Review Article

Recent advances in identification of potential drug targets and development of novel drugs in parasitic diseases. Part III: Helminths

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ABSTRACT

The term "magic bullet" was chosen to explain how a drug kills the pathogen with a greater affinity and specificity to its target without effecting its orthologue in human. The ideal parasite target is a molecule essential for its viability, growth, proliferation, and differentiation. The majority of commonly administered anthelminthics target either neuromuscular elements, or metabolic pathways. On the other hand, genomics studies combined with transcriptome analyses revealed advanced information in understanding host-parasite interactions that yielded clues for new strategies in identification of new parasite targets and designing novel inhibitors. In fact, next generation sequencing technology provides unique opportunities to understand parasites molecular biology and their role in parasite-host interactions. Besides, drug repurposing is a promising approach to expand the pool of molecules with potent inhibitory activity against a specific parasite target. The present review aims to highlight the proposed potential helminth drug targets with special emphasis on the major tropical diseases such as schistosomiasis, hydatid cyst, lymphatic filariasis, onchocerciases and soil transmitted diseases. The review also highlights hypothesized potential targets for future development of novel anthelminthics utilizing aminoacyl-tRNA synthetase and the 20S core proteasome, as well as new strategies targeting parasitic response to overcome host mechanistic target of rapamycin.

Keywords: drug repurposing; drug targets; hydatid cyst; lymphatic filariasis; novel anthelminthics; onchocerciasis; schistosomiasis; soil-transmitted diseases.

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Abbreviations: ABC: ATP-binding cassette; **AchE:** Acetylcholine esterase; **CYP450:** Cytochrome P 450; **DR:** Drug repurposing; **ES:** Excretory secretory; **FDA:** Food Drug Administration; **HDAC:** Histone deacetylase; **HTS:** High-throughput screening; **MAPK:** Mitogen activated protein kinase; **miRNA:** Micro-RNA; **mTOR:** Mechanistic target of rapamycin; **nAchRs:** Nicotinic acetylcholine receptors; **PPIs:** Protein-protein interactions; **PZQ:** Praziquental; **SAR:** Structure-activity relationship; **Sir:** Sirtuin; **TK:** Tyrosine kinase.

INTRODUCTION

The relatively well-developed, highly regulated, fully functional neuromuscular system constitutes a large proportion of cells and, gene expression of the multicellular helminths due to its essentiality for motility, survival, development, and reproduction. In fact, molecules with essential roles in neuromuscular system are the prime drug targets in the majority of available antihelminthics. Notably, neuromuscular functions rely on coordinated networks of receptors, neurotransmitters, transporters, and intracellular signaling pathways. Besides, these networks are integrated with metabolic pathways required for several cellular process. An American scientist reviewed several nicotinic acetylcholine receptors (nAchRs) and ATP-binding cassette (ABC) transporters in helminths and linked them with their respective inhibitors for development of novel antihelminthics[1].

On the other hand, a British reviewer focused on glycolytic enzymes employed in metabolic pathways that utilize carbon compounds to produce energy essentially required for pathogen survival and reproduction. In addition to energy production, metabolic pathways contribute to removal of metabolites *via* chemical reactions. Specific inhibitors of enzymes responsible for removal of toxic metabolites are ideal candidates for drug development. Discussing metabolic enzymes, the reviewer suggested phosphoglycero-mutase (PGM) an ideal target because it differs in structure, sequence and mechanism of action from the host enzyme. It was concluded that comprehensive understanding of enzymes biochemical reactions would facilitate identification of novel parasite targets[2]. Recently, Italian investigators suggested Kennedy pathway as potential drug target in *Schistosoma* spp. This pathway is utilized to initiate *de novo* synthesis of phosphatidylcholine, an essential component for tegument development and its turnover in all developmental stages. *In vitro* studies showed that the anti-anginal drug, perhexiline maleate exhibited potent inhibitory activity against all developmental

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stages. When the investigators studied metabolomics, at baseline and after treatment, they identified that its mechanism of action is *via* inhibition of Kennedy pathway[3].

To encourage studies for designing novel antihelminthic drugs, three recent reports proposed novel potential drug targets in helminths.

1. In the RNA synthesis process, aminoacyl-tRNA (aa-tRNA) synthetase, known as tRNA-ligase, is the enzyme that attaches a specific amino acid to its corresponding tRNA. In humans, 20 types of aa-tRNAs are formed by 20 aa-tRNA synthetase, one for each amino acid of the genetic code. Comprehensive computational analyses of aa-tRNA synthetase (aaRS) enzyme from 27 helminths revealed that several helminth aaRSs were potential drug targets for drug repurposing (DR). Indian investigators validated their hypothesis, and demonstrated that several inhibitors, e.g. Cladosporin, Halofuginone, and Benzoborale were bound with high selectivity and affinity to the catalytic domains in the helminth aaRSs[4]. In a recent report, German reviewers tabulated advantages of utilizing DR strategy over conventional approaches used in *de novo* drug discovery and development. It is more efficient, saves time, cost, and efforts, and reduces failure risk, with increased success probability *in vitro* and *in vivo* studies[5].

2. Host mechanistic target of rapamycin (mTOR) is linked with host immune response, and autophagy, and their essential roles against several pathogens. Notably, mTOR belongs to phosphoinositide 3-kinase (PI3K)-related kinase family responsible for controlling cell signaling, and functions employed in essential cellular processes, i.e., metabolism and autophagy. To achieve its function, mTOR utilized two different intracellular protein complexes (mTORC1 localized within endosomal and lysosomal membranes, and mTORC2 at the plasma and ribosomal membranes). The main function of the former is controlling cell growth and proliferation *via* organelle biogenesis, and protein synthesis, while that of the latter is regulation of cell survival *via* maintenance of actin cytoskeleton structures and cell metabolism. Therefore, inhibitors of mTOR pathway, e.g. rapamycin were used in treatment of cancer, diabetes, organ transplantation, coronary restenosis, and rheumatoid arthritis. A team of scientists reviewed the implications of mTOR signals on pathogens and their modulation to escape or overcome host mTOR. The reviewers discussed different parasite-host interactions in which the former induces dynamic metabolic changes through upregulation of host genes expression involved in cell catabolism, i.e., altering host mTOR. Therefore, identification of the relevant parasitic interactions with their essential roles in pathogenicity, immunopathology, as well as parasites survival, and proliferation will certainly lead to recognition of potential targets and subsequent development of novel therapeutic strategies[6].

3. American reviewers proposed proteasomes, i.e., the 20S core proteases, as potential drug targets since their inhibitors were validated in treatment of tuberculosis. In all eukaryotes, ubiquitin proteasome system, known also as proteasome complex, is assigned for degradation of cellular unrequired proteins utilizing the cytosolic and nuclear 20S core proteases, i.e., nucleophile hydrolases. Notably, ubiquitin is a tag molecule that marks the target protein for degradation. Since human proteasome is a clinically validated drug target for hematologic neoplasms, the reviewers compared structures of the 20S core proteasome of *M. tuberculosis*, and the parasites causing neglected tropical diseases aiming to encourage scientists to investigate proteasome inhibitors as promising novel therapeutic agents. Regarding helminths, the reviewers reported the studies utilized proteasome inhibitors against *S. mansoni, E. granulosus, E. multilocularis,* and *T. solium*[7].

***Schistosoma* spp.**

In silico high-throughput screening (HTS) enabled the investigators to suggest five drugs approved by Food Drug Administration (FDA) predictive novel drugs in treatment of schistosomiasis for future studies. They included Aprindine, an anti-arrhythmic drug, Tetrabenazine, an inhibitor of vesicular monoamine transporter type 2, Griseofulvin, a fungistatic drug, and two calcium channel blockers (Cinnarizine and Clotrimazole)[8]. Utilizing bioinformatics tools, and employing chemogenomic analyses, Brazilian investigators searched in several online databases for FDA-approved drugs targeting energy metabolism enzymes. *In silico* DR enabled them to identify 20 potential drugs against schistosomiasis *mansoni*. Among them, the investigators proposed four drugs, Fomepizole, Desflurane, Isoflurane, and Alendronate for further *in vivo* studies and future clinical trials[9]. Utilizing a similar approach, Australian investigators predicted ten drug targets in *S. haematobium*. They recommended further studies using functional genomics tools to establish their essentiality for parasite survival[10].

