift assay (CETSA) that employs thermal protein denaturation to measure the change in its stability upon ligand binding in the context of the entire proteome. Initially, as a proof of principle, we confirm interaction of Pyrimethamine and E64d with their presumed molecular targets in P. falciparum: Dihydrofolate Reductase (DHFR) and 3 known cysteine proteases respectively. With CETSA optimized for malaria parasites, we have now identified Purine Nucleoside Phosphorylase (PfPNP) as a previously unknown common protein target of Mefloquine and Quinine. We validated the target for both drugs, providing in vitro enzymatic inhibition evidence, defining drug-target binding affinity and demonstrating specific site of binding through co-crystal structure analysis.

Currently, we are exploring the mode of action (MOA) of all known antimalarial drugs as well as new candidate and experimental compounds with the goal of identifying their respective molecular targets. The potential of CETSA as a novel tool for discovering antimalarial drug targets will be discussed.

POSTER NUMBER: 1021

Formulation, Evaluation and Antiplasmodial Studies of Solid Dispersions of Blends of Methacrylic Acid-Based Polymers and a Hydrophilic Carrier for Improved Delivery of Lumefantrine

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The aim of the study was to develop a solid dispersion system of lumefantrine - a poorly water soluble drug, using blends of Eudargit E100, RS100 and urea with a view to improving its oral bioavailability. Different batches of lumefantrine solid dispersions (SDs) were prepared by the solvent evaporation method using the Eudragits in the presence or absence of urea as hydrophilic carrier. The morphology, practical yield, stability, drug content, thermal and spectral properties were investigated. The *in vivo* anti-malarial study was carried out using Peter's four day curative test in mice. Results showed discrete, irregularly-shaped SDs with high yield and stable over a period of 6 months with no significant change ($p < 0.05$) in the drug content. The formulations containing urea, showed increase in their drug contents. Solid state characterizations confirmed formation of amorphous lumefantrine loaded solid dispersions in the different blends with no strong drug - polymer interaction. Reduction in parasitaemia caused by the quaternary batch of the SDs was significantly higher ($p < 0.05$) and more sustained than that achieved by the plain lumefantrine and marginally higher, albeit not to a significant extent than that observed with the commercial product - Coartem®.The SDs batch (SDA3 and SDB3) showed a parasitaemia reduction of 72.3 % and 81.3 % respectively. The study has shown that solid dispersions is a promising tool for the delivery of lumefantrine and should be further exploited for development of a more effective delivery of lumefantrine alone or in combination with artemisinin for treatment of malaria.

POSTER NUMBER: 2042

The Body Cube - A Multi-Organ Microphysiologic Device that can be Operated with Near-Physiologic Amounts of Liquid

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We present a cell culture platform that can co-culture multiple interconnected human tissues, while requiring low amounts of liquid to operate. The design is an improvement over state-of-the-art 2D systems, because we used 3D

integration to shorten chamber interconnects and reduce the liquid content in the device. We show that the co-culture of GI tract epithelium, bone marrow, kidney, and liver tissue can be achieved with low amounts of liquid without loss of viability for 72 h. The device will produce metabolite concentrations at near-physiologic levels, and it could be used to test drugs for toxic metabolites more accurately because it can.

Body-on-a-chip devices could potentially predict drug efficacy and toxicity in humans better than animals. Better predictions are needed, because currently only 10% of drugs that show high efficacy and low toxicity in animal trials repeat that effect in trials with humans.[1] The reasons for that discrepancy are not known, but could result from differences in physiology, and missing metabolic pathways. Body-on-a-chip devices mimic the human body via interconnected, co-cultured tissues and re-circulating blood surrogate. The most advanced devices contain up to 14 tissues.[2,3]

However, it is difficult to fully mimic the human body. One of the obstacles is the amount of liquid in the devices. A device that represents a 100,000th of the human body, should contain 50 µL to 60 µL of blood surrogate, and current devices use up to 50 times as much to operate. But the predictive power of body-on-a-chip devices will not be realized if they dilute toxic drugs metabolites.

We designed a multi-organ device in a cube format that allowed us to shorten the connections between organ chambers. The amount of liquid needed to operate the device is 190 µL. We demonstrate that tissues of four organs (GI tract epithelium, bone marrow, kidney, and liver) can be co-cultured in the cube for 72 h. That timeframe is not suitable for long-term testing of adverse chronic effects, but it allows us to test for efficacy and short-term toxicity under more physiologic conditions than previously published devices.

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POSTER NUMBER: 1022

Sesamum indicum leaf extract therapy for acute malaria symptoms: Evaluation of anti-inflammatory, antipyretic and analgelsic effects

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Natural products from plants are important sources of new drugs, owing to the wide chemical diversity of bioactive secondary metabolites. Some plants also have a long history of use in traditional medicine systems. As part of traditional medicine practice in some parts of Africa, an extract of *Sesamum indicum* leaves is drunk as a remedy for symptoms of malaria. This study was aimed at evaluating the effects of a standardized ethanol extract of *S. indicum* leaves on inflammation, fever and pain in mice and rats. The extract was prepared by maceration in 70%v/v ethanol; its total phenol content and HPLC profile were determined. Anti - inflammatory and antipyretic properties were evaluated using formalin - induced paw edema and Brewer's yeast - induced pyrexia models in rats, while analgesic effect was assessed using acetic acid - induced abdominal writhing and tail flick tests in mice. The extract had a total phenolic content of 0.088mg gallic acid equivalents per milligram, while its HPLC profile revealed gallic acid (7.75%), caffeic acid (6.08%), rutin (5.72%) and morin (0.98%) as some of its phenolic components. At 400 and 800 mg/kg doses, the extract reduced formalin-induced paw edema within 1 hour, by 38.89% ($P < 0.001$) and 33.33% ($P < 0.01$). It also significantly ($P<0.05$) reduced Brewer's yeast-induced fever within 2 hours and significantly ($P<0.05$) inhibited acetic acid - induced abdominal writhes by 31.49 - 41.35 %, similar to a dose of 300 mg/kg aspirin. In the tail flick test for analgesic activity against thermal nociception, the extract increased response time by 13.87 - 47.82% ($P>0.05$), compared to morphine (10 mg/kg) which significantly ($P < 0.01$) increased response time by 72.75%. These findings show that *S. indicum* leaf extract possesses anti-inflammatory, antipyretic and analgesic effects; which may be due to the presence of bioactive phenolic compounds contained therein. These activities may likely be mediated by peripheral inhibition of prostaglandins. This study supports its use as remedy for malaria symptoms and indicates its potential as a candidate for further development.

POSTER NUMBER: 1023

A 384 well plate screening platform for interrogating compound activity across the entirety of *P. berghei* liver stage development

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In the malaria elimination and eradication era, safe and mechanistically diverse antimalarial drugs for chemoprophylaxis are required. The asymptomatic liver stage of the malaria parasite lifecycle is the ideal target for chemoprophylaxis, as halting the parasite lifecycle before it reaches the blood would prevent both human disease and parasite transmission. Asexual parasite replication in the liver is not cyclic as it is in the blood, thus reducing the chance that drug resistance will emerge to liver stage-targeting drugs, but also complicating the development and interpretation of *in vitro* assays to detect liver stage actives in compound screens.

We have found that single endpoint assays detecting only liver stage parasite biomass provide an incomplete description of compound activity. Here, we report the development and implementation of a 384 well plate screening platform using the *P. berghei*-HepG2 infection model, which integrates a vital biomass readout with subsequent high content imaging of late liver stage maturation markers. We have implemented a flexible segmentation, feature extraction, and data analysis pipeline using CellProfiler and KNIME, both freely available and open-source, that allows us to take an unbiased, multiparametric approach to activity classification in a primary screen, but also generate EC50 values across any meaningful single parameters in validation studies. Using this platform, we are able to reliably detect known liver stage active antimalarials and differentiate several known MoA's, in addition to detecting novel liver stage actives with a range of phenotypic profiles.

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POSTER NUMBER: 1024

Repurposing opportunities for the treatment of bacterial enteric infections

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In 2016, 4.4 billion diarrhoea cases and 1.6 million deaths were reported, 40% of these deaths were children under the age of 5. Bacterial enteric infections account for more than 0.5 million deaths annually, >93% of these are caused by *Shigella*, *Vibrio*, *Salmonella*, *Campylobacter*, *ETEC* and *EIEC*. Ciprofloxacin has been the first line treatment for bacterial enteric infections but resistance development has rendered it inefficacious in some regions of Asia. Azythromicin is the alternative treatment but resistance to this drug already exists and is spreading. There is a clear need for alternative drugs to treat dysenteria and severe diarrhoea.

Using *Shigella* as efficacy driver, the GSK Global Health Incubator Unit in collaboration with Tres Cantos Open Lab Foundation[a] aims to develop drugs to treat moderate and severe diarrhoeal cases with bacterial etiology. Besides drug discovery of new chemical entities, repurposing strategies can allow fast delivery of treatments because safety information as well as chemical material are readily available.

This work summarizes the identification of a straightforward repurposing opportunity to treat MDR shigellosis and other bacterial enteric infections. Compound has proven efficacious *in vitro* and *in vivo* in several models of *Shigella* infection, it is safe and approved for pediatric use. Current available data could support progression to a proof of concept in patients.

[a]www.openlabfoundation.org

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POSTER NUMBER: 1025

Dissecting the pathway for rhodoquinone biosynthesis in *C.elegans* : it all starts at the beginning

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Parasitic Helminths infect around a quarter of all humans — they are one of the major human pathogens. Most parasitic helminths (PHs) undergo major shifts in their metabolism following host infection. While PHs use standard aerobic metabolism during their free-living stages, many PHs live in hypoxic conditions in their host — to survive they use unusual metabolic pathways to make ATP. In particular they rely on Rhodoquinone (RQ) as a key electron carrier. While RQ is very similar to Ubiquinone, humans do not make RQ — RQ synthesis and RQ-utilizing anaerobic pathways are thus perfect drug targets but (a) no commercial drugs exist that target RQ synthesis (b) the key enzymes required for RQ synthesis are unknown.

Here I will present three key advances we have made over the last two years in understanding RQ synthesis. This work all uses *C.elegans,* a well-studied and genetically tractable non-pathogenic nematode, to understand the basic pathways underlying RQ synthesis and as a tool for drug discovery. First, we present a powerful platform to measure movement of *C.elegans* or other worms. This provides a rapid readout of the effect of drugs on worm movement and energy metabolism and can be applied to any pathogenic helminth. Second, for the first time we describe the genetic pathway for the synthesis of RQ — this is a key step forward and identifies key potential drug targets. This work combines mass-spec metabolomics with genetic and gene expression analysis. Finally, we present the results of a first generation of drug screens to identify compounds that specifically block the ability of worms to use RQ-using pathways.

We believe that this combination of a powerful platform for drug screening with the first identification of the true pathway of RQ synthesis is a real advance in understanding how parasitic helminths survive inside their hosts and how to screen for drugs that block these helminth-specific metabolic pathways.

POSTER NUMBER: 2043

Targeting intracellular pathways to generate broad-spectrum inhibitors against intracellular toxins, viruses, bacteria and parasites

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A phenotypic screen was applied in order to identify small chemical compounds capable of rescuing cells from ricin toxin intoxication.Two molecules named Retro-2 and ABMA were found among the hits (Stechmann et al. 2010; Wu et al. 2017). Retro-2 blocks the retrograde transport of ricin toxin, preventing progression from early endosome into the trans-Golgi network. ABMA alters the endo-lysosome pathway by inducing the accumulation of late endosome/multivesicular bodies and delaying access to the lysosomes. As a consequence, each of these compounds prevent a large number of toxins and intracellular pathogens using one or the other route to enter and infect cells.
In vivo efficacy was demonstrated by us and other for the following toxins and pathogens:
- Retro-2: ricin toxin, O104:H4 Shiga toxin-producing *E. coli,* vaccinia virus, enterovirus 71 and *Leishmania amazonensis* and *L. donovani infantum*.
- ABMA: ricin toxin and Herpes simplex virus 2.
In vitro, these compounds showed activities against a greater number of pathogens according to their mechanism of cell entry. These include, for one or the other drug, lethal toxin from anthrax, *Clostridium difficile* toxin B, *Clostridium sordellii* lethal toxin, filoviruses, rabies virus, dengue-4 virus, polyoma- and papilloma viruses, chlamydiales…

Retro-2 and ABMA have been optimized by medicinal chemistry into compounds with remarkably low toxicity and improved activities in the nano molar range.
We propose the development of a set of drugs targeting intracellular pathways used by toxins and pathogens as broad-spectrum counter measures.

