Spotlights on new publications

Sherif M Abaza

Medical Parasitology Department, Faculty of Medicine, Suez Canal Uinversity, Ismailia, Egypt

Corresponding Author: Tel.: 00201005243428, **E-mail:** smabaza@hotmail.com

Received: 28 April, 2019**, Accepted:** 18 May, 2019.

Print ISSN: 1687- 7942, **Online ISSN:** 2090 -2646, **Vol. 12, No. 2, August, 2019**

New drug targets - X

Malaria: It is well known that proteinases catabolize proteins and polypeptides with or without formation of a transient covalent bond. Proteinases with covalent bond use either catalytic oxygen of serine or threonine, or sulfur of cysteine. Other proteinases use activated oxygen of water (either aspartyl- or metalo-proteinases). Species of *Plasmodium* utilize proteinases'arsenal mainly for hemoglobin degradation employed in protein synthesis and subsequently for development of further life cycle stages. In a review article, **Manasi Mishra** and her colleagues from India discussed the importance of *Plasmodium* proteinases of interest as potential drug targets. Role(s) played by six cysteines (two falcipains; FLCs, two serine repeat antigens; SERAs, and two dipeptidyl aminopeptidases; DPAPs), four aspartyls (plasmepsins; PLMs), two aminopeptidases (MAPs), and one threonine (HsIV) were discussed. It is worth mentioning that catalyzing polypeptides were *via* a catalytic type of cysteine in FLCs and DPAPs, or threonine in HsIV, or without formation of transient covalent bond in PLMs and MAPs. According to MEROPS website a none-peptidase homologue is assigned as a catalytic type for SERAs. Besides, three *P. falciparum* subtilisin-like proteases, also called subtilases (SUBs), have an essential role in processing proteins, required for the cascade of invasion, egress through ruptured infected hepatocytes and/or RBCs, and *de novo* RBCs invasion. The reviewers linked their discussion to potential inhibitors reported in the last two decades. The objective of the present review is to focus for future promising anti-malarial drug targets.

Both *Plasmodium* exoneme and mononeme are newly characterized organelles identified as specialized secretory apical organelles of merozoites. Exoneme is identified as a store for mature SUB1 to be expressed in parasitophorus vacuole (PV) due to increase of intracellular Ca^{2+} during late schizont maturation (pre-RBCs egress), while mononeme is identified as a single thread-like merozoite apical organelle where rhomboid-1 (ROM1) is immunolocalized. In the present compilation, the reviewers presented two figures showing the process and steps of egress and *de novo* invasion cascade. From the second figure, it is quite apparent that 1) while SUB1 is involved in merozoites egress, SUB2 is required for merozoites *de novo* RBCs invasion, 2) *Plasmodium* organelles are implicated hwere exoneme, microneme, and mononeme express SUB1, SUB2 and ROM1, respectively, 3) degradation of PV membrane and breakdown of both RBC' membrane and cytoskeleton is essentially done by SUB1, SUB2 and ROM1 *via* release of several proteins, and 4) serine repeat antigens 5 and 6 (SERA5 and SERA6), and merozoite surface protein 1 (MSP1) are released from SUB1, while SUB2 releases MSPs 6 and 7. On the other hand, ROM1 contributes with SUB2 to catalyze the intermembrane cleavage of merozoite adhesin named apical membrane antigen (AMA1) leading to *de novo* RBCs invasion.

Although SUB1 of all species of *Plasmodium* share only ~30% similarity and sequence identity in their catalytic domain, their prodomains are different from other known SUBs. In addition, the reviewers observed that the crystal structure of *Plasmodium* SUB1 showed scissile bond allowing unusual interaction of its active site with substrate residues on both prime and non-prime sides. The reviewers hypothesized that SUB1 has metal-binding sites to accommodate Ca^{2+} for its maturation and activation. Accordingly, they attributed the activation of *Plasmodium* SUB1 to two factors; its specific Ca^{2+}-dependent regulation as a specific substrate and its catalytic domain. Therefore they suggested mature *Pf*SUB1 as a novel drug target. In this regards, several low molecular weight compounds as well as natural compounds were investigated and showed significant inhibition of *Pf*SUB1, blockage of RBCs egress, and *de novo* merozoite invasion. Peptidyl alpha-ketoamides and quinolylhydrazone 2 as *Pf*SUB1 inhibitors or difluorostatone-based inhibitors as peptidomimetic inhibitors of prodomains were evaluated as anti-malarial novel drugs. Computational analysis with *in vitro* assessment was suggested to assist molecular scientists identifying the structural requirements for validation of novel chemotherapy.