On the other hand, microRNAs (miRNAs) are endogenous, non-coding single-stranded RNAs (18–25 nucleotides) that regulate several cellular processes, e.g., cell division, differentiation, apoptosis, and immune metabolism, as well as embryonic development. Due to their specific and complex regulatory effects, miRNAs are potential drug targets. It was observed that *S. japonicum* miRNAs exhibited different expression patterns of miRNA-124-3p at

different developmental stages, significantly higher in cercaria than in schistosomula, juveniles than in adults, in males than in females, and in immature females than in mature ones. Utilizing RNA interference, a study was conducted to validate the essentiality of *Sj*miR-124-3p encoding gene for *S. japonicum* survival, growth, development, and reproduction. The investigators claimed that the balanced expression of *Sj*miR-124-3p in different developmental stages was required for development and reproduction through regulation of neurosecretory cells releasing essential molecules for survival. In contrast, its overexpression inhibited parasite antioxidants production leading to growth impairment[11].

Nucleic acids synthesis and gene expression: Pyrimidine *de novo* biosynthesis in schistosomes differs from that in the host in type of pyrimidine nucleobase and nucleoside transporters. Therefore, exclusive schistosome enzymes, their substrate specificity, and molecules involved in pyrimidine *de novo* synthesis are potential drug targets. However, several alternative pathways exist in schistosomes that challenge development of one specific inhibitor to overcome schistosome creativity and ingenuity to escape pyrimidine biosynthesis blockage[12].

A phenotypic screening of *S. mansoni* with series of benzoxaboroles identified a number of human cyclic nucleotide phosphodiesterase (PDE) inhibitors inducing parasite hyper-motility and degeneration. In the last step of purine and pyrimidine biosynthesis, PDEs hydrolyze cyclic adenosine and guanosine monophosphates (cAMP, and cGMP) to produce 5'-AMP and 5'-GMP, respectively. Benzoxaboroles are FDA-approved drugs for treatment of chronic diseases, e.g. obstructive lung diseases and Alzheimer. American investigators identified, characterized, and sequenced four genes encoding PDEs (A-D) in *S. mansoni*. To validate *Sm*PDE4A a potential drug target, the investigators cloned *Sm*PDE4A to express its C-terminal catalytic domain utilizing the nematode model (*C. elegans*) as a transgenic expression system[13]. Recently, eleven genes encoding PDEs identified in *S. mansoni* genome were sequenced and the investigators succeeded in cloning ten of them in the transgenic expression system utilizing *S. cerevisiae* and *T. brucei*. Their expressions in male and female adults, juvenile flukes, and schistosomula were determined. The study confirmed that PDE1, PDE4A, PDE8, PDE9A and PDE11 were involved in cyclic nucleotide degradation. While PDE11was mostly specific for cGMP, PDE4A displayed a dual activity for cAMP and cGMP degradation. It was concluded that the obtained results were essential to facilitate development of selective inhibitors of these regulatory key enzymes[14].

On the other hand, a series of aryl-thiazole derivatives was synthesized and investigated for their inhibitory activity against DNA biosynthesis in schistosomes *in vitro*. Evaluating parameters included schistosomula and adult viability, motility, mortality, and scanning electron microscopy (SEM) study for tegumental alterations. Expression of genes involved during egg biosynthesis and production were also evaluated using quantitative PCR. Results demonstrated that thiazole derivatives were promising novel anti-schistosomal drugs, however further studies were recommended to lower their cytotoxic effects on human cells[15].

Histone modifying enzymes: Four FDA-approved drugs, Vorinostat, Panobinostat, Belinostat, and Romidepsini were investigated as histone deacetylase (HDAC) inhibitors in treatment of schistosomiasis *mansoni* in comparison to praziquental (PZQ). *In vitro* efficacy against schistosomula, and *in vivo* studies in experimentally infected mice investigating worm and egg burdens were conducted. Variable results were obtained, however Romidepsin showed similar PZQ potency against adult worms viability and egg production withiout efficacy against juveniles[16]. A HTS of 1500 HDAC inhibitors identified three compounds with lethal potency against viability of both schistosomula and adults[17]. Virtual screening against *Sm*HDAC8 crystal structure enabled the same group of investigators to identify two inhibitors (spiroindolines and thieno[3,2-b]indoles). Both showed *Sm*HDAC8 inhibition in low micromolar concentration with minimum selectivity and affinity toward human HDACs, reduced the viability of schistosomula, juveniles, adults, and egg burden; and induced lethal morphological changes in the reproductive system[18].

Recently, a group of British investigators conducted three studies to validate that modulation of schistosome' histone methylation homeostasis offered a promising new generation for anti-schistosomal drugs. Homology modelling of histone demethylase (HDM), i.e. lysine specific demethylase 1 (*Sm*LSD1, *Sm*p_150560) and *In silico* docking studies, identified ten compounds with specific binding activity to *Sm*LSD1 in comparison to that of *Homo sapiens*. They were tested against schistosomula, and the most active inhibitor (Pirarubicin) was subsequently tested against juveniles and adults. It significantly suppressed egg production with impaired motility, and tegumental alterations, however, moderate cytotoxicity was recorded[19]. Utilizing a similar study design on histone methyltransferase (HMT); i.e. mixed lineage leukemia-1 (*Sm*MLL-1; *Sm*p_138030), the investigators demonstrated *Sm*MLL-1 essentiality for miracidia → sporocysts transformation, schistosomula survival, adult motility and egg production. Furthermore, they observed that all identified selective *Sm*MLL-1 inhibitors contained a 1,3,5-triazine core linked to a piperazine ring, as a chemical scaffold[20]. Previously, the investigators utilized structural genomics consortium to select 34 epigenetic probes (EPs) and

three epigenetic inhibitors (EIs) targeting *S. mansoni* HMT and HDM. After *in vitro* screening against schistosomula phenotype and motility, only thirteen Eps, and one EI were selected for further screening on adults, and cytotoxicity studies. Accordingly, two EPs targeting HMT (*Sm*p_000700) and HDM (*Sm*p_034000) were subjected to gene knockdown. Results revealed that both targets were essential for adult motility and egg production for the former, and oviposition and vitelline glands for the latter[21]. On the other hand, fourteen HDMs were described in *S. mansoni* that exhibited crucial activities in cercarial transition, and sexual differentiation of adults. Utilizing molecular docking and molecular modeling studies, the investigators selected *Sm*p_034000, and its predictive specific site was identified. A specific *Sm*HDM inhibitor showed significant *in vitro* potency against adult worms and schistosomulae. Ultrastructural results revealed marked reduction in adult motility and viability as well as loss of muscle fibers architecture[22].

Regarding *S. mansoni* sirtuin (SIR2), two inhibitors against human SIR1/2 (Sirtinol and Salermide) were investigated. As observed in DNA fragmentation, both inhibitors induced schistosomula and adult worm death with reduced egg production[23]. Another SIR selective inhibitor was identified (TCMDC-143295) and a series of analogs were developed that showed improved potency and selectivity without cytotoxicity[24].

Protein kinases (PKs): A team of American and Brazilian investigators conducted a large-scale RNA interference analysis accompanied with experimental phenotypic study to identify the functions of only 20% of *S. mansoni* genes. Interestingly, ~10% (250 genes) were essential for the parasite survival including neuromuscular functions, and tissue integrity. The investigators ranked drugs with potent inhibitory activity against these gene targets, and pointed out two genes encoding PKs as promising epigenetic targets, serine-threonine PK (STK25), and TAO[25]. Notably, the latter is termed so since it has a thousand and one amino acids.