POSTER NUMBER: 1026

Subcellular antibiotic visualisation reveals a dynamic drug reservoir in *Mycobacterium tuberculosis*-infected macrophages

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Mycobacterium tuberculosis (Mtb) can replicate in multiple intracellular compartments, from phagosomes to autophagosomes to the cytosol[1]. The unique properties of these compartments generate a distinctive set of pharmacokinetic parameters that will modulate antibiotic efficacy. Recent evidence obtained from mass spectrometry imaging revealed the heterogeneity of drug distributions at the tissue level[2]. However little is known about the subcellular localisation of antibiotics and how sequestration and trafficking by host cells affects antibiotic efficacy.
We have developed a correlative imaging workflow incorporating live-cell confocal fluorescence microscopy, electron microscopy and secondary ion mass spectrometry imaging to map the intracellular distribution of antibiotics in human monocyte-derived macrophages infected with Mtb. Using bedaquiline (BDQ) to establish our correlative approach, we discovered that this lipophilic antibiotic accumulates within both intracellular bacteria and host lipid droplets (LD), and to a lesser extent on other organelles such as mitochondria.
By altering host lipid metabolism, we further demonstrate a dual role for LD in the intracellular pharmacokinetics of BDQ. At first, LD sequester BDQ. However, as growing bacteria consume host LD, they also take up the sequestered BDQ. Contrary to our expectations, LD levels in macrophages correlate positively with BDQ efficacy. These results challenge the belief that host sequestration of lipophilic antibiotics such as BDQ necessarily reduces their efficacy, and demonstrate a mechanism by which lipid droplets can act as nature's nanoparticles, facilitating transfer of a drug to intracellular bacteria. Given the wide variety of pathogens that interact with host lipid droplets these results have relevance to drug and therapy development that extend well beyond tuberculosis.

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POSTER NUMBER: 2049

Evaluation of Stabilized Prefusion Coronavirus Spike Trimers as Vaccine Antigens

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Coronaviruses (CoVs) thrive in animal reservoirs and represent a constant threat to human health as most recently exemplified the 2012 emergence of MERS-CoV, which is responsible for 2229 reported cases and 791 deaths worldwide. In anticipation of the next CoV outbreak, there is an imminent need for a vaccine solution. CoV spike (S) proteins mediate cellular attachment and membrane fusion and are therefore the primary target of protective antibodies. Instability and low expression of full-length CoV S proteins has historically hindered their development as vaccine antigens. Stabilizing other enveloped viral class, I fusion proteins (e.g. RSV fusion (F) glycoprotein) in the functional prefusion conformation has resulted in highly immunogenic protein subunit candidate vaccines. To that end, we sought to evaluate stabilized prefusion CoV S trimers as vaccine candidates. Using structure-guided protein engineering, stabilizing mutations were identified to maintain several CoV S proteins across genera as trimers in their prefusion conformation (pre-S). To date, we have stabilized S proteins of 5 CoVs that infect humans: MERS, SARS, HKU1, OC43, and 229E. This presentation details our efforts to characterize the immunogenicity of MERS pre-S in mice. We show pre-S elicits more robust neutralizing antibodies to multiple MERS strains than S1 monomer or wild-type versions of S trimers and protects mice from lethal challenge at low dose. Dissection of MERS pre-S immune mouse serum reveals MERS pre-S vaccination induces neutralizing antibodies to multiple domains of the

trimer including to conserved regions outside of the receptor-binding domain. Additionally, we have optimized our MERS pre-S design for mRNA vaccine delivery, which yields robust neutralizing antibody responses. Looking forward to the next CoV outbreak, we are developing antigen design and vaccination strategies to target diverse neutralization-sensitive sites on pre-S and identifying sites that can elicit broadly neutralizing antibody responses. Our findings suggest that it may be possible to identify a generalizable solution for designing vaccine antigens for newly-emerging coronaviruses.

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POSTER NUMBER: 1027

Epigallocatechin-3-gallate from dried leaves of green tea inhibited platelet adhesion and aggregation in addition to the concomitant aspirin, clopidogrel or ticagrelor treatment

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Aim: Although epigallocatechin-3-gallate (EGCG), which is found in high contents in the dried leaves of green tea, has been reported to have an anti-platelet effect, synergistic effects of EGCG in addition to current anti-platelet medications remains to be elucidated.

Methods: Blood samples were obtained from 40 participants who took aspirin (ASA, n=10), clopidogrel (CPD, n=10), ticagrelor (TCG, n=10) and no anti-platelet medication (Control, n=10). EGCG was obtained from Sigma-Aldrich, and stock solution was prepared in dimethyl sulfoxide as 10 mM concentration. Ex vivo platelet aggregation and adhesion under various stimulators were analyzed by multiple electrode aggregometry (MEA) and Impact-R systems. PAC-1 and P-selectin expressions in human platelets were analyzed by flow cytometry.

Results: In MEA analysis, ADP and TRAP-induced platelet aggregations were lower in the CPD and the TCG groups; arachidonic acid (AA)-induced platelet aggregation was lower in the ASA group; whereas, collagen (Col)-induced platelet aggregations were comparable among 4 groups. EGCG significantly reduced ADP and Col-induced platelet aggregation in dose-dependent manner (ADP, $p=0.04$; Col, $p<0.01$) but showed only marginal inhibitory effects on TRAP and AA-induced platelet aggregation in the control group (TRAP, $p=0.10$; AA, $p=0.10$). There were no additional suppressions of platelet aggregation stimulated by AA in the ASA group, and by ADP in the CPD and TCG groups. Moreover, EGCG suppressed shear stress-induced platelet adhesion assessed by Impact-R, and had no effect on P-selectin and PAC-1 expressions.

Conclusion: Ex vivo treatment of EGCG inhibited platelet adhesion and aggregation without changes in P-selectin and PAC-1 expression. There was no additional suppressions in platelet aggregation stimulated by AA in the ASA group and ADP in the CPD and TCG groups.

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POSTER NUMBER: 1028

Anti-cancer flavonoid glycosides from *Bryophyllum crenatodaigremontiana*

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Four new flavonoid glycosides, crenatosides A-D (1-4) together with five known flavonoid glycosides (5-9), were isolated from *Bryophyllum crenatodaigremontiana* (Crassulaceae). The structures of these compounds were elucidated on the basis of 1D and 2D NMR spectra analyses. Compounds 1, 2, 4, 5, 6, and 8 were tested for their inhibition of the growth of H226 (lung cancer) cell line. We found that sagittatin A (5) significantly impeded the H226 lung cancer cells proliferation in the dose- and time-dependent manners. Additionally, 5 treatment triggered apoptosis and autophagy in H226 cells. The apoptosis induction was related to loss mitochondrial membrane potential (MMP) and increase caspase-3/-8/-9 cleavage. 5 also induced autophagy as evidenced by the formation of

acidic vacuoles (AVO), up-regulation of LC3B-II and Beclin-1. These results suggest that 5 might be a potential candidate for development of antitumor drug targeting lung cancer.

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POSTER NUMBER: 1030

mCED490 and mCEC417 as clinical candidates for the treatment of onchocerciasis

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Anti-wolbachial therapy with the prototype doxycycline leads to permanent sterilization and slow killing of filarial nematodes causing lymphatic filariasis and onchocerciasis. However, 4-6 weeks of doxycycline treatment and its contraindications limit its broad use, urging research for drugs with an improved potency and safety profile. Within a collaboration between Calibr and academia, we identified two potential clinical candidates, which deplete *Wolbachia* endosymbionts of filariae.
Out of ~300k compounds two candidates were identified using a screening cascade assessing *in vitro* efficacy against *Wolbachia, ex vivo* efficacy against *Brugia pahangi,* the ADMET and PK profile and the *in vivo* efficacy against the rodent filarial nematode *Litomosoides sigmodontis*. Single oral administration (200mg/kg) of mCED490 and mCEC417 in *L. sigmodontis* infected mice achieved a 99.9% *Wolbachia* depletion in female adult worms in comparison to vehicle controls. Long term efficacy on *Wolbachia* reduction and the impact on microfilaremia was further shown in *L. sigmodontis* infected, microfilariae-positive jirds. Oral treatment for 7 (mCED490 75mg/kg BID) and 4 days (mCEC417 50mg/kg QD) reduced *Wolbachia* by >99.9% and 99.8%, respectively, and completely cleared the microfilaremia in all animals studied by 18 weeks post treatment start. In contrast, two weeks treatment with the human bioequivalent dose of doxycycline was not sufficient to maintain *Wolbachia* depletion. Analysis of the female adult worm embryogenesis further demonstrated that remaining embryonal stages of mCED490 and mCEC417 treated animals were all degenerated, whereas doxycycline treatment prevented the occurrence of later embryonic stages. First toxicological profiling showed no safety concerns of both compounds.
These results indicate that mCED490 and mCEC417 treatment provides an improved potency in clearing the *Wolbachia* endosymbions of filariae compared to doxycycline, leading to a long-term *Wolbachia* reduction (>99%) and loss of peripheral microfilaremia.

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POSTER NUMBER: 1039

Prevalence and sensitivity patterns of methicillin resistant *Staphylococcus aureus* (MRSA) from hospitals and laboratories in Nsukka Urban

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A contemporary challenge to the appropriate treatment of hospital acquired infections is the global burden and spread of Methicillin resistant *Staphlococcus aureus* (MRSA) with the adherent resistance to available antibiotics therapy. The prevalence and the antibiotic sensitivity pattern of MRSA in some hospitals and medical laboratories in Nsukka, Urban was thus studied. *S aureus was* isolated using mannitol salt agar and the resultant colonies were characterized using microscopy and standard biochemical tests. The characterized isolates were subjected to antibiotic sensitivity test using disc diffusion method. A total of 40 isolates of *S. aureus* were confirmed from 100 samples obtained from the palms and nostrils of hospitalized patients and clinical specimen at the laboratories from various body sites of infection including blood, sputum, wound, urine and others, using standard isolation methods. Out of these, 27 isolates (67.50%) were found to be Methicillin-resistant *Staphylococcus aureus* (MRSA) using the Cefoxitin disc - sensitive method.
The antibiotic resistance pattern showed the resistance to be 100% with Cefoxitin and Cefaclor, 92.59% with Cloxacillin, Cefuroxime and ceftazidine, Oxacillin 85.18%, Ampicillin 62.96%, Erythromycin 51.85%, Amoxicillin/Clavulanic acid 48.15%, Ciprofloxacin 37.03%, Amoxicillin 25.92%, Ceftraizone 25.92% and Vancomycin 11.11%. Isolated MRSA organisms were mostly sensitive to Vancomycin, Ceftriazone and Ciprofloxacin. The inhibitory zone diameter (IZD) of the selected antibiotics against 95%, 90% and 50% (IZD_{95}, IZD_{90} and the IZD_{50}) ranged from 0 - 42.1, 0 - 40 and 0 - 27 mm respectively. This study therefore confirms that MRSA continues to pose a threat to

hospitalized patients as well as patients wound sites. And resistance to available antibiotics continues to increase which ultimately increases health care expense.

Key words: Methicillin-resistant *Staphylococcus aureus,* antibiotic sensitivity, antibiotic resistance

POSTER NUMBER: 1031

Plasmodium falciparum Niemann-Pick Type C1-Related Protein is a Druggable Target Required for Parasite Membrane Homeostasis

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Plasmodium parasites possess a protein with homology to Niemann-Pick Type C1 proteins (*Plasmodium falciparum* Niemann-Pick Type C1-Related protein, PfNCR1). We isolated parasites with resistance-conferring mutations in PfNCR1 during selections with three diverse small-molecule antimalarial compounds and show that the mutations are causative for compound resistance. PfNCR1 protein knockdown results in severely attenuated growth and confers hypersensitivity to the compounds. Compound treatment or protein knockdown leads to increased sensitivity of the parasite plasma membrane (PPM) to the amphipathic glycoside saponin and engenders digestive vacuoles (DVs) that are small and malformed. Immuno-electron microscopy and split-GFP experiments localize PfNCR1 to the PPM. Our experiments show that PfNCR1 activity is critically important for the composition of the PPM and is required for DV biogenesis, suggesting PfNCR1 as a novel antimalarial drug target.

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POSTER NUMBER: 1032

Mesenchymal stem cells (MSCs) offer a protective niche to _Mycobacterium tuberculosis_ for drug-tolerance

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Latent *Mycobacterium tuberculosis* infection poses serious challenge to TB control due to its role in disease spread, drug resistance etc. Bone Marrow - mesenchymal stem cells (BM-MSC) was shown to be a site for latent *Mtb* infection. However we lack the mechanistic insights into how MSCs provide an alternative niche to *Mycobacterium tuberculosis.* In this study, we show that *Mtb* can grow with ease within adipose tissue-derived mesenchymal stem cells (ADSCs) and can tolerate anti-TB drugs like isoniazid and rifampicin much more efficeintly within these cells in comparison to when residing in the macrophages. Mechanistically, using whole transcriptome analysis and gene knockdown approach, along with FACS and confocal microscopy we show the involvement of unique innate immune signalling pathways being differentially regulated in these cells that support the bacterial survival. We verified the presence of *Mtb* bacilli in sorted mouse lung MSCs which was comparable to bacillary load in mouse alveolar macrophages. Moreover, we also observed their presence in and around granulomas in the biopsy samples from intestinal tuberculosis patients. While the fact that MSCs can be the site for mycobacterial persistence is well known, our study for the first time establishes the mechanistic details which allow MSCs to serve as such a favorable host for *Mycobacterium tuberculosis.* This study could have immense translational value, as it unravels some unprecedented aspects of bacterial life-style within mesenchymal stem cells.