Plasmodium genomic analysis showed possession of four FLCs, nine SERAs, and three DPAPs (clan CA, family C1). Their expression levels vary in different

 DOI: 10.21608/puj.2019.12468.1044

developmental stages that are involved in several biological processes; hemoglobin degradation, synthesis of protein required for stage development, egress from hepatocytes and infected RBCs, and *de novo* RBCs invasion. In all prodomains of papain-like cysteine proteases, the N-terminal part directs trafficking to the food vacuole, while the C-terminal part inhibits the mature domain. However, *Plasmodium* FLCs have two unique structural features; short N-terminal extensions to increase folding of the mature domain to bind more with active enzyme, and a unique motif in the C-terminal, called hemoglobin binding domain. Although motif size is fixed in all plasmodial FLCs, it varies in amino acids' sequence. Both features make FLCs more efficient for its main hemoglobin degradation function. It was found that FLC-2 and FLC-3 are homologous to each other with 68% sequence identity between their catalytic domains. Interestingly, both are the major vacuolar hemoglobinases. However, knockout gene encoding FLC-2 resulted in a transient block in hemoglobin degradation with increased parasite' sensitivity to cysteine protease inhibitors.

Nine *P. falciparum* SERAs were identified to have cysteine protease motifs, however, the active site cysteine is replaced by a serine in SERA-5. As previously described in the present compilation, SERA 5 and 6 are released from SUB1 and have an indispensable role in the cascade involving egress and *de novo* invasion. However, molecular studies showed that their effects were not *via* catalytic proteolysis but through their regulatory roles on the development of parasitic stages. On the other hand, DPAPs are cysteine proteinases with a papain-fold. Previous reports hypothesized hemoglobin hydrolysis has an essential role of DPAP1 due to its localization in the food vacuole, whereas contribution in egress cascade was attributed to DPAP3. However, recent molecular studies showed that DPAP3' activity is essential and critical for efficient *de novo* RBCs invasion.

With some limitations, several studies reported the inhibitory efficacy of several general cysteine proteinase inhibitors, e.g. E-64, vinyl sulfones, furanone derivatives, and pyrimidine nitrile derivatives against FLCs, SERAs and DPAPs. Using structure-guided virtual screening, previous studies identified several non-peptidyl cysteine protease inhibitors. Also, use of natural product-based inhibitors such as Gallinamide A/Symplostatin 4 and their synthetic derivatives showed potential inhibitory efficacies. The reviewers claimed that all the above-mentioned inhibitors were not specific, and directed the attention to design a peptidomimetic compound to directly act on the unique hemoglobin motif. It is worth mentioning that the size of hemoglobin motif varies in FLCs, SERAs and DPAPs (14, 10 and 8 amino acids, respectively). The reviewers also suggested allosteric inhibitors (previously described; New drug targets IX, PUJ 2019; 12(1):68-71) as an alternative strategy to design an efficient inhibitor.

Among ten aspartyl proteinases (clan MH, family M18), the reviewers assigned four PLMs; II, V, IX and X as promising drug targets. The crystal structure of PLM II shows a flap region covering the binding cavity with a proline rich flexible loop to accommodate inhibitors of variable molecular sizes. Also, the crystal structure of PLM V shows another unique conserved feature with relative evolutionary distance from host' aspartyl proteases. Therefore, both PLMs have gained popularity as potential anti-malarial drugs. Despite the identification of several naphthoquinone derivatives as potent inhibitors of PLM V activity, previous studies recommended further chemical modifications to establish their anti-malarial potentiality. On the other hand, the role of PLMs IX and X in egress and *de novo* invasion was recently identified in 2017 using conditional knockout technology. It was found that PLM IX acts as a maturation factor for rhoptery proteins, while the latter controls SUB1 maturation. Search for their inhibitors revealed a pleiotropic peptidomimetic competitive inhibitor that showed significant efficacy against *P. falciparum in vitro* and *P. berghei in vitro*. Except for PLM V, *Plasmodium* PLMs unfortunately share varying sequence homology with human aspartic proteinases which means that searching for PLM inhibitors remains a difficult challenge.