Gonads of *S. mansoni* possess two tyrosine kinases (TKs) known as Abelson murine leukemia (Abl), *Sm*Abl1 and *Sm*Abl2. Since Imatinib was used in cancer therapy, i.e. it has a potent affinity to bind with human Abl-kinase inhibiting cell growth and replication; it was suggested as novel anti-schistosomal drug. However, the results were unsatisfactory because host albumin (SA) and α-1 acid glycoprotein (AGP) negatively influenced Imatinib binding affinity. Therefore, *in vitro* studies were conducted to evaluate the effect of SA and AGP on Imatinib efficacy against adult worms and schistosomula. Accordingly, the investigators recommended further studies solving this problematic blockage before validation of Imatinib in treatment of schistosomiasis[26]. An innovative computational DR approach accompanied by experimental *ex vivo* phenotypic screening enabled the investigators to validate several TKs in schistosomes potential drug targets. Results revealed several anti-cancer drugs; Imatinib was the most potent inhibitor, and several kinase inhibitors such as Bosutinib, Crizotinib, Nilotinib, Vandetanib, Saracatinib, Tideglusib, and Dasatinib came next with variable inhibitory activity against schistosomula, juveniles and adults. Kinomics analyses of the three *Schistosoma* spp. demonstrated that calcium/calmodulin-regulated kinase (CAMK) was the most abundant followed by AGC group, while TK-like (TKL) was uncommonly identified. The investigators listed several *S. japonicum* and *S. mansoni* PKs assigned as validated drug targets for 16 FDA-approved drugs[27]. A study was conducted to investigate the role of *S. mansoni* TK (Feline sarcoma, FES), highly expressed in miracidia and cercariae. Results revealed its essentiality for miracidia viability, schistosomula development and survival, adults pairing, and female maturation[28]. Wu *et al*.[29] claimed in their review that *Schistosoma* TKs were identified in two types: receptors, and non-receptors (cytosolic TKs). The first included epidermal growth factor receptor (EGFR), a transmembrane glycoprotein, with high tegumental transcriptional expression activity. Two homologs of insulin receptor (IR1 and IR2) were characterized with different expression activities. While *Sj*IR-1 was mainly expressed in the tegument basal membrane and muscles, *Sj*IR-2 was expressed in female vitelline tissue and male parenchyma. Therefore, to develop a safe drug utilizing *Sj*IR inhibitors, it is important to explore their roles in relation to host IRs[29].

In *Schistosoma* spp., *Sm*p38 mitogen activated PK (MAPK) was observed with high transcriptional expression activity during cercarial shedding, suggesting its essential role in host skin penetration. Its essential role was also documented by production of antioxidants to neutralize host oxidative stress factors. Therefore, *Sm*p38 MAPK was suggested as a promising attractive therapeutic target for the treatment and control of schistosomiasis[30]. Besides, *Sj*CAMK II was significantly associated with increased PZQ resistance, whereas three members of cyclin-dependent kinases (AGC group) were also identified without assigned specific functions. The latter includes cyclic adenosine, guanine, and cytosine MP-dependent PK (PKA, PKG, and PKC, respectively). Atypical PK (Riok-2) was essentially involved in RNA biogenesis and cellular processes. Its transcription level was localized in the vitellarium and ovary, and its bioinformatics analysis revealed its high potentiality as drug target. The reviewers recommended further studies exploring AGC signaling pathways and functions, and claimed that combined application of CRISPR-Cas9 technology with virtual screening would accelerate development of novel anti-schistosomal drugs[29].

Because *S. mansoni* polo-like kinase 1 (SmPLK1) was highly expressed in sporocysts and adults reproductive

organs, anti-cancer agents were investigated. Selective human PLK1 inhibitors exhibited reduction in oocysts and spermatocytes in female and male reproductive organs, respectively. Utilizing structure-activity relationship (SAR) enabled the investigators to identify BI2536, a benzimidazole thiophene inhibitor that showed high efficacy against schistosomula and juveniles[31]. Chlorambucil, an alkylating agent with anti-cancer activity, exhibited *in vitro* and *in vivo* anti-schistosomal properties. The drug showed significant decrease in worm burden, intestinal and hepatic egg counts, and progressive reduction of viability of both juvenile and adult schistosomes[32]. Future studies to investigate other PKs inhibitors used as anti-cancer drugs in treatment of schistosomiasis *japonicum* were recommended including Genistein, Sorafenib, Bosutinib, Crizotinib, Nilotinib, and Dasatinib[29]. Notably, among 52 FDA-approved drugs, 21 are multi-kinase inhibitors[33].

Cytochrome P 450 (CYP450): Genomic sequence analysis of *S. mansoni* revealed a single *cyp450* gene with only 22% sequence identity to that identified in human. It is worth mentioning that CYP450s are heme-containing monooxygenases involved in interaction with cellular oxygen and relevant substrates. Therefore, it is essential for synthesis of membrane sterols, cholesterol and ergosterol, and metabolism of prostaglandins and fatty acids, essentially involved in cell signaling. American investigators observed that its open reading frame has a heme-binding region in its catalytic domain and an overall structural conservation. To validate its essentiality for parasite survival, double stranded RNA silencing was performed in schistosomula that led to complete death. Adults and developing eggs treatments with a CYP450 inhibitor (Miconazole) resulted in *S. mansoni* death, and arrest of embryonic development, respectively. Furthermore, a SAR study was conducted and revealed that Miconazole activity and selectivity could be improved by a rational drug design[34].

Transporters: Since ABC efflux transporters, e.g. P-glycoprotein (ABCB1), are linked with multidrug resistance, an American reviewer recommended combining PZQ with ABC inhibitors to increase schistosome susceptibility to PZQ. He also hypothesized that inhibition of ABC transporters might alter several essential physiological functions such as permeability barriers, transport of signaling molecules, excretory activity, reproduction, and host immunoevasion as well, i.e. acting as anti-schistosomals on their own[35]. On the other hand, tegumental molecular targets (potential proteins and receptors) were reviewed in schistosomes to identify the targeting compounds. The reviewers hypothesized that surface functionalization of nanoparticles with inhibitors would specifically bind with tegumental molecules. Schistosome glucose transporters (SGTP-1 and SGTP-4) and aquaporin-4 were suggested as potential drug targets since they facilitate glucose uptake for energy production, and control osmotic regulation, respectively[36].

Proteases: A recent study evaluated the efficacy of two cathepsin peptidomimetic inhibitors (vinyl sulfones, WRR-286 and WRR-391) against *S. mansoni* cathepsin B1 (*Sm*CB1). Using crystallographic analyses, the investigators identified their binding mode with *Sm*CB1. Furthermore, the inhibitory potency of both vinyl sulfones was tested against human cathepsin B, and cultured newly transformed *S. mansoni* schistosomula. Since WRR-286 exhibited an extensive degree of selectivity for *Sm*CB1, and significant degenerative effects with better plasma stability, it was proposed a lead compound for future optimization[37]. South African reviewers hypothesized that identification of protein–protein interactions (PPIs) in *Schistosoma* spp. would facilitate developing inhibitors to decrease transmission, and prevent or cure schistosomiasis. Essential roles played by several molecules expressed by multiple developmental stages revealed identification of cercarial elastase (CE), a serine protease, as potential drug target. It was observed that cercaria in contact with human skin, release CE from the acetabular gland complex to digest dermal elastin, keratin, fibronectin, laminin and collagen IV and VIII, facilitating skin invasion. Since Elafin is a novel substrate for elastase, i.e. a selective serine protease inhibitor, the reviewer recommended its application on the skin prior to water source contact[38].

Endogenous protease inhibitors: A number of genes encoding serpins, endogenous serine protease inhibitors, differ in number among *Schistosoma* spp., one, three and eight genes in *S. haematobium*, *S. japonicum*, and *S. mansoni*, respectively. South African reviewers proposed three of them promising drug targets. The first is schistosomula endogenous serpin (SrpQ) expressed immediately after skin invasion regulating CE expression and protecting schistosomula from its own elastase. Second, schistosomula and adult protease inhibitor (PI56) was reported to degrade host neutrophil elastase that plays a crucial role in development of innate immune response. To survive long time in host venous plexus, *Schistosoma* spp. express KI-1, a Kunitz-type protease inhibitor. Its tegumental localization confirmed its essential function; protecting adults from the continuous contact with host coagulation factors as well as host immune mediators. Expression of KI-1 in eggs was also observed and it was attributed to egg protection either in mesenteric veins (against host immune mediators) or in intestine (against host digestive enzymes). Therefore, use of selective KI-1 inhibitors results in egg digestion, and prevention of fibrotic schistosomiasis sequelae. On the other hand, *Sj*B10 was expressed in all developmental stages, but because its function is still hypothetical, the reviewers recommended further studies elucidating its role as potential drug target. Although genes encoding schistosomal serpins showed

low similarity (~50%) to their host orthologues, and were functionally characterized since a decade, no further studies were conducted investigating their use as potential drug targets[38].