POSTER NUMBER: 1033

PEGylated lipid nanocontainers improve the pharmacodynamics of artemisinin-based combination therapy in mice infected with *Plasmodium bergei*

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Given the global socio-economic impact of malaria, increasing resistance to first-line fixed-dose combination drug (artemether-lumefantrine, AL) approved by World Health Organization and the emergence of new parasites, drug formulation scientists are seeking new ways to enhance the delivery of AL, such as the use of PEGylated solid lipid nanocontainers (PEG-SLNs), a formulation technique that has shown improved drug bioavailability and patient compliance. This study explored the potential of AL-loaded PEG-SLNs as an improved oral malaria chemotherapeutics. The PEG-SLNs were prepared from tailor-made hydrolipophilic bases containing beeswax and Phospholipon® 90H with increasing amounts of PEG 4000, using high shear hot homogenization, and thereafter evaluated *in vitro* for physicochemical performance. *In vivo* antimalarial activity was done using a standard suppressive protocol in Wistar mice, while hematological and histological studies were performed on major organs implicated in malaria. The results of solid state characterizations revealed proper solubilization of AL in the hydrolipophilic bases and thus enhanced loading in the PEG-SLNs, as well as compatibility of the drugs and excipients employed in the preparation of the PEG-SLNs. The PEG-SLNs were stable, with size ranging from 57.93 to 496.3 nm with varied polydispersity indices. Furthermore, the PEG-SLNs had higher clearance of parasitemia and more sustained antimalarial activity than Coartem ® (commercial sample of AL) and chloroquine phosphate tablets with minimal effect on the hematological parameters tested, and also ameliorated the liver and kidney complications of the malariogenic mice. The study confirmed that PEG-SLNs can be pursued as a new sustained-release delivery system for improved oral malaria chemotherapy. Further studies would seek to investigate cellular drug uptake and long-term stability of the PEG-SLN.

POSTER NUMBER: 1035

Repositioning the antihistamine drug Astemizole as an anti-plasmodial agent

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Malaria caused by the *Plasmodium falciparum* parasite is a life-threatening disease. The emergence of multidrug resistant and extremely drug resistant parasites strains have reduced the efficacy of current drug regimens, posing a great challenge to malaria prevention, treatment and eradication efforts. The discovery and development of anti-malarial agents with novel modes of action and activity against resistant strains of the parasite thus remains an urgent need.

The antihistamine drug Astemizole (AST) was identified as a promising anti-malarial agent in a previous high throughput screen. However, the cardiotoxicity liability of AST would preclude its further development as an antimalarial. In this study, the repositioning of AST was investigated using a medicinal chemistry approach. A number of analogues were synthesized towards addressing the cardiotoxicity (hERG) liability and enhancing solubility while maintaining and/or improving anti-plasmodial activity. Structure activity and structure property relationship studies have revealed some structural features important for anti-plasmodial activity, solubility and inhibition of the hERG channel. These lay a foundation for an optimisation programme towards repositioning of AST as an anti-malarial agent.

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POSTER NUMBER: 1034

Target-based triage and prioritization of novel chemical entities from phenotypic screening of *Mycobacterium tuberculosis*

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Antimicrobial resistance (AMR) is a global health crisis with huge human and economic costs. Multiple-drug resistant tuberculosis (MDR-TB) is the largest contributor of AMR, with predictions that by 2050 MDR-TB will be the cause of a quarter of the 10 million deaths due to AMR. With severe attrition of candidate drugs through the discovery process, we need a much larger set of novel chemical entities (NCEs) to maintain a robust pipeline of drugs in earlier stages of development and guarantee an adequate number of new clinical candidates. We performed a high-throughput phenotypic whole-cell screen of *M. tuberculosis* against a diverse chemical library of approximately 100,000 compounds in the AbbVie collection. Here we describe the chemotypes of various hits from this screen and efforts to progress them into new leads for TB drug discovery. Limited resources force us to choose carefully which compound series to pursue in the drug discovery pipeline as early as possible. New drugs must be effective against pre-existing resistant strains of *M. tuberculosis* and have mechanisms of action and resistance that are distinct from other chemotypes in the development pipeline. We used a set of mono-resistant isolates of *M. tuberculosis* to build a panel of phenotypic assays that provide information about mechanisms of resistance. These target-based phenotypic assays, in addition to other key chemical and biological data, allowed us to prioritize compound series early in the discovery process.

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POSTER NUMBER: 2001

Effects of *Plasmodium falciparum* haemoglobinases changed by antimalarials in clinical sites and *in vitro* long-term resistance selections

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Haemoglobin digestion is an intricate process performed by the intraerythrocytic stage of the parasite *Plasmodium falciparum*. The importance of the process is evident by the redundancy and abundance of globin proteases and haemozoin synthesis factors. Recent genetic surveys revealed increased copy number of the genes encoding the haemoglobinases Plasmepsin II and Plasmepsin III. The plasmepsin gene amplifications have been linked by genome-wide association studies and in clinical trials with resistance to piperaquine, a partner drug for artemisinin, implicating a role for plasmepsin in either the mode of action of piperaquine or the resistance mechanism. However, we demonstrate that overexpression of Plasmepsin II and Plasmepsin III alone failed to change the susceptibility of *P. falciparum* to piperaquine, as well as artemisinin and chloroquine by a standard dose-response analysis and a piperaquine survival assay. Haemobloginases were also implicated as a possible target of another antimalarial compound, MMV029272. Long-term resistance selections with MMV029272 (a 1-(3,4-dichlorophenyl)-3-[5-[4-(1H-indol-3-yl) piperidin-1-yl]-2-phenylpentyl]urea) revealed multiple independent mutations in dipeptidyl aminopeptidase 1 (DPAP1, PF3D7_1116700), a globin peptidase. The drug-selected parasites showed a 4-5 fold change in IC_{50} in comparison to the parental line. Notably, all selected parasite lines also gained a copy number polymorphism of an ABC transporter, ABCI3, (PF3D7_0319700). The DPAP1 mutations were shown to confer resistance to MMV029272 by episomal overexpression of mutant forms of the gene. The effect of DPAP1 mutations on the mode of action of the MMV029272 compound will be further characterized, as will the effect on other antimalarials that target the haemoglobin-digestion process.

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POSTER NUMBER: 2002

Evaluation of efficacy of novel antimalarial combinations

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Plasmodium falciparum malaria-endemic areas present increasing symptoms of resistance to the current first line treatments, compromising artemisinin combination therapies. Therefore, new compounds with a different mechanism of action must be developed for use in combination with existing therapies to combat resistance. New compounds that undergo redox cycling have been identified, which amplifies oxidative stress levels within the erythrocyte and generates ROS when oxidized back to the original molecule. Therefore we must develop new drugs based on these compounds for use in combination with existing therapies to combat resistance. In part of a PhD project and larger MRC Ship project, the aim of this study was to investigate *in vitro* ADME, as well as *in vivo* pharmacokinetics of novel antimalarial compounds and assess candidates in new combinations. *In vitro* ADME experiments are first conducted to predict physiological characteristics of compounds. Favourable *in vitro* activity and ADME profiles encourages further examination in an *in vivo* mouse model. Mice are administered orally and or intravenously with compounds at 20 mg/kg and 5 mg/kg, respectively to determine pharmacokinetic parameters. Strong *in vitro* activity and good *in vivo* exposure are two main factors determining the compound's fate to progress as viable combination candidates with existing antimalarials (artemisone) and novel artemisinin-like compounds. The promising *in vitro* activity (IC50: 15nM in NF54) and ADME results obtained prompted further *in vivo* investigation of one compound in particular, namely PhX6, which is a derivative of Methylene blue. It presented a good bioavalability (>60%) and long half-life (8hrs) when tested in mice. This compound, among others, will be combined with artemisone, as well as novel non-DHA forming artemisinin derivatives to investigate their interaction. Optimal pharmacokinetic parameters are critical to achieve significant efficacy levels for *in vivo* parasite suppression. Therefore we selected the compounds that presented the most desirable ADME and pharmacokinetic parameters to be investigated in novel combinations. These compounds are very similar to methylene blue and literature suggests that they will have a synergistic interaction when combined with an artemisinin, as this was found to be true for methylene blue and artemisinin combinations. Compounds presenting synergy will finally be tested in a SCID mouse model to test efficacies against the true parasite target.

Funding: The project is funded by the DAAD-NRF Joint In-country Scholarship (Doctoral Scholarship to L. Laing)

POSTER NUMBER: 2003

Parasite-specific cyclic nucleotide phosphodiesterase inhibitors to target Neglected Parasitic Diseases (PDE4NPD)

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The PDE4NPD consortium brought together efforts to tackle kinetoplastid diseases (human African Trypanosomiasis (sleeping sickness), leishmaniasis, Chagas' disease) and one major helminth disease (schistosomiasis). The consortium has established a generic cyclic nucleotide phosphodiesterase (PDE) drug discovery platform to tackle these (and other) Neglected Parasitic Diseases (NPDs). The approach builds on insights and technologies that have been developed in the highly successful therapeutic targeting (e.g. Viagra®, Daxas® or Otezla®) of various members of the 11 human PDE families in the human genome. The PDE4NPD platform has cloned all genes encoding PDEs in studied (de)validated a number of parasitic PDEs as drug target via both *in vitro* and *in vivo* parasitology approaches. PDE4NPD has screened PDE-focused and fragment libraries by employing various target-centric biochemical, biophysical and pharmacological PDE studies. To support various medicinal chemistry programs PDE4NDP has invested strongly in structure-based approaches, resulting in the generation of a large number of new PDE x-ray structures, next to a fragment screening campaign using one of the optimized x-ray systems. Complementary to these molecular approaches, the consortium has also performed phenotypic screening on a large number of parasites, including the malaria parasite *P. falciparum*. The phenotypic testing of PDE4NPD compounds (> 1000) has resulted in a number of *in vivo* active compounds that are currently actively pursued for further development.
In conclusion, PDE4NPD has established a generic PDE drug development platform for tackling a wide variety of parasitic diseases and delivering PDE-based drug development candidates

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POSTER NUMBER: 2004

Studies on the secondary metabolites and their anti-dengue virus activities from native *Metarhizium anisopliae*

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Metarhizium anisopliae is a kind of entomopathogenic fungi on insect. It is distributed all over the world. The main application for the fungi is as a pest control for crops, acting as a biological pesticide. In previous studies, *Metarhizium anisopliae* was able to produce a large quantity of secondary metabolites containing anticancer and antiviral activities. In this study, *Metarhizium anisopliae* MA-126 was cultured into solid-state fermentation and liquid state fermentation. After partition extraction where both had a concentration of 50 μg/ml on the ethyl acetate layer, they had an inhibition rate of 35% and 10% for the NS2B protein. The ethyl acetate layer was purified by LH-20 column, high performance liquid chromatography (HPLC) to yield five compounds including four known compounds: Destruxin A (1)Destruxin B (2)Destruxin E chlorohydrin (3)Destruxin E diol (4), the other is a new compound MAF (5). All structures were elucidated by NMR spectra techniques, including ^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, and NOESY, with physical data (mass spectrometry, optical rotation, IR, ultraviolet spectrum), also compare with the compound data in the literature to establish.

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POSTER NUMBER: 2005

Faropenem and Clavulanate exhibit synergy against *Mycobacterium abscessus in vitro*

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Mycobacterium abscessus is an increasingly common pathogen responsible for a wide range of lung and soft tissue infections. Due to its inherent multi-drug resistant phenotype, treatment is difficult and usually involves a cocktail of antibiotics that have no proven efficacy against *M. abscessus*. Faropenem, a carbapenem, has weak activity against *M. abscessus* as it is hydrolysed by the Ambler Class A ß-lactamase present in *M. abscessus*. In this study we aimed to identify synergy between faropenem and clavulanate, a ß-lactamase inhibitor, against *M. abscessus*. Synergy was found with a fractional inhibitory concentration (FIC) of 0.08 and there was a 20-fold reduction in the MIC of faropenem.

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POSTER NUMBER: 2006

Integrating artificial intelligence (AI) methods in structural-dynamics based drug discovery workflow: case studies on amylin, protein tyrosine phosphatase 1B and Ras superfamily proteins

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Protein structures are intrinsically dynamic. The motions of their side chains, secondary structures and domains, contribute to conformational diversity. For example, we have recently observed that a specific pocket of Rab11, where small molecules (potential drug candidates) can bind to, has different sub-conformations across Rab11 multiple structures [1]. This dynamic property of proteins necessitates the analyses of sufficiently diverse structural conformations to identify potential drug binding sites.

To address this challenge, we propose a workflow involving artificial intelligence (AI) methods, to identify potential drug binding sites, particularly allosteric sites, in disease-associated proteins. The initial step considered the protein flexibility through examining its multiple high-resolution structures from a curated database and/or by generating its multiple conformations *in silico*. Next, we derived representative structures in different conformational states, for mapping binding sites.