In *P. falciparum*, there is only one threonine proteinase (*Pf*HslV) belonging to clan PB, family T1, and nine aminopeptidases (MAPs) assigned in clan MA, with different families. The reviewers recommended *Pf*HsIV potentiality as a promising drug target for several reasons. Among them were high expression in schizont and merozoite stages, highly conserved in all *Plasmodium* species, and with unknown sequence analog in human host. On the other hand, MAP sarecytosolic exopeptidases also known as a metallo enzymes that contain zinc in its active site. Two MAPs were functionally characterized; alanyl (*Pf*M1AAP), and leucyl (*Pf*M17LAP) aminopeptidases belonging to families 1 and 17, respectively. Studies showed that both MAPs have tightly bound zinc ion in their active site. Therefore, zinc ion chelating from the active site inactivates the enzyme, leading to lethal effects on *Plasmodium in vitro* and *in vivo*. The reviewers discussed several recent studies that reported strategies to the design of inhibitors for both MAPs, such as compounds containing hydroxyamic acid and amino-benzosuberone derivatives.

Finally, the reviewers concluded that the proteinases of *Plasmodium* provide excellent opportunities as potential drug targets as well as a documented strategy to design specific inhibitors as novel anti-malarial drugs. To design a specific inhibitor, the majority of the previous studies focused on homology modeling and molecular docking approaches, ignoring the high-resolution crystal structure-based strategy that might show its promising significant role in the near future. Based on this strategy, the reviewers recommended

further studies to design: 1) compounds to block SUB1 activity utilizing its specific Ca^{+2}-dependent regulation and its catalytic domain, 2) broad-spectrum specific inhibitors for hemoglobin motif sites in FLCs, SERAs and DPAPs, 3) a specific inhibitor of PLM V as it is the only one that doesn't share sequence homology with human aspartic proteinases, and 4) compounds that efficiently chelate zinc from M1AAP and M17LAP' active sites. Compiled from **"Structural insights into key *Plasmodium* proteases as therapeutic drug targets." Front Microbiol 2019 Mar 5; 10:394.**

Cryptosporidiosis: It is well known that tRNA is a link between mRNA and the amino acid machine for protein synthesis (ribosome). Therefore, tRNAs are necessary translation molecules to synthesize new proteins according to the genetic code. Aminoacyl-tRNA synthetases (KARs), encoded by *kars* gene, are cytoplasmic enzymes that charge tRNAs with their cognate amino acids. KARs are also involved in signaling pathways *via* binding to transcription factors (MITF and USF2) and through this binding, they can influence their transcriptional activities. In *P. falciparum*, two KRS copies were identified for cytoplasm amino acid sequence (*Pf*KRS1) and apicoplast (*Pf*KRS2), whereas *C. parvum* encodes only one copy (*Cp*KRS). In addition, 96% identity was observed within the active-site region of *Pf*KRS1 and *Cp*KRS. Meanwhile, two reports with four years interval initiated the main objective of the present compilation. In 2012, screening a natural product library, the investigators identified cladosporin, a fungal secondary metabolite, as potent KARs inhibitor and showed significant *in vitro* activity against erythrocytic as well as hepatic stages of *P. falciparum*. In 2016, using virtual 3D-screening of synthetic compounds, bicyclic azetidines were identified with potential inhibitory efficacy against cytosolic *P. falciparum* phenylalanyl-tRNA synthetase. **Beatriz Baragña** with 77 contributors from UK, USA, Switzerland, Spain, Canada, Cambodia, and India decided to validate apicomplexan KRSs as promising drug target for malaria and cryptosporidiosis.

To achieve their goal, they selected an inhibitor of both *Pf*KRS1 and *Cp*KRS using a structure-based approach and library screening and their own synthesized compounds. First, they characterized and analyzed the KRS activities for *P. falciparum*, *C. parvum* and human (*Hs*KRS) by cloning their recombinant forms. Screening r*Pf*KRS1 against GlaxoSmithKline library (~13,000 compounds) identified a compound (No. 2). However, it displayed high metabolic instability. To optimize its stability, the investigators identified the major sites of hydroxylation and succeeded to synthesize compounds No. 3, 4 and 5. In each synthesized compound, the investigators evaluated its inhibitory potency regarding competition with ATP and the selectivity ratio for *Hs*KRS/*Pf*KRS1. Compound no. 5 showed efficient inhibitory potency with excellent oral bioavailability, moderate half-life, and a good profile in *in vitro* assays. The selected compound also showed high stability, confirmed by both structural and thermal shift assays.