Heat shock proteins (HSPs): In their review to identify molecules involved in PPIs in *Schistosoma* spp., the reviewers indicated that HSP60, accompanied with its co-chaperonin HSP10, were expressed in all developmental stages to overcome stress conditions. Notably, HSP60 is responsible for protein folding *via* stabilizing peptides, a crucial step in developmental stages differentiation. The reviewers recommended combination of selective HSP60 inhibitors with PZQ however; they claimed absence of reports regarding *Sm*HSP60 crystal structure. Therefore, no inhibitors targeting *Sm*HSP60 were developed yet[38].

Tetraspanins (TSPs): They were identified in the schistosomal tegument to maintain its plasma membrane structure and, were suggested potential drug targets. The reviewers recommended surface functionalization of nanoparticles with TSPs inhibitors for development of a new anti-schistosomal drug[36]. In addition to maintenance of tegument integrity, the TSEs through their interaction together, mediate regulation of signaling pathways for migration of the juvenile flukes. Therefore, TSE inhibitors were proposed promising novel anti-schistosomal drugs[38].

Specific targets

Apical tegument proteins: Adult schistosomes possess a hepta-laminate tegumental surface, continuously repaired and replaced in order to survive against the host immune responses. In addition to TSPs identified in tegumental plasma membrane, annexins (Anxs) are abundant molecules in *Schistosoma* proteome. Due to their involvement in membrane turnover and maintenance, they are interesting drug targets and/or a vaccine candidate. In their review, Leow *et al*.[39] discussed members of Anxs family capable to bind with acidic phospholipid membranes in several eukaryotes. It is worth mentioning that *S. mansoni* possess 13 Anxs expressed differentially throughout its body surface, and structures internal epithelia. Among them are Anx-B7a, B22 and B30 in the syncytial tegument, B7 and B22 in the gut lining epithelium, and B5 in the vitelline gland. Since the crystal structure of *Sm*-Anx-B22 which is strongly localized in adult's apical regions of the tegument, demonstrated an α-helical segment, a cysteine residue in its motif loop, and canonical calcium binding sites, the reviewers proposed it a promising ideal drug target[39].

Neuromuscular transmitters: Acetylcholine esterase (AchE) is known to control neuronal functions through its rapid breakdown of acetylcholine (Ach), a neurotransmitter in both central and peripheral nervous systems of eukaryotes. Schistosome tegument utilizes nAchRs to regulate host glucose-uptake. Bioinformatics analyses identified several AchE isoforms in *S. haematobium* (10), and *S. japonicum* (4). However; *S. mansoni* possesses the highest number of cysteine-loop motifs in nAchRs (13), as binding catalyzing sites, in comparison to *S. haematobium* (6) and *S. japonicum* (3). Accompanied with the availability of extensive genomic data of AchE and nAchRs in *Schistosoma* spp., the reviewers recommended further studies to discover novel drugs against schistosome nAchRs[40]. Australian researchers investigated ruthenium compounds, AchE potent inhibitors that cause Ach accumulation leading to unorganized neuromuscular functions and death due to respiratory paralysis. Synthetic ruthenium compounds were *in vitro* tested against schistosomula and adult, as well their effects on egg hatching and development. Based on the obtained results, two inhibitors (Rubb7-tnl and Rubb12-tri) were selected for further investigations on worm burden, egg burden and hatching, and glucose-uptake ability, as well as a cytotoxicity study. Although the results were satisfactory in comparison to PZQ, the investigators recommended further studies to modify ruthenium synthetic compounds for development of better selective inhibitors[41]. Later, surface functionalization of nanoparticles with AchE, and nAchR antibodies was strongly recommended as novel therapeutic candidate against schistosomiasis[36].

Since schistosome neuromuscular transmitters include biogenic amines (BAs), a Canadian study was conducted to immunolocalize BAs major component, octopamine (OA) and its precursor tyramine (TA) in *S. mansoni* adults and schistosomulas. Results revealed that OA was strongly localized in two pairs of ganglia in schistosome head, nerve chords along the gynecophoric canal flaps in males, ovary and embryo within the *Schistosoma* egg in females. They tested 29 drugs against schistosomulas motility and length, and according to their phenotypic studies, they selected only three drugs, Chlorpromazine (CPZ), Carvedilol (CAR) and Propranolol (PR) to conduct concentration-response assays. Results revealed that CPZ gave the best results where motility and length dramatically increased followed by complete paralysis. The investigators recommended further *in vivo* studies as well as widening the search for more compounds for future investigation against schistosomulas and adult schistosomes[42].

Thioredoxin glutathione reductase (TGR): It is an essential enzyme for schistosome' survival and was identified in the genomes of *S. mansoni* and *S. japonicum*. In fact, TGR plays a central role in redox regulation and homeostasis as it maintains sufficient levels of glutathione and thioredoxin required for several biochemical processes. To survive for a long time in their host, schistosomes produce antioxidant molecules to neutralize reactive oxygen species (ROS) generated either from activated host immune response or during stress conditions. American reviewers

claimed that antioxidants in schistosomes included cytosolic superoxide dismutase (SOD), thioredoxin, glutathione-S-transferase (GST), glutathione peroxidase (GPx), TGR, and peroxiredoxin. Since glutathione and thioredoxin were synthesized *de novo* in schistosomes, and all antioxidants were investigated as vaccine candidates, the reviewers recommended further studies investigating compounds with selective inhibitory activity against glutathione and/or thioredoxin biosynthesis pathways as alternative chemotherapeutic agents in PZQ-resistant strains[43]. Utilizing X-ray crystallography combined with docking studies, an allosteric site of *S. mansoni* TGR was identified. Notably, members of Flavin adenine dinucleotide (FAD)/nicotinamide adenine dinucleotide (NAD)-linked reductase family are fundamental enzymes maintaining redox homeostasis in several eukaryotes, with a similar catalytic mechanism to that identified in TGR. Besides, they are well-known targets of anti-cancer and anti-inflammatory drug development. Therefore, the investigators recommended further studies to design selective TGR inhibitors utilizing FAD/NAD reductase family members[44]. Since previous studies validated the selective inhibitory activity of oxadiazole-2-oxide analogs against *Sm*TGR and *Sj*TGR of juvenile and adults *in vitro*, a Chinese study investigated 39 novel synthesized furoxan derivatives in treatment of schistosomiasis *japonicum*. Docking and SAR studies revealed that several new derivatives exhibited greater affinity toward recombinant *Sj*TGR binding site. Significantly, compound (6d) with trifluoromethyl on the pyridine ring has the highest activity and the investigators recommended its validation for the phase I clinical trial[45].

Proteasome complex: Utilizing large-scale gene expression microarrays, Brazilian researchers investigated the effects of a proteasome inhibitor (MG-132) on gene expression of *S. mansoni* adults *in vitro*. The investigators performed *in silico* functional analyses of the affected genes, followed by a scanning electron microscopy study to record the molecular events underling the phenotypical changes in *S. mansoni* development and sexual differentiation, as well as its tegumental alterations. Two issues were observed, first, MG-132 not only inhibited the 20S core proteases, but also activated gene expression of 26S proteasome; a result suggesting that the treated flukes recovered proteasome function *via* synthesis of new proteasome units. Second, MG-132 also downregulated the proteasome maturation protein gene (*Sm*p_074160), two chaperone molecules involved with proteasome regulatory complex (Hsm3 and Nas6), and a number of genes encoding ion channels. This, in return, suggested failure of the proteolytic activity recovery with subsequent cellular damage[46].

Universal stress proteins (USPS): They are proteins that enable schistosomes to tolerate diverse stress condition. Interestingly, no genes encoding USPS are characterized in the human genome, rendering them potential drug targets in *Schistosoma* spp. It was hypothesized that schistosomula utilize USPs to avoid oxidative stress expressed by host nitric oxide and hydrogen peroxide production. Search or development of USPs inhibitors might be promising novel anti-schistosomal drug[47].