We have been refining our workflow and testing it on amylin, protein tyrosine phosphatase 1B (PTP1B) [2] and Ras superfamily (particularly Rab [1]) proteins, to discover novel allosteric binding sites. We performed virtual screening and identified small molecules that can preferentially bind to different conformations of those sites. In our PTP1B study, we also considered drug repurposing by searching for binding sites in other proteins that are homologous to PTP1B, as their ligands may potentially bind to the novel PTP1B binding sites. This proposed workflow could lead to novel contributions in the drug discovery field.

References:
1. Kumar AP, Lukman S. Allosteric binding sites in Rab11 for potential drug candidates. PLoS One 2018; 13:e0198632
2. Kumar AP, Nguyen MN, Verma C, et al. Structural analysis of protein tyrosine phosphatase 1B reveals potentially druggable allosteric binding sites. Proteins 2018; 86:301-321

POSTER NUMBER: 2007

Profiling the *Plasmodium falciparum* resistome using *in vitro* directed evolution and chemogenomics

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The emergence of antimalarial-resistant *Plasmodium* parasites poses a significant threat to malaria eradication. Resistance strategies encoded by the malaria parasite "resistome" include mutations in multidrug resistance genes and genes of drug-targeted pathways that result in suboptimal inhibitor binding. An experimental approach that integrates *in vitro* directed evolution of resistant *P. falciparum* parasites, whole genome sequencing, and bioinformatics analysis can identify these resistance mutations and potential antimalarial drug targets. To date, analysis of *P. falciparum* strains treated with 48 different compounds has revealed 1,789 single nucleotide variants (SNVs), 1,248 insertion-deletion events (indels), and 204 copy number variants (CNVs) that developed during resistance acquisition. Approximately 1/3 of all drug resistance acquisition events observed thus far have been attributed to gene amplifications, highlighting the importance of copy number variation in antimalarial resistance. For example, selections with multiple structurally diverse compounds resulted in amplifications of the ABC transporter *pfabcI3*, suggesting a possible role in conferring multidrug resistance. Importantly, several potential drug targets appear to be promising candidates for future drug development efforts, including thymidylate synthase, farnesyltransferase, isoleucyl-tRNA-synthetase, acetate CoA ligase and dipeptidyl peptidase. Compounds are continually added to this experimental pipeline as phenotypic screening efforts yield novel chemical scaffolds with demonstrated antimalarial activity. While further investigation is required to define the clinical relevance of genes identified using this strategy, this ongoing systematic characterization of the resistome will enhance understanding of resistance mechanisms, inform future drug design, and advance the malaria elimination campaign.

POSTER NUMBER: 2047

Promising malaria transmission blocking activity of Malaria Box hit 1,2,3,4-tetrahydroisoquinoline-4-carboxamide derivatives against all asexual and sexual stages of *Plasmodium falciparum*

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Identification of new antimalarial compounds is an everlasting assignment to researchers to tackle *Plasmodium* parasites, tirelessly evolving resistant strains and thus keeping on knocking out the drugs in use. Transmission blocking components (TBC) are a potentially strong weapon to decrease intensity of malaria transmission, thus slowing down the diffusion of resistant strains and supporting elimination of the disease. Most research on TBC is focused on gametocytocidals, however early sporogonic stages, developing in the mosquito during the first 48 hours after feeding, should not be neglected as a target, considering that molecules interfering with the development of these stages may contribute to the overall transmission blocking efficacy of a combination treatment. In order to increase the chance of success for identifying compounds acting on gametocytes and/or early sporogonic stages, we selected compounds from Medicines for Malaria Venture (MMV) malaria box, all with confirmed *in-vitro* activity against asexual blood stages of *P. falciparum*. The compounds MMV 006427; 085583; 000642; 665876; 006429; 665827; 000662; 019738; 006767 and 019266 were selected and screened for *in-vitro* activity against *P. falciparum* asexual stages and mature gametocytes using a luciferase assay (3D7elo1-pfs16-CBG99 strain) and against early sporogonic stages employing the *P. berghei* ookinete development assay (Pb.CTRP.GFP strain). Of these, 3 showed high antiplasmodial and gametocytocidal activity with a 50% effective concentration (EC_{50}) value below 5 µM, namely, MMV000662 (EC_{50}= 3.57 µM), MMV006429 (EC_{50} = 4.42 µM), and MMV000642 (EC_{50} = 3.43 µM). The same 3 molecules were the most active in the ookinete development assay, counts at the fluorescent microscope revealed 50% inhibitory concentration (IC_{50}) values between 20 and 40 µM. Interestingly, at 50 µM evident morphological alterations were noted and fully developed, elongated ookinetes could not be detected. These findings clearly demonstrate the existence of chance factor for the development of transmission-blocking drugs that can eliminate *Plasmodium* parasites from a vertebrate population and have significant implications for the future design and implementation of transmission-blocking interventions within the field. Detailed findings will be presented.

Funding: This study was funded by MMV through the Malaria Challenge Grant.

POSTER NUMBER: 2009

Development and validation of an *Onchocerca ochengi* adult male worm in gerbil model for macrofilaricide development

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Background: Onchocerciasis currently afflicts an estimated 37 million people and is the second leading infectious cause of blindness world-wide. The development of a macrofilaricide to cure the disease has been hindered by the lack of appropriate small laboratory animal models. This study was therefore, aimed at developing and validating an *O. ochengi* (the closest in phylogeny to *O. volvulus*) adult male worm model in the Mongolian gerbils.

Methodology/ Principal Findings: Mongolian gerbils (*Meriones unguiculatus*) were each implanted with 20 *O. ochengi* male worms from cattle in the peritoneum, followed by drug and placebo treatments starting three days after. The implanted worms were recovered from the animals and analyzed for viability. In control animals, the recovery of the worms was averagely 35 %, with 89 % of these being 100 % motile. Treatment of the infected animals with flubendazole (FBZ) significantly reduced worm burden to 6.0 % versus 27.8 % in controls, and worm motility to 0.0 % in treated group versus 91.1 % in controls. Using this model, we tested a related drug, oxfendazole (OFZ) and found it significantly reduced worm burden to 20.3 % in the treated group versus 43.0 % in the control group, and worm motility to 22.7 % in the treated group versus 95.0 % in the controls.
Conclusions/Significance**:** We have developed and validated a gerbil *O. ochengi* adult male worm model for testing new macrofilaricides *in vivo*, and used it to determine the efficacy of oxfendazole *in vivo*.

Keywords: onchocerciasis, gerbil, macrofilaricide, male worms

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POSTER NUMBER: 2018

Therapeutic RNA Aptamer Inhibition by Antibodies

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Despite the advances in biomedical research there remains a disconnect between translating new drugs from the bench to the bedside. Though biopharmaceuticals offer advantages to small molecules, they often require modifications to achieve therapeutic efficacy. The most popular approach involves the conjugation of polyethylene glycol (PEG), which until recently, was considered a "non-fouling" chemical moiety. Our laboratory explores the use of RNA aptamers to control coagulation in surgical settings. Recently, a PEGylated RNA aptamer designed in our laboratory was tested in clinical trials with preliminary success. However, administration of the PEGylated RNA aptamer, Pegnivacogen, elicited severe allergic reactions causing early termination of the Phase III trial. In contrast to the general assumption that PEG is non-immunogenic, animal studies clearly demonstrate that PEGylated compounds can evoke the development of anti-PEG antibodies. The ubiquity of PEG in medical and commercial products and the severe adverse events observed in the aptamer clinical trial prompted us to investigate whether anti-PEG antibodies can affect the anticoagulant activity of a PEGylated aptamer. *Here, we demonstrate that anti-PEG antibodies directly bind to and inhibit aptamer function both in vitro and in vivo. Addition of anti-PEG IgGs decrease aptamer-mediated clot time extension in an aPTT and reduced antithrombotic activity in a murine model of thrombosis. In addition, we detected the presence of anti-PEG IgGs in nonhuman primates after only a single injection of PEGylated aptamer.* To our knowledge, this is the first study to address aptamer inhibition by anti-PEG antibodies. These findings inform drug development as a whole by offering insights into the PEG-hypersensitivity phenomenon in the hopes of bridging the gap between drug design and drug efficacy.

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POSTER NUMBER: 2035

Organelle dynamics and proteomic profiling of *Plasmodium falciparum* under different stress conditions

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Mitochondria and Endoplasmic Reticulum are two key organelles of a eukaryotic cell. In *Plasmodium falciparum*, ER is involved in folding and translocation of nascent proteins along with maintaining cellular Ca^{2+} homeostasis. Similarly, in addition to housekeeping function of mitochondrion, it also plays crucial parasite specific role in *Plasmodium falciparum* such as heme biosynthesis. Because of their key roles in maintaining cellular homeostasis and involvement in parasite specific processes these two organelles have been suggested as potential targets for development of novel anti-malarials. However, cascades which follow the disruption of these organellar functions are poorly understood. In this study, we followed the sequence of events which occur in *Plasmodium falciparum* upon induction of organelle specific stresses. For better understanding, we also observed the changes in organelle dynamics as well as in the proteomic profile of the parasite, with a view to identify the major pathways involved. Our study demonstrates that induction of cellular stress leads to disruption of asexual cell cycle of the parasite; the treated parasites were found unable to grow beyond trophozoite stage. Further, both ER and mitochondrial stresses have shown to induce a cascade of reactions in the parasite leading to apoptosis-like cell death. However, in both the cases when stress stimulus is withdrawn before a specific time period of stress, a majority of parasites tend to recover and progress to subsequent cell cycle whereas withdrawal of the stimulus after this time period fail to recover the parasite growth and it undergoes cell death. This indicates a specific time period during the stress where parasite gets committed to cell death. Next, in order to decipher the possible mechanisms regulating this commitment of parasite towards cell death, we, by employing proteomics approach, are currently investigating the associated proteomic changes in parasite before as well as after parasitic commitment towards cell death. Identifying and validating these mechanisms could be a source for development of new anti-malarial drugs.

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POSTER NUMBER: 2048

Targeting protein kinases for the development of novel drugs for trematode infections

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There are no effective vaccines against the economically important global human and animal pathogenic trematodes *Schistosoma mansoni*, *S. japonicum*, *S. haematobium*, *Fasciola hepatica*, *Clonorchis sinensis* and *Opisthorchis viverrini* are. Indeed, current treatment and control of these trematode species relies mainly on chemotherapy with praziquantel and triclabendazole. However, the increasing development of parasite resistance to these drugs makes the search for novel drugs a compelling priority.

Protein kinases which regulate a myriad of vital cellular processes such as metabolism, cell cycle, development and apoptosis are highly attractive targets for drug discovery. Indeed, several kinase inhibitors have already been approved for clinical use. Moreover, some approved kinase inhibitors have been shown to possess microfilaricidal activity illustrating the potential of repurposing this class of drugs for the management of trematode infections. The inhibition of unique essential trematode kinases therefore is a potentially viable approach for the development of novel drugs for trematodes. The proposed study aims at deciphering druggable conserved essential trematode protein kinases and evaluating their inhibition by a panel of approved kinase inhibitors and novel chemicals as a strategy for the identification of novel leads for the development of new drugs for trematode infections. The validity of this approach will be confirmed by carrying out an *in vitro* phenotypic assay.

Methods:
Computational biology approaches will be employed to identify the full complement of protein kinases in *C. sinensis, F. hepatica, O. viverrini, S. haematobium*, *S. japonicum and S. mansoni*. Subsequently, phylogenetic analysis and clustering techniques will be applied on the identified kinases so as to identify the conserved trematode kinases. These will then be mapped onto pathways to ascertain their essentiality. Kinases identified as essential will then be cloned, expressed and probed with a panel of approved kinase inhibitors together as well as novel small chemicals obtained from DrugBank. Chemicals with inhibitory activity will be further evaluated in an *in vitro* phenotypic screen at the various developmental stages of the trematodes.

POSTER NUMBER: 2010

Evaluation of Rubella Antibodies among Pregnant Women Rivers State and Abia State, Nigeria

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In a hospital-based cross-section survey, one hundred and eighty (180) pregnant women attending clinics in Braithwaite Memorial Specialist Hospital Rivers State (BMSH) and Abia State University Teaching Hospital Aba (ABSUTH) Nigeria were examined randomly to evaluate the prevalence of rubella virus antibodies. Enzymes linked immune-absorbent assay (ELISA) was used to detect the Rubella virus antibody (IgG and IgM) in the samples obtained. Prevalence of Rubella virus antibodies was determined using the following socio-demographic factors such as Age, Marital status, Educational status, Occupation, Residence (urban and rural), History of Vaccination and Gestational period. Out of 180 pregnant women assessed, 155 (86.1%) tested positive for Rubella virus IgG antibodies while 14 (7.8%) were positive for Rubella virus IgM antibodies. Ninety (90) serum samples examined from BMSH, IgG 71(78.9%) and IgM 5(5.55%) and ninety (90) serum samples from ABSUTH recorded IgG 84 (93.3%) and IgM 9 (10.0%) respectively. The highest seropositivity test results for IgG and IgM antibodies in this study population was found among pregnant women with age groups of 39 - 43years and 44 - 48years. While age groups between 19 - 23yers (71.4 % and 0 %) had the lowest prevalence of rubella IgG and IgM antibodies respectively. Pregnant women in their third trimester showed IgG (94.7 %) and IgM (15.8 %). Pregnant women of the secondary educational level were observed to have the highest prevalence for IgG (93.4 %) while the highest prevalence for IgM (22.2%) antibodies was found among the primary level of education. pregnant women that were traders had a prevalence of rubella IgG and IgM antibodies of (92.6 % and 9.8%) respectively. Married pregnant women were found to have the highest seropositivity for rubella IgG and IgM antibodies. A high seropositivity for rubella IgG and IgM antibodies was observed among pregnant women from an urban area (87.7 % and 8.3%) compared with rural pregnant women (81 % and 4 %) respectively. Vaccinated pregnant women were found to have the highest prevalence for rubella IgG and

IgM antibodies (87.4 % and 8.4 %) respectively. There is a significant risk of acquiring major or recurrent rubella virus illness in pregnancy; it is hence suggested that rubella virus serological examination of women should be added as a routine screening during pregnancy in the study areas. This will help to discover those at the threat of contracting the infection and strategies on how to prevent the infection and to protect the foetus.