Because compound (5) showed toxicity in mice at oral dose of 50 mg/kg, the investigators proceeded to investigate its *in vitro* and *in vivo* assays in lower oral doses. It showed *in vitro* activity against *Pf*KRS1 (half maximal inhibitory concentration; IC_{50}=0.015 vs 1.8 and 49 *u*M for *Hs*KRS and HepG2 cells, respectively). IC_{50} was also excellent when the compound was tested against chloroquine-and atovaquone-resistant trains (0.51 and 0.52 μM, respectively) as well as drug sensitive strain (0.39 μM). Moreover, it showed half maximal effective concentration; EC_{50} = 0.95 *u*M, when tested against *P. vivax* liver schizont. On the other hand, *in vivo* studies revealed 90% reduced parasitemia after four oral doses of 40 mg/kg daily. For cryptosporidiosis, the investigators evaluated compounds 2 and 5 as well as the natural metabolite (cladosporin) against two *Cryptosporidium* species affecting man (*C. parvum* and *C. hominis*), *in vitro*. Results revealed inhibition of parasite growth with EC_{50} of 1.2, 2.5, and 0.7, respectively. However, compound (5) showed variable EC_{50} against both species (1.3 and 6.0 μM, respectively). Both cladosporin and compound (5) showed parasite elimination at consistent rate when compared to other investigated protein synthesis inhibitors. Accordingly, the investigators extended their study to confirm that compound (5) was bound in the same manner to *Cp*KRS as to *Pf*KRS1.

To show its bioavailability in the gastrointestinal tract, the investigators conducted *in vivo* studies using two models (NOD SCID γ and INF-γ-knockout mice). Mice were infected with oocysts and Nluc-expressing transgenic oocysts and treated with oral dose of 20 mg/kg once daily for 7 days started on day 7 and 4, respectively. Monitoring parasite reduction was also differently estimated. Next day after therapy completion, individual quantitative PCR for estimation of oocysts/g stool was conducted for the first model. Luciferase measurements in pooled stool samples of the entire cage was conducted in the second model three times, with one week interval, to estimate gene expression at the transcriptomic level. Results compared to paromomycin therapy revealed 96% reduction on NOD SCID γ infected mice, and complete reduction below detection level in the second model, for 3 weeks follow up. Molecular dynamics of the selected compound showed high affinity toward *Cp*KRS. Moreover, parasite KRS selectivity vs *Hs*KRS was observed.

Therefore, the investigators validated structural-based screening approach to identify molecular drug targets of apicomplexans. This approach succeeded to modify compounds towards the best stability, solubility and bioavailability, and to identify a valuable compound

(No. 5). They recommended further studies to optimize it regarding efficient potency and selectivity. Compiled from **"Lysyl-tRNA synthetase as a drug target in malaria and cryptosporidiosis." Proc Natl Acad Sci (USA) 2019 Apr 2; 116(14):7015-7020.**

Giardiasis: Potassium channels are proteins characterized by formation of pores through cell membrane to allow potassium passage for its action in specific regulatory cellular function such as proliferation, differentiation and motility. Based on their biophysical and structural properties, K^+ channels are divided into four families; voltage-gated, calcium-activated (KCa), inward-rectifier and two-pore-domain. Previous studies identified and characterized K^+ channels in *P. falciparum*, *T. cruzi*, and *T. brucei* and showed their significant role in growth as well as parasite' response against stress factors. Screening for an efficient inhibitor of *T. brucei* K^+ channels identified fluticasone propionate which was validated as a new drug for African sleeping sickness. The role of K^+ channels in *G. lamblia* was reported in several publications, however, its identification in the genomic analysis of *G. lamblia*, assemblage E, encouraged **Lissette Palomo-Ligas** and her colleagues from Mexico to produce the present compilation. Due to several reports of metronidazole resistance, their main objective was to characterize K^+ channels in *G. lamblia* to discover a new drug target for treatment of giardiasis.

Using *in silico* strategy, a recent methodology to identify protein functions aided with a structure-based computational approach, the investigators succeeded in identifying and characterizing a new K^+ channel as a membrane protein in *G. lamblia* genome (*Gi*K). With the lack of known *Gi*K crystal structure, the investigators utilized homology model approach with some modification to construct *Gi*K. After validation of the structural quality of their model (three dimensional *Gi*K), they could predict 14 flexible regions suggestive of being blockers binding sites. In addition, sequence analysis of their model revealed low homology with human proteins. Out of 290 K^+ blockers selected from drug bank, Sigma profile and Zinc databases, the investigators succeeded in identifying 110 inhibitors binding to *Gi*K. Among them, 39 drugs specifically interacted with three spots in its pore region; S5, S2–S4 and C terminal. A number of these drugs were previously reported to affect *in vitro* or *in vivo* growth of *T. brucei, L. donovani, P. falciparum, T. gondii* and *T. vaginalis*, and only one compound (trifluoroperazine) showed inhibition of *G. lamblia* excystation.