Cestodes

Cestodes lack the ability to synthesize fatty acids and cholesterol *de novo*; hence, they possess highly expressed genes involved in uptake and transport of lipids from the host such as fatty acid binding proteins, and transporters. Molecules expressed in the excretory/secretory (ES) products, e.g., HSPs, glycosylphosphatidylinositol (GPI)-anchored proteins, and antigen B, proved their essential role in evasion of host immune response. Besides, other factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF), and insulin-signal transduction cascades proved their role in signaling pathways[48]. Recently, Argentinian researchers investigated *in vitro* twenty HDAC inhibitors (classes I and II) against *Mesocestoides vogae* larval stage, as a cestode model. Results showed significant viability decrease with loss of integrity and extensive tegumental damage. Two benzhydroxamate derivatives (Class I) were selected (TH65 and TH92) due to their low cytotoxicity effects for further assays. Both compounds significantly exhibited lower IC_{50} values in comparison to albendazole. In addition, both compounds were used in combination with ABZ, and the results revealed potent cestocidal effects in a time-dependent manner. Further studies were recommended to evaluate their efficacy in treatment of murine models of hepatic hydatid cyst and neurocysticercosis[49].

***Echinococcus* spp.**

Comparative genomic analyses of *E. granulosus*, *E. canadensis*, and *E. multilocularis* showed genetic diversity, and occurrence of single nucleotide polymorphism sites (SNPs) in the genes encoding drug intake and metabolic enzymes. Although *E. granulosus* and *E. canadensis* belong to *sensu lato* complex, SNPs in the genes encoding drug intake and metabolism were observed more frequently than those recorded in *E. canadensis* and *E. multilocularis* (different species complex). The investigators also observed that amino acids sequences of certain proteins in *E. canadensis* were more different in *E. granulosus* than in *E. multilocularis*. This poses an opportunity for novel drug development for tratement of hydatid cyst in endemic areas with several *Echinococcus* spp. These proteins included leucine-like protein, ubiquitin-conjugating enzyme, and glycosyl transferase; essential enzymes for PIPs, cell signaling, and glycan biosynthesis, respectively[50].

Nucleic acids synthesis and gene expression: Recent evolutionary technology enabled the investigators to characterize, on molecular basis, the germinal layer.

Expressions of several genes that allowed mitosis, developmental transitions and asexual proliferation were demonstrated. It is worth mentioning that tubulin is the drug target of benzimidazole (BNZ)-based chemotherapy with limited effects on the germinal layer, a fact that explains the high recurrence rates. An extensive characterization of the stem cell system in *E. multilocularis* germinal layer revealed differentiated cells, e.g., tegumental, glycogen and nerve cells, while proliferating cells increased during early brood capsule and protoscolex development with sharp reduction in number after complete development. This indicates that a large number of germinal layer cells are present in the mature protoscolex, but with slow cell cycle kinetics as long as the protoscolex remains within the metacestode stage. Accordingly, ribonucleotide reductase inhibitors combined with BNZ was proposed as a novel strategy for treating echinococcosis targeting both germinal layers cells, and tubulin[48].

Since miRNAs play a major role in gene expression regulation in several biological cellular processes, a group of investigators characterized miRNA expression profiles in *E. granulosus*[51], *E. canadensis*[52], *Mesocestoides vogae*[53], *Taenia* spp.[54], and *Hymenolepis* spp.[55]. Recently, the same group of investigators[56] analyzed the expression profile of miRNAs in *E. multilocularis in vitro*, and the results revealed five points. First, expression of 37 miRNAs, among them miR-71, miR-4989, miR-9, let-7, miR-10, and miR-1 were highly expressed. Second, the first two miRNAs were absent in the host. Third, miR-71 was expressed in the germinal layer cells of metacestodes cultured *in vitro*. Fourth, MiR-4989 was involved in hydatid cyst survival and development. Finally, all highly expressed miRNAs were conserved in other cestodes, suggesting essential roles in development, survival, and host-parasite interaction. According to *in silico* functional analyses of *E. multilocularis* miRNAs, the investigators concluded that these predicted targets certainly would help to identify selective novel therapeutic drugs for treatment and control of alveolar hydatid cysts[56].

Histone modifying enzymes: A higher density of gene markers (CpG sites) was observed in *Echinococcus* spp. than detected in the mammalian genomes. Notably, CpG sites occur with high frequency in genomic regions called CpG islands, i.e., DNA regions (at least 200 bp) in which guanine and cytosine exceed 50%. Characterization of these CpG sites in *Echinococcus* spp. revealed lower numbers of histone DNA methyltransferases, and methyl-CpG binding domain proteins than observed in the mammalian hosts[50]. Wide transcriptional expression levels throughout several developmental stages of *E. granulosus* and *E. multilocularis* revealed HDAC8 essentiality in parasite' survival and growth as well as hydatid cyst development. Both *Eg*HDAC8 and *Em*HDAC8 were expressed in adults during egg production and in hydatid cyst wall during protoscolices formation. Sequence analysis of the encoding genes showed high similarity with more than 55% divergence from that of *Homo sapiens*. The inhibitory potency of trichostatin A (TSA), as a pan-HDAC inhibitor was investigated in comparison with PZQ and ALB, and it showed tegumental alterations and viability loss[57].

Molecular machinery of programed cell death (apoptosis and autophagy): It is well known that endoplasmic reticulum (ER) is the central structure in eukaryotes that participates in calcium handling, and storage essentially required for proteostaisis (protein hemostasis), synthesis, and transportation. In fact, disturbance in ER functions generate ER stress and activate intracellular signal transduction pathway, i.e. unfolded protein response to restore proteostaisis An Argentinian study investigated the efficacy of Bortezomib (Bz), a proteasome inhibitor, on *E. granulosus* protoscoleces and metacestodes *in vitro*. Results revealed that Bz exhibited potent pharmacological and dose-dependent effects and killed 50% of protoscoleces and metacestodes after 96 h treatment. The investigators showed that Bz induced ER stress, autophagy and subsequent death. Utilizing Western blot and *in toto* immunofluorescence labeling, the investigators demonstrated increased transcripts of the autophagy related genes (ATGs 6, 8, 12, 16). Accordingly, the investigators suggested that Bz treatment activated autophagy process in both larval stages, and subsequently proposed proteasome inhibitors novel drugs for treatment and control of cystic echinococcosis[58].

Cytochrome C complex (Cyt c): Enzymatic assays showed that mitochondria of *E. multilocularis* possessed two genes encoding Cyt c reductases for the aerobic pathway. Both reductases (NADH, and succinate) were assigned on Cyt c I and II complex, and Cyc c II and III complex, respectively. Japanese investigators observed that combined atovaquone (ATV), a complex III inhibitor, and atpenin, a quinone binding site inhibitor of complex II, completely killed protoscoleces *in vitro*; while ATV oral administration significantly reduced primary alveolar hydatid cyst development *in vivo*. Therefore, Cyt bc1 complex was proposed a potential drug target for development of novel therapy against alveolar hydatid cysts[59]. In the same year, the same investigators demonstrated that neither PZQ nor artemisinin killed *E. multilocularis* protoscoleces under both aerobic and anaerobic culture conditions. Under only aerobic conditions however, combined therapy of PZQ or artemisinin with ATV induced significant elimination activity, and reduced the time required for hydatid cysts elimination compared with ATV alone. The investigators also recorded that pyrvinium pamoate, an antihelminthic against pinworms, inhibited the growth of protoscoleces (100%) at 6 d, and 95% at 7 d under aerobic and anaerobic conditions, respectively[60].