Keywords: Evaluation, Rubella IgG, Rubella IgM, Antibodies, Pregnant Women, Nigeria

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POSTER NUMBER: 2037

Using a drug-sensitive yeast strain as a model system to identify targets of antiparasitic and antineoplastic compounds through *in vitro* evolution and whole genome sequencing

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Identifying the cellular targets and mechanisms of action (MOA) of active small molecules discovered through phenotypic screens is an important and challenging task in drug discovery. Chemogenomic profiling in yeast can reveal compound-target interactions and resistance mechanisms. A very effective strategy to directly identify the target of a small molecule is through *in vitro* drug-resistance evolution and whole genome analysis (IVIEWGA). The mutated amino acid residues also offer insight into the mechanism of drug binding and enzymatic activity. We have successfully used this technique to delineate drug-target interactions and drug resistance in Plasmodium and in model organisms such as yeast.
Here we report on an extensive chemogenetic characterization of the yeast resistome through IVIEWGA. We identify genes/pathways targeted by therapeutic drugs or small molecules with unknown MOA and elucidate their resistance mechanisms. We determined drug susceptibility to over 1000 small molecules and performed IVIEWGA experiments for >100 compounds. We describe the targets of novel antiparasitic compounds in light of the corresponding genetic mutations. Our work also allows us to identify genes involved in multidrug resistance. In addition, the analysis of resistance-conferring amino-acid changes and the resulting predicted changes to protein structures provide insight into compound-protein interactions at amino-acid resolution. We show that targets are conserved across species and demonstrate that *S. cerevisiae* is a tractable model for drug target discovery. Our comparative-genomics approach expands on the limited number of tools available for analyzing compound-target interactions and can be applied to studies of other eukaryotic antimicrobials and chemotherapeutics.

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POSTER NUMBER: 2011

Assessment of artemisinin-induced ring stage dormancy in *Plasmodium falciparum*

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Emergence of artemisinin (ART) resistant *P. falciparum* threatens the existing methods to control malaria, especially in SE Asia. Recently, polymorphisms in the Kelch “K13-propeller” protein were associated with reduced susceptibility to ART drugs; however, the mechanism of drug resistance is not fully understood. Aside from K13 mutation-associated resistance, there are other factors responsible for treatment failure. ART has the unique ability to cause growth arrest in the ring stage of *P. falciparum*. This dormant stage has various physiological differences from normal rings and is characterized morphologically in both sensitive and resistant parasites by condensed chromatin and reduced cytoplasm following treatment with 700nM of DHA. Remarkably, ART is a unique compound that causes dormancy upon drug exposure in the first asexual cycle. After drug removal, the parasites recover and continue normal growth, potentially causing treatment failure marked by recrudescence in patients. Here we address the dormancy phenotypes by assessing a qualitative and quantitative method to describe arrested development. By using the ImageStream X Mark II Imaging Flow Cytometer, we analyze dormancy in SHAM-treated and ART-treated parasites by observing and quantifying the ratio of parasites that enter dormancy and eventually recover. We used D10 parasites that express GFP in the apicoplast (D10 ACP_{LEADER}-GFP) and CellTrace Violet (CTV) cell proliferation dye that stains the cytoplasm in order to discriminate between parasite multiplication and growth.

Also, we use the hemozoin content, which is the parasite insoluble crystalline form of heme, as a stain-free parameter to monitor parasite growth. As a proof of concept, we have verified the growth of parasites stained with CTV without any toxicity for up to 8 generations, allowing us to identify and discriminate between dormant rings, rings, trophozoites, and schizonts. This methodology will enhance the quantitative and temporal assessment of dormancy and survival rates by using new imaging-based technology. Our studies will provide insights in the phenotypes associated with ART resistance and drug discovery.

POSTER NUMBER: 2012

Malaria therapeutic drug-design: what is the risk of cross-resistance between novel endoperoxide-based compounds and artemisinin?

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The decline in the efficacy of Artemisinin-based Combination Therapies (ACT) for the treatment of *Plasmodium falciparum* malaria and the spread of artemisinin resistance across South-East Asia highlight the need for the assessment of the risk of cross-resistance between artemisinin and novel antimalarial drug-candidates. On the one hand several antimalarial drugs with an endoperoxide function, like in artemisinin and its derivatives, are in advanced research or development. On the other hand, in Asia the prevalence of isolates harboring K13 non synonymous polymorphisms linked to artemisinin resistance is highly and constantly increasing. Considering the mode of action of artemisinins based on Radical Oxygen Species (ROS) generation by homolytic O-O bond cleavage of the endoperoxide, whether a possible cross-resistance between artemisinins and novel compounds with also an endoperoxide-based mode of action was investigated.

To address this issue, several hybrid molecules containing an endoperoxide moiety, including current combinations used in the field, were studied. The efficacy of these molecules has been studied on artemisinin-resistant laboratory strains and/or on clinical isolates from Southeast Asia. Specific chemosensitivity assays revealed a strong cross-resistance with artemisinin for some of these hybrid molecules. Structure-activity relationships demonstrated that the ability of hybrid chemical structures to avoid artemisinin resistance, could depend on the pharmacophore associated to the endoperoxide part. The phenotypic and genotypic impacts of drug pressure with one of these endoperoxide-based molecules evidenced a worrisome selection of parasites harboring K13 mutation.

These results underline the risk of cross-resistance between artemisinins and endoperoxide-based compounds and advocate for considering this aspect during antimalarial drug development.

POSTER NUMBER: 2013

Identification of novel anti-tubercular agents using multiple approaches

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Mycobacterium tuberculosis causes over 1 million deaths every year. There is an urgent need for new drugs, which is hampered by our lack of knowledge of vulnerable pathways/enzymes with potential to be drug targets. We have taken a number of approaches to identify novel target and novel anti-tubercular series. These include simple growth assays under standard conditions, using variation in media components and growth conditions, use of non-replicating conditions, and more complex assays using hypomorphs, recombinant strains, and intracellular assays, high content screening, and combination assays. We have identified a number of series with promise for development, as well as several promiscuous targets, and a small number of novel drug targets. In particular, we have discovered the target of the aminothaizoles as a key enzyme of glycolysis.

POSTER NUMBER: 2014

Corallopyronin A - an antibiotic active against helminths, STIs and Staphylococci

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Corallopyronin A (CorA) inhibits bacterial DNA-dependent RNA polymerase, sterically blocking the switch region, not the active site. Thus, it kills rifampicin-resistant *Staphylococcus aureus*. We have shown *in vitro* and *in vivo* CorA activity against Gram-negative *Wolbachia* endosymbionts, targets for controlling filarial infections causing lymphatic filariasis and onchocerciasis. Antiwolbachial therapy results in worm sterility and slow killing of worms. Importantly, CorA also targets *Rickettsia* spp., *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and multi-resistant *S. aureus* (MIC 0.0625-1 µg/mL; nM range). The latter three are WHO Sustainable Development Goals and Priority Pathogen List targets requiring new antibiotics. PK/PD and ADME results have not thrown up red flags to halt development. CorA is better than the comparator rifampicin, having activity against multi-resistant *S. aureus* with no induction of drug-drug interactions that could negatively affect CorA administration with other treatments, e.g. HIV drugs, and not drive rifampicin-resistant tuberculosis. It is lipophilic and stable in liver microsomes from dogs and humans. We have overcome a crucial hurdle frequently encountered with natural products, cost-efficient production, for which we have developed heterologous production in *Myxococcus xanthus* and a downstream process yielding CorA at >95% purity, confirmed by quantitative NMR and HPLC. CorA, 90% purity, has lowered the effective regimen to the TPP recommended 7-14 days and is the only antiwolbachial drug showing consistent reduction of adult worms in animal models used by the MacDA and DND*i* consortia for pre-clinical development of macrofilaricides. The final production protocol will provide CorA at >90% purity for non-GLP pre-clinical studies, genotoxicity, hERG and non-GLP toxicity tests in 2018 and 2019. We are engaged with the competent German regulatory authority for advice on CorA specification for GMP production by a CMO in 2019 to conduct GLP-tox studies and a phase 1 trial.

POSTER NUMBER: 2015

Discovery of FUMR as the death receptor in sepsis

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Sepsis is the 10th leading cause of death with an estimated fatality of more than 6 million per annum world-wide. Sepsis causes more deaths than breast, prostate and colo-rectal cancers and HIV/AIDS combined. Ageing population and the emergence of antibiotic resistant bacteria are the two most important compounding factors. There is no effective treatment for sepsis with more than 100 drug trials (mostly anti-inflammatory) failing in the last 25 years. Immune paralysis, a consequence of apoptosis, is the major reason for most fatalities associated with sepsis (which accounts for more than 80% of fatalities). Through a genome-wide CRISPR screen, we identified the receptor that causes immune cell death during sepsis. Genetic ablation of this receptor in mice offers almost absolute protection from polymicrobial inflammatory sepsis as well as from the secondary pneumonia. We also have identified the human equivalent of this receptor. Developing blocking antibodies to this receptor will lead to an effective treatment for sepsis.

POSTER NUMBER: 2038

New antitubercular compounds inspired on isoniazid

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A library of N-substituted tosyl N´-acryl-hydrazones was prepared with a novel and efficient one-pot aza-Michael reaction. Starting from commercial aldehydes, including aliphatic and isoprenyl chain, aromatic and carbocyclic to cover a broad spectrum of steric demand, thirty compounds were prepared. The collection was tested against Mycobacterium tuberculosis H37Rv strain with nine analogs displaying MIC below 10 μM. The most active members have a MIC of 1.25 μM, being exclusively *E* isomers. Also, we performed the chemoinformatics analysis of these libraries using Molinspiration and Osiris to calculate the physicochemical properties and possible cytotoxicity risk. To validate the action mechanism the acrylates the activity of the most active compounds we compared with an InhA overexpressing strain. The assayed reveled that all the analogs evaluated doubled the MIC obtained on the wild strain. Those results suggested that the enoyl CoA reductase could be the actual target of the new isoniazid mimics, validating its mechanism of action. A molecular docking was performed to study the ligand-protein interaction. This new acrylates are promising starting point to develop new antitubercular drugs.

POSTER NUMBER: 2016

Discovery and development of trophocidal and cysticidal compounds for the treatment of *Acanthamoeba* infections

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The pathogenic free-living amoeba, *Acanthamoeba*, cause several diseases including a severe brain disease known as Granulomatous Amoebic Encephalitis (GAE), with a 90% mortality rate. *Acanthamoeba* has also been found to cause Amoebic Keratitis (AK), in association with poor contact lens hygiene, which may result in blindness. The current drug regimens were not originally discovered for *Acanthamoeba* infections but have shown *in vitro* and *in vivo* potency against these organisms. The high mortality shows that these regimen(s) are not the best indicative treatment for the patients. New specifically developed drugs for these diseases need to be discovered, developed and implemented into the current drug regimen(s) to give patients a better chance of survival or loss of eyes. Herein, using high-throughput screening methods we screened several large compound libraries from Calibr (11,968 compound ReFrame library), an FDA approved drug library (1,134 drug library - Dr. Kiplin Guy formerly at St. Jude Children's Research Hospital) and Medicines for Malaria Ventures (MMV Malaria Box (400 compounds) and Pathogen Box (400 compounds)) in search for new active chemical scaffolds. In our search we identified 67 compounds to possess sub micro to nanomolar potency against *Acanthamoeba* trophozoites, confirmed through dose response secondary screening. Fifty two of these compounds are newly described to have any activity against these amoebae, 15 compounds have been previously recorded within the literature and/or currently used therapeutics. Nanomolar potency compounds were tested for cysticidal activity in a newly developed 30 day high-throughput cysticidal screening method developed in our lab. Through collaborations with Dr. David Boykin at Georgia State University, using bis-benzimidazole amidine and diamidine scaffolds through a structure activity relationship (SAR) study we discovered several compounds that possess anti-trophozoite activity. Only the nanomolar inhibitors were further tested for cysticidal activity. Forty two compounds mainly from the SAR studies were discovered to have cysticidal or cystistatic activity against three clinical isolates of *Acanthamoeba*.