The Mexican investigators concluded that *Gi*K is a KCa channel with low homology similarity to human proteins, and proved to be an attractive drug target for giardiasis. The investigators recommended further studies to confirm their conclusion and to perform *in vitro* and *in vivo* assays investigating the efficient active drugs against *G. lamblia*. Compiled from **"Identification of a novel potassium channel (*Gi*K) as a potential drug target in *G. lamblia*: Computational descriptions of binding sites." Pee J 2019 Feb 27; 7:e6430.**

Hydatid cyst: WHO included cysticercosis and hydatid cyst, caused by the larval invasion of *T. solium* and *Echinococcus* spp., respectively among 17 neglected tropical diseases (NTDs). Almost all NTDs acquire this grouping due to inefficient chemotherapy. Only three drugs; mebendazole, albendazole (ALB), and praziquantel (PZQ) proved to have moderate potency; e.g. ALB is ineffective to treat ~40% of patients with hydatid cysts. With exception of *Hymenolepis* spp., life cycle of cestodes involves two or more hosts and requires significant events to allow cestodes to accommodate and withstand living in variable host' organs. These several processes including cell proliferation, differentiation and reproduction are hypothesized to be controlled by a complex system of gene expression. Using bioinformatics and genomic analyses, scientists were able to identify novel therapeutic targets. Like *Caenorhabditis elegans*, a free-living and experimental model for nematodes, *Mesocestoides corti* is a validated cestode experimental model. It inhabits mice' peritoneal cavity and does not infect man and is easily cultured. On the other hand, histone deacetylase (HDAC) enzymes, validated drug targets for cancer, are known to directly affect cellular chromatin structure leading to the regulation of gene transcription. It is well known that DNA expression is regulated by acetylation and de-acetylation. HDAC enzymes remove acetyl group from histone permitting histone to tightly wrap DNA, and inhibit DNA expression. According to their sequence and structure similarity, HDACs are grouped into four classes in which classical HDACs include classes I, II, and IV with similar catalytic domains having zinc as cofactor, whereas class III, known as sirtuins, includes enzymes related to yeast. Class I includes HDACs 1-3 and 8, while class II includes two subtitles; IIa for HDACs 4, 5, 7, and 9, and IIb for 6 and 10. Only HADC 11 is assigned to class IV. **Hugo R. Vaca** *et al.*, from Argentina were intrigued by reports utilizing HDAC enzymes in treatment of schistosomiasis as well as reports characterizing HDACs in *E. multilocularis* genome. This prompted them to identify and characterize genes encoding classes I and II HDACs in cestodes. Using *M. corti* larval stage as an experimental model, the researchers attempted to demonstrate the inhibitory potency of Trichostatin A (TSA), as a pan-HDAC inhibitor.

Using bioinformatics, the investigators were able to identify genes encoding 6 and 7 classical HDACs enzymes (three from class I and 3-4 from class II). Their wide transcriptional expression levels throughout several developmental stages demonstrated their

essential role in parasite' survival and growth. Furthermore, the investigators observed different transcriptional expression levels within a single genus. In *E. granulosus, Egr*HDAC1 is the most copious with the highest transcript in all developmental stages, while *Egr*HDAC8 is only expressed in adults during egg production (onchosphere), and in hydatid cyst wall during protoscolices formation. While in *E. multilocularis*, *Emu*HDAC1 is the most abundant with the highest transcript only in oncospheres and cyst wall developmental stages. *Emu*HDAC8 transcriptional expression levels particularly increased in activated oncospheres; an observation that suggested an essential role for HDAC8 in development and survival of *Echinococcus* spp. Accordingly, complete nucleotide sequences of genes encoding HDAC8 in *E. canadensis*, *M. corti* and *Homo sapiens* were conducted. Results revealed particular structural features in both parasites' HDAC8 with more than 55% divergence from that of *Homo sapiens*. HDAC8 was previously reported as a drug target in the treatment of schistosomiasis *mansoni*. Similarly, the investigators strongly validated HDAC8 as a drug target in cestodes.