Protein kinases (PKs): Protein kinase inhibitors, e.g. pyridinylimidazoles (targeting MAPK)[61], and Imatinib (targeting Polo-like kinase)[62] exhibited inhibitory activities *in vitro*. It is worth mentioning that MAPK pathway is a conserved signal transduction pathway utilized to transmit extracellular signals, e.g. cytokines, hormones, and growth factors. It is activated through consecutive phosphorylation reactions[63]. In 2018, a Chinese study identified two MAPKs in *E. granulosus sensu stricto* (*Eg*MKK1 and *Eg*MKK2) expressed at the larval stages. Both MAPKs exhibited binding activity with a substrate of myelin basic protein, e.g. sorafenib tosylate (PD184352), and U0126-ethanol (EtOH). Both inhibitory substrates suppressed MAPKs phosphorylation leading to cytolytic effects on protoscoleces *in vitro*. The investigators recommended further studies to increase their oral bioavailability[64].

Transporters: Recently, Amahong *et al*.[65] proposed glucose transporter 1 (GLUT1), responsible for the transmembrane transport of glucose, a promising drug target in *E. granulosus*. They cloned *Eg*GLUT1 encoding gene from *E. granulosus sensu stricto* and observed its essentiality for glucose uptake and protoscoleces viability. They also demonstrated that WZB117, a reversible competitive inhibitor, blocked glucose uptake and decreased ATP level with subsequent inhibition of metacestode viability *in vitro*. In experimentally infected mice, a 10 mg/kg dose of WZB117 exhibited significant reduction of number and size of hydatid cysts as efficiently as albendazole. In addition, no change was observed in either body weight or blood glucose level in the treated mice for 4 weeks[65].

Tetraspanins (TSPs): To characterize TSPs in *E. granulosus*, a Chinese study cloned uroplakin-I-like TSP and immunolocalized *Eg*TSP1 in the tegumental surface of adults and larvae using specific polyclonal antibody. Utilizing RNA interference, the investigators observed *Eg*TSP1 essentiality for the tegument biogenesis, development and maturation, as well as stability and maintenance of the membrane structural integrity. They also assessed the cytokine profiles in experimentally infected mice previously immunized with recombinant *Eg*TSP1. Significant elevated levels of IgG1, IgG2a, interferon (IFN)-γ and interleukin (IL)-12 were recorded, suggesting that *Eg*TSP1 elicited a T helper1-mediated immune response[66]. Using dsRNA-based interference technology, similar results were obtained when a study conducted in Iran demonstrated that silencing expression of *Eg*TSP1 led to significant reduction of protoscoleces viability with dramatic tegumental changes. Besides, after three days of dsRNA interference, the investigators performed an ultrastructural study revealing impairment of the highly specialized microvilli covering the entire cestode surface, i.e., microtriches, with vacuolated tegument compared to the control adults, not treated with dsRNA interference[67].

Specific targets

Excretory/secretory (ES) products: Since thioredoxin-glutathione reductase (TGR) was one of the major components of ES proteins in *Echinococcus* spp., a study conducted in Uruguay showed that TGR inhibitors exhibited satisfactory results against hydatid cyst at concentrations of 20 μM[68]. Later, a Chinese study identified thioredoxin peroxidase (TPx), an antioxidant enzyme, expression in ES proteins of all developmental stages of *E. granulosus*. After intraperitoneal infection of mice with *E. granulosus* larvae, the investigators demonstrated that *Eg*TPx significantly induced peritoneal macrophage recruitment and activation *via* an alternative (PI3K/AKT/mTOR) signaling pathway. As previously described, PI3K is phosphoinositide 3-kinase (PI3K)-related kinase family, and AKT is PK B. Since the activation of PI3K/AKT/mTOR pathway was suppressed by pre-treatment with an AKT/mTOR inhibitor, the investigators suggested that the alternative activated macrophages might play an essential role in hydatid cyst establishment in the host[69]. On the other hand, computational analysis of *E. multilocularis* ES proteins identified 617 out of 673 putative proteins, i.e. 91.7% supported by transcription analyses[70]. This simply means that ES proteins play an essential role in survival, growth and development of hydatid cyst, hence potential drug targets. A Chinese study identified 383 ES targets with no sequence similarity with host proteins, and the investigators proposed them ideal diagnostic markers, drug targets and vaccine candidates[70].

Nematodes

The majority of commercially available nematocidal drugs, i.e. Levamisole, Pyrantel, Ivermectin, and Monepantel, activate members of ligand-gated ion channels superfamily. Utilizing molecular docking studies on five genes encoding acetylcholine gated chloride (AGC) channels in *C. elegans* as representative nematode model, Canadian investigators validated AGC channels potential drug target[71]. On the other hand, metabolic enzymes are potential drug targets in almost all pathogens, as previously discussed. Utilizing chemogenomics screening against the developed homology model of nematode carnitine palmitoyltransferase 2 (CPT2), the investigators identified several compounds with potent inhibitory activity on mammalian CPTs. It is worth mentioning that CPTs are mitochondrial membrane enzymes facilitating the transport of cytoplasmic long chain fatty acids into the mitochondrion to be oxidized with subsequent energy release. The investigators selected only three hit compounds and synthesized several analogs for further *in vitro* evaluation. Using molecular modeling studies and SAR analyses, their efficacy were evaluated against a broad variety of nematode species with different modes of parasitism: *T. muris, A. ceylanicum, Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* as intestinal nematodes, and *Brugia pahangi* as a tissue nematode. Results revealed variable

inhibitory potency that was attributed to variability in the cuticle membrane penetration and uptake. The study recommended further studies to optimize their pharmacokinetic properties to be efficiently used in treatment of both intestinal and tissue nematodes[72].

Filarial nematodes

***Wuchereria bancrofti* and *Brugia* spp.**

In 2012, *B. malayi* transcription profile and gene expression analyses in different life cycle stages revealed that 27% of the identified transcripts were expressed in all stages studied. Among them, the study identified novel five transcription patterns associated with parasite lifecycle stages or gender. The investigators validated their essentiality in several biological functions involved in *B. malayi* development, survival, and reproduction, as well as lymphatic filariasis establishment and maintenance. It was concluded that changes in gene expression were highly regulated and finely tuned across life cycle stages. In microfilaria transmitted from mosquitoes to host, there was increased expression of genes encoding molecules required for host invasion, larval molting, and immunoevasion. There was increased expression of genes encoding reproduction of adults residing in their host. The investigators recommended further proteomic studies and genes sequencing to identify potential drug targets[73].

Due to difficulties in cultivation of filarial worms *in vitro*, a British investigator and her colleagues[74] developed a novel approach to perform HTS for anti-parasitic drugs against several *B. malayi* potential drug targets using yeast cultures (engineered *Saccharomyces cerevisiae* strains). Based on this approach, the investigators replaced essential yeast genes with filarial or human genes to assess drug efficacy without harmful effects on the host. Yeast strains were labeled with different fluorescent proteins to identify the drug with specific inhibitory activity on *B. malayi* drug targets, not on its human orthologue. Accordingly, 15 *B. malayi* enzymes were selected, cloned, and transferred to yeast strains after deletion of their target genes. Yeast strains were cultivated and monitored for their growth in the presence of *B. malayi* and human genes to identify enzymes controlling yeast growth. The perfect enzyme, i.e. promising drug target, was the one that inhibited yeast growth with expression of *B. malayi* genes, but not human orthologues. Results revealed eight *B. malayi* enzymes that were screened again against 400 inhibitors. The investigators succeeded to identify nine specific inhibitors among them five exhibited *in vitro* anti-filarial activity against *B. pahangi*. Using this yeast-based screening platform approach proved efficient to specify inhibitory compounds against filarial enzymes only[74].

Recently, a study recommended Oxfendazole, a complementary treatment of choice to eliminate lymphatic filariasis, in particular the endemic foci with lower prevalence. The investigators claimed that short-term treatment with Oxfendazole, a macro-filaricidal drug, could considerably reduce the timeframe of mass drug administration programs[75]. In fact, the Horizon 2020/EU project (www.eliminateworms.org) is currently planning for Oxfendadazole use in phase I clinical trial. More recently, since *B. malayi* female head region contains cells and tissues controlling secretory, sensory and reproductive behaviors, American investigators conducted a RNAi and tissue-specific (spatial) transcriptomics study to map gene expression patters at the host-parasite interface. Results revealed membrane-anchored antigens expressed in the intestinal tract, and receptors associated with ES pore, and vulva. The investigators proposed these receptors potential novel drug targets due to their essential role in controlling neuromuscular functions, host-parasite communication, and *B. malayi* fertility[76].