POSTER NUMBER: 2017

Compounds with potential activity against *Mycobacterium tuberculosis*

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Background: Mycothiol, ergothioneine and gamma-glutamylcysteine are low molecular weight thiols that are synthesized by *M. tuberculosis*. Thiols are molecule with a functional sulfhydryl group, generally known for their ability to protect against oxidative stress. In this study, we sought to investigate compounds that could be combined with potential thiol biosynthesis inhibitors to achieve effective tuberculosis treatment.

Approach: Libraries of off-patent compounds and natural compounds were screened against thiol-deficient *M. smegmatis* mutants. Few of the compounds that selectively inhibited these mutants were further expounded in *M.tuberculosis*, namely azaguanine (Aza), sulfaguanidine (Su), bacitracin (Ba) and fusaric acid (Fu). Their mode of action was investigated.

Results and discussions: The compound Ba was able to inhibit the growth of strains that were unable to excrete thiols and/or have an altered membrane lipid profile. Further investigations revealed that extracellular thiols were more likely to be involved in the detoxification of Ba and the efficiency of Ba is greatly influenced by its permeability through the thick lipid layer of mycobacteria membrane. Su was found to inhibit folate biosynthesis and consequently induced the production of oxidative stress, which consequently affected the mutants that were thiol-deficient. Fu inhibited the production of thiols, consequently affecting mutants that were already deficient in a thiol or two. Aza inhibited the growth of the MSH-deficient mutant. Aza seemed to affect the biosynthesis of ergothioneine, consequently inhibiting the MSH-deficient mutant that relies mainly on ergothioneine biosynthesis for detoxifications.

Conclusion: In this study, we proposed and provided a rationale for the investigation of the suitability of a few compounds in combination with inhibitors of thiols production in tuberculosis regimen.

POSTER NUMBER: 2039

Structural studies of human antibody responses against leading malaria vaccine antigen PfCSP

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Malaria is a complex, mosquito-borne disease estimated to cause over 300,000 childhood deaths annually. Plasmodium falciparum (Pf), the major parasite causative of malaria, accounts for approximately 88% of malaria mortality, and demonstrates considerable resistance to current drug therapies. It is therefore imperative to develop a vaccine to Pf to reduce malaria morbidity and mortality. Reverse vaccinology holds promise to design effective immunogens for the development of malaria vaccines. This concept is based on interrogating the B cell repertoire of infected or vaccinated subjects to identify inhibiting antibodies that will guide immunogen design. The circumsporozoite protein (CSP) is the major surface antigen of Pf sporozoites and a leading malaria vaccine antigen. Here, we structurally and functionally characterized protective and non-protective antibodies to PfCSP from four healthy adults living in the malaria-endemic area of Lambaréné, Gabon, and from eight vaccinated European donors who underwent immunizations with aseptic, purified, cryopreserved Pf sporozoites (PfSPZ Challenge) under chloroquine prophylaxis (PfSPZ-CVac), which resulted in protection against controlled human malaria infection. Our structural delineation of protective and non-protective epitopes highlights key differences of B cell responses during

natural exposure and vaccination. In addition, we provide the molecular mechanism underlying clonal selection and affinity maturation of human B cells expressing protective antibodies. Collectively, this data provides the blueprints to engineer optimized antigens that can be tested as pre-erythrocytic subunit vaccines.

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POSTER NUMBER: 2019

Self-emulsifying delivery system for DHA oil: A pre-emulsification approach can be used for improving the bioavailability for various clinical uses

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Oral delivery of the lipophilic compound suffers from the limitation of their effective drug delivery and manifestation of their potential therapeutics. Solublizing the hydrophobic's in (gastro-intestinal tract) GIT medium is required in order to increase the permeation, thus absorption from GIT. Docosahexaenoic acid (DHA) is an omega-3-fatty acid, an essential structural component of the cell membrane of neuronal tissues. Thus, in addition to its role in neurodegenerative disorder, It has anti-inflammatory and antiviral properties by virtue of its metabolite Protectin D1 and can be used to potentiate antiviral therapy. But due to liquid and hydrophobic nature, it is highly vulnerable to oxidation and has very low aqueous solubility. Thus, developing a delivery system which can increase its oxidative stability, dissolution profile, shelf life and permeation through GIT, is a needful task for developing a pharmaceutically acceptable formulation to manage various clinical conditions.
Self nano emulsifying drug delivery (SNEEDS) was used as a carrier for increasing the aqueous solubility of DHA oil and developing a stable formulation. It is a preconcentrate of DHA oil along with surfactant and co surfactant which upon agitation with GIT fluid form nanoemulsion. Further the prepared liquid preconcentrate was spray dried using a hydrophilic carrier in order to increase its shelf life and oxidative stability. The prepared powdered formulation was characterized for *in vitro* parameters viz. flow properties, shelf life stability, peroxides formations and self-emulsification time. The toxicity profile and permeability studies of the developed formulation were accessed by *ex-vivo* Caco 2 cell line and intestinal permeability studies.
The prepared solid SNEDDS were of spherical shape and had good flow properties. Reduced peroxide formation (15.2 meq/kg) was observed in comparison to liquid SNEDDS (24.8 meq/kg) after 3 months of stability studies. Nanoemulsion formed was found to be stable in 0.1 N HCL and water with no change in morphology was observed in TEM imaging. Also, no chemical interaction was observed with the used carrier in ATR analysis. Ex vivo studies depicted nontoxic nature of the prepared delivery system with no change in TEER value and paracellular permeability and have > 90% cell viability, also higher concentration of DHA was permeated to Caco2 cell from the prepared formulation in comparison to DHA oil.
Thus, developed delivery system improved the oxidative stability, bioavailability and shelf life of the DHA oil. As this approach mimics the natural emulsification process of oil absorption, it acts as a promising and cost-effective pharmaceutical approach in order to improve therapeutics in neurodegenerative disorder and antiviral therapy.

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POSTER NUMBER: 2020

Evaluation of the Pharmacokinetic-Pharmacodynamic Relationship of Praziquantel in the *Schistosoma mansoni* Mouse Model

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After more than 40 years of use, Praziquantel (PZQ) still remains the drug of choice for the treatment of intestinal and urogenital schistosomiasis. Its anti-parasitic activity resides primarily in the (*R*)-enantiomer. Hitherto neither the molecular target nor the pharmacokinetic-pharmacodynamic relationship have been fully elucidated. Here we investigated the efficacy and pharmacokinetics of PZQ in the *Schistosoma mansoni* mouse model to determine the key factors that drive its efficacy.
Dose-response studies with racemic PZQ with or without addition of an irreversible pan-cytochrome P450 (CYP)

inhibitor, 1-aminobenzotriazole (ABT), were performed. In addition, efficacy of PZQ in the presence of the CYP inducer, dexamethasone (DEX), was determined. Plasma samples were obtained by tail vein bleeding at 4 time points. The (*R*)-PZQ levels were determined using a LC-MS/MS method. Non-compartmental pharmacokinetic analysis was performed using PKsolver. In addition, experiments using an enhanced *in vitro* assay were conducted. We found that the use of ABT increased (*R*)-PZQ plasma exposures in the systemic circulation by ~10 to 20 fold but the latter were not predictive of efficacy. The use of DEX decreased plasma exposures of (*R*)-PZQ in the systemic circulation by ~10 fold without reducing efficacy. We extrapolated the (*R*)-PZQ concentrations in mouse portal vein / mesenteric veins from the systemic exposures and found that a free exposure of (*R*)-PZQ of ~ 20 μM*h in the portal vein was needed to obtain a worm burden reduction >60%.
It is suggested that the high (*R*)-PZQ concentrations available before the hepatic first pass metabolism drive the efficacy against *S. mansoni* adult worms residing in the mesenteric veins. It is then possible that the current dosing regimen of 40 mg/kg in preventive chemotherapy programs may provide suboptimal concentrations in low-weight patients such as children, due to smaller total amounts of drug administered, and may consequently result in lower cure rates.

POSTER NUMBER: 2021

Tools for identifying and characterizing new anthelmintics with *C. elegans*

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C. elegans is a convenient model organism for studying drugs that treat parasitic worm infestations (anthelmintics). It has been used both to screen for new anthelmintic candidates and to better understand the mechanism of existing drugs, such as benzimidazoles, avermectins and levamisole.
We have developed a high-throughput assay for investigating time-resolved responses of nematicidal compounds in C. elegans. We show that short-term dynamics of drug responses frequently contain transient components that can reveal overlooked aspects of nematode biology. For example, we show that transient recovery from levamisole-induced paralysis in larval worms, first described 40 years ago, is driven by indirect action on muscaninic receptors.
Finally, we describe a novel application of an imaging technology borrowed from the manufacturing industry, which allows us to monitor responses of thousands of individual worms under dozens of conditions simultaneously. This has applications both in high-throughput compound screens for candidate anthelmintics and in genetic screens for their targets.

POSTER NUMBER: 2022

Malarial Killing Missile: Structure-based Hybridization of P218 and Artemisinin

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The efficacy of artemisinin combination therapy for treating malaria is compromised by parasite resistance to artemisinin and partner drugs. More effective drugs and combinations are thus needed. Heme-activated artemisinin promiscuously alkylates many parasite proteins and irreversibly damages them by forming covalent adducts. Although the mode of artemisinin action is rapid and effective for killing parasites, the short pharmacological half-life of artemisinin means that it must be combined with longer-acting drugs that act on single targets. *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) is the validated target of antifolate drugs, including the clinical candidate P218 developed in our laboratory. We hypothesize that hybrid compounds with two pharmacophores, one artemisininin-like and another antifolate-like can act as a “guided missile” to selectively alkylate *Pf*DHFR and lead to its irreversible inactivation.

A series of hybrid compounds were designed and synthesized. The prototype compound in the initial series, BION-004 inhibits wild-type *Pf*DHFR enzyme activity comparable to P218 (K_i values 0.60±0.02 and 0.43 ± 0.07 nM,

respectively). BION-004 potently inhibits parasite growth, including quadruple mutant *Pf*DHFR strain V1/S (IC_{50} = 3.5±0.2 nM). The inhibition of PfDHFR activity by BION-004 is potentiated by heme in a time-dependent manner suggestive of irreversible inactivation. X-ray structures of the liganded complex revealed binding of BION-004 to the PfDHFR active site in the expected manner. Proteomic analysis of the liganded complex revealed alkylation of *Pf*DHFR active site residues C50, M55 and C59 supporting the guided missile mechanism of inhibition. From these data, we conclude that hybrid compounds can be used to selectively alkylate *Pf*DHFR and irreversibly inhibit its activity. Future work will include synthesis of BION-004 derivatives with superior pharmacological pharmacokinetic properties.

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Structure-based drug discovery for African sleeping sickness by targeting a subpocket in *Trypanosoma brucei* phosphodiesterase B1

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Obtaining selective inhibitors is an important therapeutic objective when targeting cyclic nucleotide phosphodiesterases (PDEs). One such PDE, *Trypanosoma brucei* PDE B1 (TbrPDEB1), is a validated drug target for the treatment of African sleeping sickness. Here, we elucidate the molecular determinants of inhibitor binding and explore the P-pocket of this enzyme as an accessible parasite-specific ligand-binding region, amenable to directed design and absent from the highly homologous human PDE off-targets hPDE4B and hPDE4D. By iterative cycles of design, synthesis, pharmacological evaluation and structure elucidation of inhibitor-bound TbrPDEB1 and hPDE4B/4D complexes, we have developed the first selective TbrPDEB1 inhibitors. Subsequent treatment of parasites with these TbrPDEB1 inhibitors reveals an increase in intracellular cAMP levels and severe disruption of *T. brucei* cellular organization, chemically validating TbrPDEB1 as a therapeutictarget in trypanosomiasis. Furthermore, since all trypanosomatid PDEs are believed to possess P-pockets close to their active sites, our studies provide a generic rationale for the discovery of further selective antiparasitic PDE inhibitors.

POSTER NUMBER: 2024

Cembranoids from a cultured soft coral *Sinularia flexibilis* and immunomodulatory effect of cembranoids on dendritic cells

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In order to search for bioactive marine natural products, we have studied the chemical constituents from the organic extracts of a cultured soft coral *Sinularia flexibilis*. This study had led to the isolation of four cembranoids, flexibilisolide H (1), flexibilisolide I (2), isosinulaflexiolide K (3) and (-)-sandensolide (4). Current study indicates that compound 3 has been reported that it inhibits the expression of proinflammatory proteins, iNOS and COX-2, in LPS-induced murine macrophage cell line RAW264.7. However, 3 effects on the normal immune function remains unknown. Therefore, we evaluated the effect of 3 on the lipopolysaccharide (LPS)-induced murine bone marrow-derived dendritic cell (DC). Our experimental results reveal that phenotypical and functional maturation of DCs stimulated by LPS were profoundly reduced by 3 in a concentration-dependent manner, such as the expressions

of co-stimulatory molecules (CD40, CD80, and CD86). Moreover, 3 reduced TNF-α, IL-6, IL-12 and NO release from LPS-activated DCs, decreased their abilities to stimulate allogeneic T cell proliferation, and inhibited LPS-induced MAPKp38 and Erk pathways. Finally, we also found that 3 can reduce contact dermatitis related to dendritic cell activation. These findings offer a new insight into the immunopharmacological function of 3 and its impacts on the DCs.