Investigations of the effect of TSA in different concentrations, as pan HDAC inhibitor, on the viability of *M. corti* larval stages in comparison with PZQ and ALB showed similar results regarding tegmental alterations and viability loss. Utilizing Western blot with anti-pan-acetylated-protein antibodies, the investigators observed hyper-acetylation of histone H4 six days after TSA treatment, coinciding with decrease of *M. corti* larval stage viability. In addition, they observed two bands (~14 kDa) indicating hyper-acetylation of histone H2A or H3 in *M. corti* larval stage. Finally, the investigators recommended further studies to develop new HDAC inhibitors to be investigated in the treatment and control of hydatid cyst, one of the neglected tropical diseases. Compiled from **"Histone deacetylase enzymes as potential drug targets of neglected tropical diseases caused by cestodes." Int J Parasitol Drugs Drug Resist 2019 Apr; 9:120-132.**

Filariasis: Among the neglected tropical diseases (NTDs) documented by WHO, onchocerciasis caused by *O. volvulus*, and lymphatic filariasis caused by *W. bancrofti*, *B. malayi*, and *B. timori* are controlled by mass drug administration (MDA) including three drugs; diethylcarbamazine (DEC), ivermectin, and albendazole. However, in sub-Saharan Africa where co-infection with loiasis is common, and much concern should be taken for fear of fatal encephalopathy commonly occurring with ivermectin. Besides, the diverse side effects resulting in severe skin reactions with DEC in lymphatic filariasis, and blindness in onchocerciasis necessitate development of novel drugs. Both diseases are grouped in NTDs due to scarcity of alternative chemotherapeutic agents that specifically target only *O. volvulus*, *W. bancrofti*, and *Brugia* spp. It is documented that these nematodes depend on *Wolbachia* bacterial endosymbiosis for survival and fecundity. Adult permanent sterilization followed by interruption of disease transmission with slow killing of adults without adverse fatal effects, were reported in prolonged tetracycline regimens.

Accordingly, **Rachel H. Clare** with 30 contributors from UK, USA and Sweden hypothesized that screening inhibitory compounds targeting *Wolbachia* will enable them to develop a novel drug against onchocerciasis and lymphatic filariasis. Meanwhile, the industrial scale collaboration between anti-*Wolbachia* (A·WOL) association and Astra Zeneca's global high-throughput screening (HTS) center validated using an insect cell line (C6/36) infected with *Wolbachia* to be screened against 1.3 million compounds simultaneously. It is worth mentioning that contributors of the present compilation were chemists. The investigators used *Aedes albopictus* derived cell line (C6/36) infected with *Wolbachia pipientis* for screening and human monocytic THP-1 cell line for monitoring cytotoxicity. First, chemist investigators excluded compounds with well-known unwanted characters (toxic or risky explosive). Based on molecular weight and solubility, chemists classified compounds into 57 clusters at least three compounds each. Then, these clusters were screened to identify IC_{50}. Only the most potent clusters were screened against microfilaria collected from *B. malayi*-infected gerbils. Utilizing the strategy of industrial scale anthelmintic NTD HTS campaign, the investigators spent only two months instead of four years to screen 1.3 million compounds. They were able to identify nine hit series as novel antibacterial chemotypes. Among them was 4-piperidino pyrimidines, selective inhibitors for cholesterol biosynthesis pathway. Interestingly, *Wolbachia* are known to induce cholesterol homoeostasis in the infected cells. Furthermore, profiles of the 9 HTSs were investigated regarding potency, physicochemical properties as well as drug metabolism and pharmacokinetic (DMPK) properties. The investigators recommended future development of these multiple chemotypes to deliver improved, safe and more selective filaricidal drugs. Compiled from **"Industrial scale high-throughput screening delivers multiple fast acting macrofilaricides." Nat Commun 2019 Jan 2; 10(1):11.**

Schistosomiasis: It is well established that mitogen-activated protein kinase (MAPK) signaling pathways contribute in regulation of cellular processes such as growth, metabolism, apoptosis, and immune responses. MAPK signaling pathways, stimulated with extracellular stimuli, induce sequential phosphorylation to activate other proteins, causing cellular changes according to the transcriptional profile. Among four subfamilies of MAPK signaling pathways, p38 MAPK interacts with several regulatory mechanisms related to homeostasis resulting in several events such as stress responses,

inflammation and apoptosis. Among nine MAPKs detected in *S. mansoni*, *Sm*p38 MARK was identified in two isoforms (*Sm*p38.1 and *Sm*p38.2). It was found that stimulation of *Sm*p38 MARK by extracellular stimuli such as light and temperature during cercarial shedding, is followed by transcriptional changes responsible for host invasion *via* skin penetration. Also, the essential role played by *Sm*p38 MARK is documented by production of antioxidants to neutralize humoral and cellular cytotoxic factors as well as oxidative stress factors generated by either mammalian definitive or snail intermediate hosts. Therefore, **Livia das Graças Amaral Avelar** *et al*. from Brazil decided, in the present compilation, to elucidate role(s) of *Sm*p38 MAPK signaling pathways in their mammalian hosts aiming to identify their target gene(s). They hypothesized that the gene(s) could be drug targets for schistosomiasis.