Nucleic acids synthesis and gene expression: Recently, Brazilian investigators utilized yeast-based approach to determine the crystal structure of deoxyhypusine synthase (DHS) that catalyzes the translation factor 5A (eIF5A) in *B. malayi*. It is worth mentioning that eIF51 is essentially required for initiation, elongation and termination of protein synthesis translation. *In vitro* phenotypic assays were conducted utilizing HTS to identify new efficient DHS inhibitors. The study succeeded to identify spermidine analogues that exhibited DHS inhibitory activity. Spermidine, originally isolated from semen, is a polyamine compound found in ribosomes and living tissues with various metabolic functions. Unfortunately, *Bm*DHS crystal structure showed high similarity with the human orthologue that might challenge development of selective DHS inhibitors[77].

Protein kinases (PKs): In 2015, *in vitro* assessment of efficacy of Imatinib, Dasatinib, and Nilotinib, tyrosine kinase inhibitors, on *B. malayi* males, females, L3, and microfilariae was performed. Three-dimensional modelling showed that Dastinib exhibited inhibitory activity against the assayed stages with a lower LC_{50} value. Imatinib showed more or less similar LC_{50} against microfilaria and L3 larvae, but higher LC_{50} values against adult stages. In contrast, Nilotinib exhibited inhibitory activity with higher concentrations for all stages except for males (higher than Dasatinib, but lower than Imatinib). In spite of the obtained results, the investigators recommended *in vivo* assessment of Imatinib due to its human safety administration as anti-cancer therapy[78].

N-myristoylation enzymes: Due to high similarity of functional genomic data, and genome sequence conservation between *C. elegans* and *B. malayi*, a study validated N-myristoyltransferase (NMT) potential drug target. The investigators conducted a detailed

molecular study on the NMT enzymes from both nematodes. Utilizing chemical inhibition studies with the NMT inhibitor (DDD85646) and other related derivatives strongly supported repurposing NMT inhibitors for development of novel drugs against lymphatic filariasis[79].

Transporters: Notably, Emodepside is marketed in two drugs, Profender© and Procox©, in combination with PZQ, and Toltrazuril, for deworming control of dogs against helminthic and protozoal infections, respectively. An attempt to explore its efficacy and mode of action in treatment of lymphatic filariasis was conducted recently. Results evidenced that Emodepside targeted potassium channels (SLO-1) and it was more potent on *B. malayi* males than females. Knockout studies showed that females expressed two SLO-1 isoforms (F and A) while males expressed only the F isoform. Expression of both isoforms in *Xenopus laevis* oocytes showed that SLO-1F was more sensitive to Emodepside than SLO-1A alone or in its combination with SLO-1A. The study emphasized that sex-dependent effects should be considered on evaluating he efficacy profile of a new drug candidate[80].

Proteases: In 2014, a study characterized the effects of *Wolbachia* endosymbiosis (number and viability) on the expression levels of two cysteine proteases (CP1 and CP6) in *B. malayi*. *In vitro* killing of *Wolbachia* using tetracycline treatment resulted in differential expression of both CPs, with subsequent alterations in *B. malayi* survival and growth. Accordingly, the investigators proposed CP1 and CP6 potential drug targets[81].

Specific targets

Ecdysone receptors (EcRs): They are specific drug targets in filarial nematodes. Ecdysteroids and EcRs are essential for larval development during which their levels significantly vary, i.e. levels are high before the third and fourth molts. Screening approach with cytotoxicity studies identified only two compounds (Ponasterone A and Muristerone A). Both drugs showed a significant increase of aborted eggs, embryos and microfilariae in comparison to 20-hydroxyecdysone, a potent EcRs agonist[82].

Calcium-binding protein (calumenin, CALU): Korean scientists proposed the CALU potential drug target in filarial nematodes due to its essential role for cuticle development, and its malfunction increased drug entry. Besides, amino acid sequences of filarial CALUs shared well-conserved structural features, but not with human CALU. Molecular docking with structure-based virtual screening using commercially available FDA-approved drugs were conducted. Itraconazole, an inhibitor of sterol biosynthesis as well as anti-fungal agent, was selected as a potential drug to bind and specifically inhibit nematode CALUs[83].

***Wolbachia* endosymbiosis** is another potential drug target specific for filarial nematodes. Since bacteria contain a transcription elongation factor, also known as transcription cleavage factor (GreA), a study hypothesized that *Wolbachia* GreA a potential drug target for treatment of lymphatic filariasis. The gene encoding GreA in *Wolbachia* that exists only in prokaryotes was sequenced, cloned, overexpressed, and characterized. Its three-dimensional structure was predicted, and the investigators demonstrated that *Wolbachia* GreA had an essential role in filarial survival, development, and pathology[84]. A HTS was conducted against a cell line infected with *Wolbachia*, and human monocytic cell line for monitoring cytotoxicity. Among the potent compounds (nine hits) that were screened against *B. malayi* microfilaria, 4-piperidino pyrimidines exhibited a potent inhibitory effect as a filaricidal drug[85]. To analyze the metabolic pathways in *B. malayi*, the investigators generated a network representation with the previously published stage-specific transcriptomic data for both *B. malayi* and its *Wolbachia* endosymbiont. Utilizing flux balance analysis, Kyoto encyclopedia of genes and genomes (KEGG) reaction database, online database for predicting biodegradation and biosynthesis, followed by several *in silico* knockouts enabled the investigators to predict 102 metabolic reactions involved in cellular metabolic processes essential for parasite survival. Three known drugs; Fosmidomycin, MDL-29951 and Tenofovir were investigated to inhibit isoprenoid precursor biosynthesis, gluconeogenesis, and purine metabolism, respectively. Besides, all drugs were tested against *B. malayi* adults *in vitro*, and the results revealed significant reduction of *Wolbachia* number/worm. Fosmidomycin and Tenofovir reduced fecundity with significant decrease in microfilariae number/worm, however, only Fosmidomycin decreased microfilariae motility[86].

Intestinal cell adhesion protein (LAD-2): In 2019, American investigators[87] utilized proteomic analyses of *B. malayi* body wall, gut, and reproductive tract to identify nine proteins with high homology in other filarial adults and a specific function in adults' viability and survival. Among them was uridine 5'-diphospho (UDP) glucuronosyltransferase that was inhibited by Probenecid, a drug used in gout treatment. This enzyme is a microsomal metabolic enzyme required for LAD2 biosynthesis, a cell adhesion molecule (CAM) that serves as phosphorylation site in signal transduction[87]. Later, the same group of investigators performed *lad2* gene knockout (RNA interference) and the results revealed significant decrease in motility and reduction of microfilaria release. The study demonstrated complete loss of both microvilli and pseudocoelomic fluid with untied mitochondrial cristae in the intestinal epithelium. The investigators hypothesized that suppression of CAM (LAD2) disrupted the intestinal tract tight junction leading to loss of pseudocoelomic

fluid, a lubricant medium for nutrition exchange and cellular signaling during mitotic cell proliferation, i.e. blocking cell proliferation with subsequent induction of apoptosis[88].

Trehalose-6-phosphate phosphatase (T6PP): This enzyme is required for the synthesis of trehalose and is utilized by bacteria, fungi, plants, and invertebrate animals as an energy reserve to protect them against oxidative and osmotic stress. In *C. elegans*, T6PP proved essential for the parasite survival since its inhibition resulted in T6PP accumulation, i.e. toxic effects on *C. elegans*. American investigators[89] determined *Bm*T6PP crystal structure and utilized structure-guided mutagenesis, combined with kinetic analyses to identify its conserved residues for binding sites. The investigators claimed that identifying T6PP binding sites supported possible development of broad-spectrum inhibitors with high binding affinity to T6PP pocket[89].