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POSTER NUMBER: 2025

***In vitro* and *in vivo* anti-*plasmodial* properties of tetracyclic iridoids: a novel compound isolated from *morinda lucida* benth**

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Malaria remains a global health issue due to the continuous presence of the vectors and parasites. In spite of increasing efforts to control malaria, the disease possesses danger to public health and economic development of both the tropical and subtropical regions in the world. The purpose of this study was to investigate the anti-plasmodial properties of three Tetracyclic iridoids; Molucidin, MLF-52 and ML2-3 isolated from Ghanaian Medicinal Plants, *Morinda lucida*. *In-vitro* anti-plasmodial assay was performed on 3D7, Dd2, and field isolates (MISA011) sampled from the Greater Accra region of Ghana. Molucidin, ML-F52, and ML-2-3 had activity with IC_{50s} values of 3.5μM, 1.5μM and 2.5μM against 3D7 strain, 27.6μM, 15.7μM, and 23.2μM against Dd2, and 8.9μM, 12.2μM and 6.6μM against MISA011 respectively. Also Molucidin, MLF-52 and ML2-3 showed significant inhibition on the schizont rupture against 3D7 strain with IC_{50s} of 0.12μM, 0.07μM and 0.10μM, respectively. Phenotypic study of Molucidin treated schizont stage of parasites revealed that Molucidin inhibited the maturity process of schizont stage with intact inner and outer membranes. *In vivo* efficacy of the anti-plasmodial properties of the Molucidin was determined using *P. yoelli* on ICR mice. Although Molucidin was found to suppress parasitaemia significantly, it failed to show 100% cure with 5 days of 30mg/kg daily shot. Our study showed that tetracyclic iridoids; molucidin, ML2-3 and ML-F52 can be a potential lead compound for the development of new chemotherapy.

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POSTER NUMBER: 2026

***In vitro* biotransformation and enzyme kinetic characterization of R- and S-praziquantel metabolism: predicting and rationalizing *in vivo* drug clearance and drug-drug interactions**

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Background: Praziquantel (PZQ) is the only drug available for the treatment of all forms of schistosomiasis. New pediatric formulations for the active enantiomer R-PZQ and the racemate PZQ are currently under development. There is however limited drug metabolism data on PZQ available to support these initiatives. Detailed knowledge of PZQ metabolism will enables the use of physiologically based pharmacokinetic (PBPK) modeling to determine appropriate doses for the new formulations in pediatric patients and to predict risks for drug-drug interactions in mass drug administration (MDA) use of the drug.

Methods: Biotransformation studies on Praziquantel were conducted in human liver microsomes and recombinant CYPs. Structure elucidation was inferred from mass spectra. Enzyme kinetic studies to determine the Michaelis-Menten kinetics, Km and Vmax, of the formation of the main metabolites were determined by LC-MS/MS.

Results: CYP reaction phenotyping studies with HLM and r-CYPs indicate the major involvement of CYP1A2, 2C19, 2D6 and 3A4/5 in the metabolism of R-and S-PZQ. Biotransformation studies showed that PZQ is metabolized to cis-4-OH-PZQ mainly by CYP1A2 and CYP2C19. CYP3A4/5 on the other hand metabolize PZQ to a mono-hydroxyl metabolite currently referred to as X-OH-PZQ whilst CYP2D6 metabolizes PZQ to minor novel mono-hydroxyl

metabolite referred to as Y-OH-PZQ, both pending structural elucidation by NMR. R-PZQ was more rapidly metabolized than SPZQ.

Discussion & Conclusions: The differential role of CYP1A2 & CYP2C19 and of CYP3A4 & CYP3A5 in the formation the 4-OH-PZQ and the novel X-OH-PZQ respectively are intriguing findings as the X-OH-PZQ has not been reported before in humans. NMR studies are required to establish the chemical structures of X-OH-PZQ and Y-OH-PZQ. In the in vitro conditions, cis and not trans 4-OH-PZQ formation has been observed contrary to many in vivo reports in humans which indicate that the trans 4-OH-PZQ as the main metabolite. These data will enable us to understand the rapid clearance of PZQ and predict potential drug-drug interactions in the use of PZQ and the potential role of genetic and environmental factors in the inter-individual variability of PZQ pharmacokinetics.

POSTER NUMBER: 2027

Open Source Malaria: Lead Optimisation of a Triazolopyrazine Series

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Annual deaths from malaria have plateaued since 2015, yet still the disease claims the lives of an estimated 445,000 people each year, mostly young children.[1] Frontline treatments for malaria are currently artemisinin combination therapies (ACTs). However, the inevitable reports of resistance or tolerance have already appeared and so new medicines that can replace or complement the ACTs are urgently needed.
The Open Source Malaria (OSM) consortium operates on the principles that all data and ideas should be freely shared, anyone may participate and there will be no patents.[2] Synthetic chemistry and biological evaluations are recorded in real time using online electronic laboratory notebooks.[3]

The current project focus is lead optimisation of a series based on a triazolopyrazine core (“Series 4”), which was inherited from the pharma industry and subsequently pursued by the Medicines for Malaria Venture. Many compounds with high potencies against *P. falciparum* have been discovered, some with promising physiochemical properties (good solubility, low metabolic clearance, low hERG binding). Two members of the series have shown potency *in vivo*. Mechanism of action studies have implicated as the target the ion homeostasis pump PfATP4, a putative target of several other antimalarials in development. Preliminary predictive modelling was run in 2017 for this target in order to help understand the remarkably diverse set of chemotypes that apparently bind it. In parallel, a metabolomics approach is being undertaken to provide alternative mechanism of action data. The latest developments in this series, from both the Sydney laboratory and other contributing laboratories around the world, will be presented.

[1] WHO World Malaria Report, 2017
[2] A.E. Williamson *et al.*, *ACS Cent. Sci.* 2016, 2, 687-701.
[3] M.N. Robertson *et al.*, *Parasitology* 2014, 141, 148-157.

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POSTER NUMBER: 2028

Sequential and rationale preclinical investigation of three selected anti-*M. ulcerans* natural products

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Buruli ulcer (BU) caused by *Mycobacterium ulcerans* is the third most prevalent mycobacteriosis, after tuberculosis and leprosy. The existing therapy consisting of a combination of Rifampicin-Streptomycin requires 56 days of intramuscular injection of Streptomycin (STR), daily trekking to health facilities. Patients who cannot comply with this long-lasting and demanding treatment, have herbal remedies as main option is remote endemic areas. Moreover, most of the affected are children in whom STR can cause hearing impairment. Given these limitations, there is a need for short course, more effective and safer treatment options to control BU. In an attempt of validating herbal therapies for BU, an ethnopharmacological survey was preliminarily carried out to identify plants

and field practices associated with the treatment of Buruli ulcer. A total of 98 plants from three highly affected countries in West Africa were identified. Of those, a total of 113 extracts were prepared from 13 species and screened for antimycobacterial activity. Thirteen of the tested extracts demonstrated significant activity (MIC 125 - 250 µg/mL) against *M. ulcerans*. Further bioassay-directed fractionation of promising extracts led to the purification of 11 active compounds (MIC ranging from 16 to 128 µg/mL against *M. ulcerans* and overall varied selectivity against normal Chang liver cells. From the 11 isolated *M. ulcerans* inhibitors, 3 promising candidates belonging to two chemical scaffolds, *viz*. AKC0, AKC1 (isoflavonoids), and P122 (alkaloid) were selected for progression on the basis of their antimycobacterial activity and selectivity index (MIC≤ 32 µg/mL; SI≥ 4). In addition, AKC1 delayed the development of edemas in mice model of Buruli ulcer after only 4 weeks of treatment at oral dosage of 250mg/kg b.w. These activity and safety profiles motivated further investigation that should establish a sequential and rationale preclinical investigation of the selected promising compounds (AKC0, AKC1, and P122) by using *in vitro* and *in vivo* assays on *M. ulcerans*. Specifically, 1) Derivatives of two selected active chemical scaffolds against *M. ulcerans* will be submitted to structure-activity-relationship (SAR) coupled with cytotoxicity studies; 2) The best anti- *M. ulcerans* hits (MIC < 4µg/mL4; SI> 100) will be selected and submitted to pharmacokinetics and 3) *in vivo* studies in mice model; 4) The screened-out leads will be investigated for their mode of anti-BU action. We anticipate that upon the completion of these objectives, at least 01 anti- *M. ulcerans* lead compound with appropriate efficacy and safety will be identified and further progressed to anti-Buruli ulcer drug development.

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POSTER NUMBER: 2029

Novel anti-*Wolbachia* drugs as short-course macrofilaricides for onchocerciasis

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Onchocerciasis is a priority Neglected Tropical Disease targeted for elimination. A safe, one-week curative drug regimen suitable for all target populations would dramatically shorten elimination time frames in hard-to-reach areas or where current strategies are failing. Effective (>90%) antibiotic-mediated depletion of the *Onchocerca volvulus* symbiont, *Wolbachia,* leads to gradual sterility and death of adult parasites. This mode of action is attractive because it avoids the potentially lethal host inflammatory adverse reactions to more rapid-acting nematodicidal agents. However, current registered antibiotics at recommended doses need to be taken for 4 weeks to exert macrofilaricidal activity. Here we present the current Anti- *Wolbachia* (A-WOL) Consortium portfolio of anti-*Wolbachia* based macrofilaricide candidates discovered through phenotypic screening of focused and diversity libraries (BioFocus, AbbVie, MMV, AZ) and validated in a range of *in vitro* and *in vivo* filarial infection models. Two preclinical candidates, AWZ1066 and ABBV-4083, have met the criteria for full preclinical development and ABBV-4083 has been advanced into Phase I clinical testing.

POSTER NUMBER: 2044

TDR Targets: integrated chemogenomic mining of pathogen genomes for drug discovery

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The volume of biological, chemical and functional data deposited in the public domain is growing rapidly, thanks to next generation sequencing, and highly-automated screening technologies. However, there is still a large data imbalance between model, well-funded organisms and pathogens causing neglected diseases (NDs). We developed a chemogenomics resource, (TDR Targets, tdrtargets.org), that aims to organize and integrate heterogeneous large

datasets with a focus on drug discovery for human pathogens. The database also hosts chemical and genomic data from other organisms to leverage data for comparative and inference-based queries. One of the major impacts of TDR Targets is to facilitate target and chemical prioritizations by allowing users to formulate complex queries across diverse query spaces.
In this communication we will highlight new data and functionality updates in TDR Targets. In this release, the database has been updated to integrate data on >2 million bioactive compounds; 20 pathogen genomes; and 30 complete genomes from model organisms and other related pathogens. Furthermore, the data was also used to populate a recently developed network model (Berenstein AJ, 2016) to produce i) a novel *druggability* metric for targets based on the connectivity in the network to bioactive compounds, ii) to guide new prioritization strategies for both targets and compounds, and iii) to visually aid in the navigation across target/compound spaces in the web interface. This network model connects protein (target) nodes to compounds, based on curated bioactivity annotations. It also connects proteins to other proteins based on shared annotations, and compounds to other compounds based on chemical similarity and substructure metrics. This chemogenomic network facilitates a number of inferences, such as inferring plausible targets for orphan drugs or candidate compounds for orphan targets.

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POSTER NUMBER: 2045

A yeast platform for generation of synthetic natural product-like chemical matter by combinatorial biosynthesis

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Natural products (NPs) encompass an enormous chemical diversity with privileged biological activities and have inspired the majority of drugs in current use. Over the past three decades, however, emphasis has shifted away from the use of NPs in drug discovery towards synthetic chemical libraries, due in part to isolation, dereplication, resupply and chemical tractability issues associated with NPs. Recent metagenome sequencing efforts have revealed hundreds of thousands of NP biosynthesis enzymes, which can be readily classified into different biosynthesis pathways. To solve issues traditionally associated with NP-based drug discovery, we have developed a *Saccharomyces cerevisiae* platform for heterologous production of NP-like chemical matter, termed synthetic natural products (SynNPs). We synthesized a large library of codon- and GC-content optimized NP biosynthetic genes (BSGs) from plant, fungi and bacteria and cloned the collection into BSG expression modules. A programmable yeast artificial chromosome (YAC) assembly method was used to construct highly diverse combinatorial BSG libraries predicted to generate a massive number of *in situ* generated NP-like molecules. SynNP libraries were efficiently screened for bioactive molecules in target- or cell-based positive selection screens in a yeast cell microfactory format. Chemical diversity generated by various SynNP clones was demonstrated by high-throughput metabolomics and activity guided purification (see abstract by Cook et al. for further details). The SynNP platform thus allows the exploration of diverse NP-like chemical space in a target agnostic manner at ultra-low cost and high throughput.