To achieve their goal, *S. mansoni* life cycle stages were maintained through passages between *Biomphalaria glabrata* and Golden hamsters. Sequence of *Sm*p38 MAPK was obtained, amplified, and cloned to synthesize double-stranded RNAs (dsRNAs). Schistosomula and adult worms were exposed to dsRNAs for variable time periods and their *Sm*p38 MAPK expressions among *S. mansoni* life cycle stages were measured using quantitative real-time PCR. In addition, schistosomula were observed daily for movement, color and tegument integrity, while adult worm motility was observed for 10 days and coupled worms were cultured to count laid eggs. The investigators extended their studies *in vivo* using dsRNAs-exposed schistosomula to infect Swiss mice. This was followed by counting adult worms and hepatic eggs compared to control schistosomula treated with nonspecific dsRNA. A second *in vivo* study was conducted to evaluate male and female maturation, and their morphological characteristics, as well as egg maturation stages. Swiss mice were infected and grouped into three groups; untreated schistosomula, nonspecific dsRNA-treated, and Smp38 dsRNA-treated. For evaluation of egg and miracidium viability, a third *in vivo* study was performed in which infected Swiss mice were grouped into three groups (nonspecific dsRNA, *Sm*p38.1-and *Sm*p38.2-treated).The obtained eggs were examined for viability and used to infect *B. glabrata* snails. Miracidia viability was also measured, and snails were examined for cercarial shedding. SB 203580 inhibitor that was previously tested against miracidial *Sm*p38, was used in schistosomula cultures using different concentrations. After exposure to the selected inhibitor, susceptibility of adult worms with inhibited *Sm*p38 activity against oxidative stress factors were investigated using an estimation of glutamate-cysteine ligase expressed in non-treated worms. Finally, the investigators used RNASeq approach to identify gene(s) encoding *Sm*p38 MAPK pathway.

Results revealed a significant reduction in the transcription level induced by the gene encoding *Sm*p38 MAPK. *Sm*p38 MAPK has a significant role on *S. mansoni* regarding both survival and reproduction, as well as interactions against cytotoxic and oxidative stress factors generated by its host. Tegmental damage, underdeveloped ovaries in females, and increased number of immature eggs and non-*via*ble miracidia were observed in parasites depleted of *Sm*p38 MAPK. Similar observations were obtained using SB 203580 inhibitor. In addition, results of investigating *Sm*p38 MAPK activity against oxidative stress factors confirmed that wild parasites evolved strategies to evade host immune system through regulation of several enzymes such as thioredoxin 1, glutathione peroxidase, glutathione-S-transferase, methionine sulfoxide reductase and others to act against oxidative stress factors. Using RNASeq approach to identify gene(s) encoding *Sm*p38 MAPK signaling pathway, the investigators identified the expression of several genes. These included genes encoding structural functions (synthesis of tubulin, collagen type 1 and proteins required for ribosomes, spliceosomes, mitochondria and cytoskeleton), and those encoding purine and pyrimidine metabolism, as well as genes encoding expression of enzymes utilized by *S. mansoni* to evade host immune responses. Hence, *Sm*p38 MAPK signaling pathway is a multigene drug target.

The investigators concluded that *Sm*p38 MAPK signaling pathway is essential for *S. mansoni* survival and homeostasis, as well as in regulating genes responsible for structural development, purine and pyrimidine metabolism pathways as well as antioxidant defense. Therefore, *Sm*p38 MAPK is considered a promising attractive therapeutic target for the treatment and control of schistosomiasis. Compiled from **"*Sm*p38 MAP kinase regulation in *Schistosoma mansoni*: Roles in survival, oviposition, and protection against oxidative stress." Front Immunol 2019 Jan 24l 10:21.**