POSTER NUMBER: 2046

A novel cell-free method to culture *Schistosoma mansoni* from cercariae to juvenile worm stages for *in vitro* drug-testing

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The anthelminthic treatment against schistosomiasis is limited and relies almost exclusively on a single drug, praziquantel (PZQ). Even though PZQ is potent in killing adult worms it has been shown to have limited activity against earlier developmental stages. Current *in vitro* drug screening strategies depend on newly transformed schistosomula for initial hit identification, thereby limiting sensitivity to new compounds predominantly active on later developmental stages. This study aimed to establish a highly standardized, straightforward and reliable culture method to generate and maintain advanced larval stages *in vitro*. We present here how this method can be a valuable tool to test drug efficacy at each intermediate larval stage, reducing the reliance on animal use (3Rs). Cercariae were mechanically transformed into skin stage schistosomula and successfully cultured under cell- and serum-free conditions for up to four weeks. Under these conditions, larval development halted at the lung stage. Addition of human serum propelled further development into juvenile worms within eight weeks. Skin and lung stages, as well as juvenile worms (late liver stage), were tested with known anti-schistosomal compounds such as PZQ, oxamniquine, mefloquine and artemether. Our findings showed stage-dependent differences in larval susceptibility to the tested drugs. The phenotype of juvenile worms, when exposed to reference drugs, was comparable to previously published works for *ex vivo* harvested adult worms. This *in vitro* assay can help reduce reliance on animal experiments in the search for new anti-schistosomal drugs and provide a platform for the investigation of the host protein- or cell-mediated effects on the parasite's development.

POSTER NUMBER: 2030

Targeting Aurora-like kinases in *Plasmodium falciparum*

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The intraerythrocytic development of the malaria parasite diverges from the paradigm of eukaryotic cell cycle. Novelty of *Plasmodium* cell cycle offers opportunities for development of therapeutics directed against essential components. Plasmodial kinases, such as Aurora kinases, could prove to be valuable targets for therapies because of their pivotal roles in regulating cell division. Essential *Plasmodium* Aurora-related kinases (Arks) 1, 2, and 3 are homologous to Aurora A and B, Ser/Thr kinases involved in cell division. PfArk-1 has been shown to be expressed during early schizogony. PfArk-2 contains a classical aurora kinase domain similar to that of Aurora A, and PfArk-2 and PfArk-3 have been shown to be expressed during schizogony. To discover potent and selective small molecule inhibitors of PfArks, we screened a library of optimized mammalian Aurora kinase inhibitors that have evolved from a general pharmacophore models for Ser/Thr kinases. In addition, we are also repurposing human Aurora kinase inhibitors to discover antimalarial lead compounds. We have identified novel potent inhibitors (EC_{50} < 1 μM) in cell-based screening using SYBR Green I fluorescence based assay. Selectivity of the hits against mammalian cells were determined using the MTS ((3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) cell proliferation assay. Results from our studies to assess structure-activity-relationship, specificity of inhibition, and cellular effects of the compounds will be presented.

POSTER NUMBER: 2031

Exploring the effect of plant derived small molecule to inhibit DHN melanin, a major virulence factor in *Aspergillus fumigatus*: An early stage drug discovery

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Aspergillus fumigatus, an opportunistic fungal pathogen is associated with a wide array of diseases both invasive and non-invasive. It produces 1,8-dihydroxy naphthalene (DHN)-melanin that imparts greenish grey colour to the conidia and protects it from ultra violet irradiation, ROS, antifungal drugs and host immune system. They have also been shown to provide cell wall stability and structural rigidity to the conidia. Hence, targeting the DHN-melanin pathway may be considered as a novel drug target and underlines the importance of this study.

The focus of this study was to identify small molecule/s from medicinal plants that have the potential to inhibit

DHN-melanin biosynthesis pathway in *A. fumigatus*. Initial studies involved molecular docking analysis of the major secondary metabolites from the bioactive plant extracts as identified using GC-MS and their interaction with the ketosynthase domain of polyketide synthase protein. Using this approach, MFC077 was selected for further analysis. The minimum effective concentration (MEC) of MFC077 against *A. fumigatus* was determined by CLSI broth micro-dilution method. The biochemical studies for melanin synthesis, ergosterol formation and cell surface hydrophobicity were performed using spectrophotometric methods. Electron microscopic studies were performed to evaluate the effect of MFC077 on *A. fumigatus* cell surface morphology. Differential proteomics studies were carried out to identify exclusive proteins after the treatment.

The molecular docking analysis showed that MFC077 formed proper hydrogen bonding at Asn422 residue of ketosynthase domain at low binding energy -5.71 Kcal/mol. The results indicated that MFC077 inhibited melanin production in *A. fumigatus* (91.80%), reduced ergosterol content (83.63%) and hydrophobicity of the cell (65.24%) at the MEC of 0.039 mg/mL. Electron microscopy revealed altered conidial surface, disappearance of protrusions and absence of melanin layer on outer cell surface. Differential proteomics analysis showed 108 exclusive proteins in the treated organism as compared to 80 in the control.

Compound MFC077 demonstrated potent *in vitro* efficacy in inhibiting DHN-melanin biosynthesis pathway in *A. fumigatus*. DHN-melanin is a major virulence factor hence, inhibiting this pathway would render the pathogen avirulent and would help faster and effective eradication from the host system. Further studies are warranted to establish the antifungal potential of this molecule.

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A novel screen to identify inhibitors of efflux and bactericidal compounds in 'persister-like' *Mycobacterium tuberculosis*

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New drugs are needed to reduce tuberculosis (TB) treatment duration and combat drug-resistant disease. Strategies to discover new paradigm-shifting drugs include targeting low-metabolic state 'persister-like' *Mycobacterium tuberculosis* bacilli that are phenotypically tolerant to most TB drugs or targeting mycobacterial drug efflux to potentiate current drug regimens. We have established a multiple assay screen to identify compounds from a 10,000-diversity set library that inhibited *M. tuberculosis* efflux or were cidal against low-metabolic state bacilli or log phase actively-replicating *M. tuberculosis*. Screening results were compared with toxicity data from zebrafish and murine cell screens to compile compound activity profiles. Hits and hit-analogues will be re-synthesized and re-screened to validate compounds and explore structure activity relationships, before characterizing mode of action using genomics and transcriptomics.

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Novel 4-Amino-7-Chloroquinoline Appended [1,2,3]-Triazoles can Simultaneously Target Asexual Stages and Gametocytes of *P. falciparum*

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Background: Malaria is a tropical disease which remains a global health problem despite the availability of effective tools. Drug resistance and absence of safe and effective transmission-blocking drugs are the two major roadblocks which have severely halted the progress of malaria elimination campaigns. One way to combat these problems is to develop safe and effective antimalarials. In the present work, 4-amino-7-chloroquinoline appended [1,2,3]-triazoles are designed, synthesized and screened for their ability to simultaneously target asexual stages and sexual stages (gametocytes) of *P. falciparum*.
Methodology: The study begins with development of a continuous *in vitro* culture of asexual stages and gametocytes of *P. falciparum* along with identification of gametocyte producing Indian field isolates for drug screening experiments. Further, a very simple and cost effective gametocytocidal-drug screening assay was optimized, which

is also applicable to less affluent laboratory setups and methylene blue was investigated as a gametocytocidal agent. Then, pharmacokinetic properties of newly synthesized group of quinoline-triazole hybrids were predicted using *in silico* tools and their cytotoxicity evaluated on VERO cells. Further, their antiplasmodial potential was investigated against chloroquine sensitive and chloroquine resistant *P. falciparum*.
Results: After optimizing the asexual stage culture and gametocyte production, two field isolates RKL-9 and JDP-8 were identified as high gametocyte producers and were deemed suitable to screen gametocytocidal drugs. Addition of [1,2,3]-triazoles to a 7-chloroquinoline nucleus resulted in formation of compounds that demonstrated potency in nanomolar range against chloroquine sensitive strain (3D7) of *P. falciparum* with three compounds showing IC50 of <100 nM against chloroquine resistant field isolate (RKL-9). Further, the lead compounds were also observed to be causing morphological deformations in mature gametocytes which lead to the appearance of abnormal pyknotic parasites. All compounds displayed attractive pharmacokinetic profile whilst majority demonstrated little or no cytotoxicity.
Conclusions: This is the first study which presented two gametocyte producing lines of Indian origin which can be further used in anti-gametocyte drug discovery. Our initial results prove that these newly synthesized quinoline-triazole hybrids may have the potential to be used as prototypes for the development of effective multi-stage antimalarials against *P. falciparum*. Further evaluation of the lead compounds in Standard Membrane Feeding Assays (SMFAs) and in rodent malaria model is highly recommended.
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Mechanisms of anti-cancer activity by compounds from *Severinia buxifolia*

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Lung cancer is one of the most common cancer worldwide which is by far the leading cause of cancer death because of its rapid metastasis leading to poor prognosis. While the incidence of lung cancer about 80% diagnosed with non-small lung cancer (NSCLC), developing novel anti-metastasis drug to treat with NSCLC is indispensable. Severinia buxifolia is a common folk medicine which behaves with pharmacological effects such as anti-malaria, anti-rheumatism, and anti-nociceptive in Southeast Asia. Given that hypoxia-inducible factors 1α (HIF-1α) is considered to associate with cancer metastasis by transactivation of its target genes, compounds isolated from S. buxifolia were investigated for their effects on anti-metastasis and HIF-1α activity in NSCLC cell line A549. Among them, we found compounds sbs-A and sbs-B showed better inhibitory activity on cancer invasion in both normoxia and hypoxia conditions. Sbs-A and sbs-B were found to inhibit the luciferase reporter drive by HIF-1 responsive element and the expression of HIF-1 target genes such as Twist, MMP9, c-Myc, Sox2, and CA9. Probe into the mechanism of HIF-1 inhibition we found sbs-A and sbs-B caused a two-fold decrease in HIF-1α mRNA expression but might not explain a potent inhibition of HIF-1α protein and its transactivation activity. Both PHD-2 and proteasome inhibitors cannot reverse HIF-1α inhibition by sbs-A and sbs-B. HIF-1α protein degradation rate without significant difference between cell treat with or without sbs-A. In conclusion, our results showed suppression of HIF-1α might underlie the anti-metastasis mechanism of sbs-A and sbs-B, and how the compound inhibits HIF-1α is still under study.

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Screening of Actinobacteria for Novel Antimalarial Compounds

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Introduction and Aims: The success of our first line antimalarial treatment is threatened by increased drug resistance in *Plasmodium* parasites. This makes the development of novel drugs critical to combat malaria. Historically, biological systems have been a good source of novel antimalarial compounds, and thus are an ideal place to search for potential drugs. Members of the bacterial phylum, *Actinobacteria*, are well known antibiotic producers, but their antimalarial potential has not been well investigated. This makes the actinobacteria a

potentially, valuable source of novel antimalarial compounds. The aim of this investigation was to screen actinobacteria extracts for antimalarial activity and isolate and evaluate the active compounds found.

Methods: Novel actinobacteria were cultured in liquid medium and extracted with ethyl acetate. Crude extracts were tested for *in vitro* antiplasmodial activity, using the parasite lactate dehydrogenase assay (pLDH) against the drug sensitive NF54 and the multi-drug resistant K1 strains of *Plasmodium falciparum*. Active extracts were purified by solid phase extraction (SPE) and high performance liquid chromatography (HPLC). Cytotoxicity of pure compounds was determined against the Chinese Hamster Ovary (CHO) cell line using the MTT assay. The structures of purified active compounds were then elucidated using high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) analysis.

Results: Three of seven actinobacterial strains tested had extracts with antimalarial activity. The strain that produced the most active extract was PR3 and was shown to be a member of the *Streptomyces* genus by 16S rRNA gene sequencing. Two active pure compounds have been isolated from strain PR3 both of which have IC_{50}s of less than 100 ng/ml against both NF54 and K1 strains of *P. falciparum*. Both are non-toxic to the CHO cell line and their structures are being elucidated using HRMS and NMR analysis.

Conclusion: Both compounds show excellent *in vitro* antiplasmodial activity and host selectivity making them excellent candidates for structural elucidation and *in vivo* studies.

Keywords: Malaria, drug resistance, natural products, actinobacteria

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A 2,4-dioxothiazolidine analog induces apoptosis in oral cancer cells

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Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck, and the incidence is increasing worldwide. This study was aimed at exploiting a 2,4-dioxothiazolidine analog (A5), to develop potent antitumor agents. The anti-proliferative effects of A5 were assessed by MTT assays, flow cytometry, and Western blotting. Compound A5 induced apoptosis as indicated by caspase activation and PARP cleavage. PI/Annexin V analysis demonstrated that A5 increased apoptotic cells in a dose-dependent manner. In addition, it increased reactive oxygen species (ROS) generation which was similar to that of H_2O_2. In conclusion, the ability of A5 to target mutiple aspects of cancer cell growth with higher potency suggest its value in oral cancer therapy.