Leishmaniasis: Due to adverse toxic side effects of the drugs commonly used in the treatment of *Leishmania*sis, and existence of resistant strains and genetic diversity of *Leishmania* species in some areas such as Brazil and North Africa, there is an urgent need to develop novel drugs. As described in previous compilations (PUJ 2016; 112-117 for toxoplasmosis and visceral *Leishmania*sis, and PUJ 2018; 118-121 for malignant malaria), protein kinases (PKs) have an essential role to phosphorylate amino acids contributed in several regulatory pathways and signal transduction. Therefore, PKs are essential enzymes for cell development and parasite survival and growth, hence they are potential drug targets. According to the type of phosphorylated amino acid, eukaryotic PKs are classified into two main groups, serine/threonine and tyrosine PKs. During 2002, in another proposed classification based on sequence similarity eight groups were identified, while the ninth group included several

other kinases that do not fulfill common characters of the eight groups. In *Leishmania* spp., as with eukaryotic cells, PKs not only have a conserved structure, but also have conserved function(s). **Joyce V.B. Borba** *et al*., from Brazil, USA, Ukraine, and UK, hypothesized that identification of *Leishmania* kinome, the complete set of genes encoding PKs identified by genomic analysis, will help to develop efficient inhibitor(s) that specifically act on PKs activity.

Using refined bioinformatics pipeline approach, the investigators first compared PKs detected in *L. infantum* and *L. braziliensis*, as model references for cutaneous and visceral leishmaniasis, respectively. Detected PKs were classified, and the unknowns were predicted utilizing identified kinomes of other similar kinetoplastids, e.g. *L. major, T. brucei*, and *T. cruzi*. This was followed by construction of multiple phylogenetic trees keeping only the catalytic domains for automatic multiple sequence alignment. This approach helped the investigators to confirm correct grouping of unknown PKs. After PKs classification and using Gene Ontology (GO) databases, function(s) were assigned for each PKs group. In addition, PKs present in both *Leishmania* spp., and absent in *Homo sapiens* kinome were identified to select target PKs that can be used for chemotherapy. The investigators searched for efficient inhibitory compound(s) against the identified PKs activity for the next *in vivo* evaluation (bone marrow derived macrophages of infected BALB/c mic). The investigators observed the number and viability of intracellular amastigotes, and calculated EC_{50} for each compound.

Results revealed the identification of a higher comparable number of eukaryotic PKs (224 and 221 in *L. infantum* and *L. braziliensis* genomes, respectively) representing ~2.5% of their genomes. It is worth mentioning that it was estimated to be ~2%, 1.9%, and 1.5% in other *Leishmania* spp., *S. mansoni* and *P. falciparum*, respectively. This comparison highlighted the importance of phosphorylation process undertaken in both *Leishmania* spp. It was observed that detected PKs belonged to six groups with absence of three groups; tyrosine kinase, tyrosine kinase like, and receptor guanylate cyclase groups. Assigned functions for the detected PK groups were cell growth and death (36%); signal transduction (31%); environmental adaptation (7%); metabolism (6%) and 20% for other functions; translation, transcription, motility and cell community (4% each), catabolism (2%) and morphogenesis and protein folding and sorting (1% each). In addition, comparing structural sequence with *H. sapiens* PKs revealed 61% divergence, and the investigators were able to select eight PKs as potential drug targets according to their assigned functions. Among them, seven were previously proposed as drug targets in *T. brucei*; aurora kinase 1 (essential for cell proliferation), casein kinase 1 (regulating signal transduction pathway), two cyclin-dependent kinases (regulating transcription), glycogen synthase kinase 3 (mediating serine and threonine phosphorylation), Wee-1 like kinase (the key regulator of cell cycle progression), and polo-like kinase (contributing in cytokinesis, and meiosis). Searching for compounds to inhibit PKs activity revealed only seven compounds and the selection was based on compounds that have not been previously reported in literature as anti-leishmanial drugs. Among them, three compounds were proposed. "Trametinib" showed significant potency against amastigotes of *L. braziliensis* and *L. infantum* with LC_{50} at 15 and 30 µM, respectively. Interestingly, Trametinib is used as chemotherapeutic agent in anaplastic thyroid cancer targeting mitogen-activated extracellular signal-regulated kinase 1. Also, it is the same drug target (MARK) in the previous compilation for treatment of schistosomiasis *mansoni*. Two other compounds were suggested; NMS-1286937 targeting polo-like kinase1 with excellent oral bioavailability, and R1530 targeting both PKs (aurora and polo-like kinase4).

Based on the obtained results, the investigators validated the approaches utilized in their study as an ideal model for novel drug development. They recommended future studies to identify more significant PKs inhibitors in experimental *Leishmania*sis model. Compiled from **"Unveiling the kinomes of *Leishmania infantum* and *L. braziliensis* empowers the discovery of new kinase targets and anti-Leishmanial compounds." Comut Struct Biotechnol J 2019 Feb 8; 17:352-361**.