Onchocerca volvulus

Two limitations face control and elimination programs for onchocerciasis. First is its coinfection with loaiasis in endemic African countries because Ivermectin treatment is contraindicated in patients with high level of *L. loa* microfilaremia, i.e. leading to severe meningoencephalitis. Second, identification of potential drug targets in *O. volvulus* is challenged by adult worm maintenance in culture for long time. Understanding its biology system and the factors supporting its longevity in human certainly would lead to sufficient and efficient knowledge of specific gene expression and transcriptomic profiles, with subsequent identification of novel potential drug targets. Recently, a study developed and applied a 3-dimensional culture system utilizing engineered *in vitro* human skin and adipose tissue to simulate human *in vivo* hosting. The investigators recommended further studies to optimize the culturing conditions that improve the long-term development of adults *in vitro*[90]. However, a study conducted in 2019 validated Mongolian gerbils (*Meriones unguiculatus*) an animal model for experimental infection with *O. ochengi* male adults collected from infected cattle[91].

After studying aminoacyl tRNA synthetases (aaRSs) distribution in 27 human infecting helminths, Indian investigators[4] modelled *O. volvulus* aaRSs and investigated the efficacy of selective aaRSs inhibitors: Cladosporin, Halofuginone, Benzoborale and Borrelidin. They also demonstrated the active binding sites by analysis of multiple sequence alignments. The investigators validated *O. volvulus* aaRSs potential drug targets[4].

Novel drugs: A Swiss reviewer[92] discussed the outcomes of several studies conducted in the last decade for assessment of two veterinary drugs (Moxidectin and Emodepside) in treatment and control of human onchocerciasis. Glutamate-gated chloride channels and voltage-gated potassium channels were assigned drug targets for both drugs, respectively. Influx of potassium and chloride ions resulted in paralysis and subsequent parasite death[92]. Later, a group of scientists investigated the efficacy of Emodepside on *O. ochengi*-naturally infected cattle. In a dose-dependent manner, it exhibited direct microfilaricidal activity with potent prolonged or possibly irreversible suppressing effects on fecundity[93]. In an attempt to validate Emodepside for phase I clinical trial, a group of investigators[94] evaluated the safety of its administration in two formula, i.e. liquid and tablet in two randomized groups with comparison to placebo control group. The study also assessed the pharmacokinetics of both drug formulations in 103 healthy volunteer subjects. Overall results revealed that Emodepside had acceptable safety with satisfactory tolerability and pharmacokinetics profiles with a single liquid dose up to 40 mg or 10 mg twice daily for 10 days. Since the liquid formula would not be suitable for feasible clinical use under the conditions expected for patients with onchocerciasis in endemic foci, further studies were recommended to develop better tablet formulation compatible with Emodepside biopharmaceutical properties overcoming the recorded limitations, e.g. poor absorption, and low blood concentration[94].

Flubendazole (FBZ) and Oxfendadazole (OFZ) gained much attention in veterinary medicine due to their potent inhibitory efficacy on β-tubulin, with subsequent inhibition of motility, reproduction and cell secretory processes. Since FBZ was excluded for human use due to its poor tolerance and absorption, only OFZ was allowed for human use against tissue-dwelling larval helminths. In a study conducted by Cho-Ngwa and his colleagues[91] the efficacy of FBZ and OFZ in experimentally infected gerbils was investigated. In comparison to control gerbils receiving placebo, both drugs significantly reduced worm burden, viability and motility[91]. One-year later, *in vitro* studies showed that OFZ exhibited modest to marginal motility inhibition of *O. gutturosa* adults, *O. volvulus* pre-adults and *O. lienalis* microfilariae[75].

Nematodes causing soil transmitted diseases (STDs)

In an attempt to identify novel broad-spectrum chemotherapeutic agent against STDs, an American study[95] screened 1280 FAD-approved drugs against all developmental stages of *A. ceylanicum*, and the free-living *C. elegans* (fourth larval stages and adults). Notably, *C. elegans* is often used as an alternative model for other nematodes causing STDs. The most active compounds were further investigated against *T. muris* adults. The investigators selected two pairs of compounds, sulconazole and econazole (targeting CYP450), and pararosaniline and cetylpyridinium

(targeting HSP90) for further evaluation in hamsters experimentally infected with *A. ceylanicum*. Results revealed that pararosaniline significantly exhibited potent inhibitory activity on hookworm fecundity *in vivo*. The study proposed CYP450 and HSP90 potential drug targets for development of broad-spectrum human STD drugs[95].

Strongyloides stercoralis: Since a decade, a study was conducted demonstrating the first transcriptomic analyses of *S. stercoralis* third larval stage (L3) using next generation sequencing. Based on homology, gene ontology and biochemical pathways, the investigators succeeded to characterize and identify more than eight thousands putative molecules. The investigators claimed that the majority of them served for serodiagnosis and immunization, and proposed only four potential drugs targets with no homologues in the host. They included phosphoglycerate mutase (a metalloenzyme), glutamate synthase, isocitrate lyase (an anabolic enzyme in glyoxylate cycle), and alcohol dehydrogenase I (enzyme for alcohols↔aldehydes interconversion)[96]. Later, proteins expressed in the parasitic and free-living females, and L3, with their putative roles in pathogenesis of strongyloidiasis were reviewed. Transcriptomic comparison between parasitic and free living females revealed significant upregulation of two protein families in the former, astacins, and sperm-coating proteins (SCPs). Other genes including those encoding AchE, aspartic proteases, prolyl oligopeptidases (serine proteases), endogenous protease inhibitors (cystatins, and serpins) and transthyretin-like proteins (TLPs) were also discussed for their predictive role in pathogenesis and virulence. Astacins are zinc-metalloproteases involved mainly in larvae moulting, and skin penetration. Mucosal damage, tissue degradation, and immunomodulation are the main functions for their expression in the parasitic females. It is worth mentioning that SCPs belong to the cysteine-rich secretory proteins superfamily. Similar SCPs were identified in other nematodes, e.g. venom allergen-like in *B. malayi*, and *Ancylostoma*-secreted proteins in *Ancylostoma* spp. The reviewers indicated that SCPs role in parasitism was not recognized yet, however they suggested their role in immunomodulation. On the other hand, TLPs were identified exclusively in nematodes with unknown specific role in parasitism. Therefore, the reviewers recommended further studies to identify SCPs, and TLPs roles, crystal structures, binding sites and substrate preference for development of novel therapeutic drugs[97].

***Ancylostoma* spp.:** In response to hookworm infections, reactive oxygen species (ROS) are released by host immune effector cells. Hemoglobin degradation with subsequent heme detoxification also produce toxic ROS. As a result, peroxiredoxins (PRXs) are expressed from hookworms for protection against ROS because PRXs possess a catalytic cysteine residue reducing ROS to form an intermolecular disulfide bond. Identification of *A. ceylanicum* PRX-1 crystal structure and its biophysical and biochemical analyses allowed American investigators to demonstrate the inhibitory activity of Conoidin A, a human PRX inhibitor, on *Acey*PRX-1. It was observed that Conoidin A inactivated *Acey*PRX-1 through cysteine alkylation maintaining PRX-1 in an unfolded conformation[98]. Later, another American study characterized *A. caninum* nAchR (*Acan*AchR16) by cloning and expressing the receptors in *Xenopus laevis* oocytes. They investigated the efficacy of several cholinergic agonists and antagonists for their inhibitory activity. Results revealed that *Acan*AchR16 was not sensitive to several cholinomimetic anthelmintic drugs such as Levamisole, Oxantel, and Pyrantel, while bromocytisine derivatives, e.g. DHβE and α-BTX exhibited potent and moderate inhibitory activity, respectively. Accordingly, the investigators validated AChR16 a potential drug target in hookworms[99].

***Ascaris* spp.:** A Chinese study identified two molecules essentially involved in spermatogenesis of *Ascaris* spp., a trypsin-like serine protease (TRY-5), and an endogenous serine protease, i.e., serpin (SRP-1). The investigators conducted their study on *A. summ* and demonstrated that *As*TRY-5 was responsible for sperm activation, while two functions were assigned for *As*SRP-1, supportive element for the major sperm protein-based cytoskeletal assembly, and regulatory inhibitory activity on *As*TRY-5 expression. Therefore, it was concluded that both molecules were potential drug targets[100]. In 2021, an American reviewer[101] summarized the comparative genomics and chromosome evolution in *Ascaris* spp., discussing the complete germline genome related to its somatic genome, and transcriptomic datasets. Interestingly, genomic analysis of *Ascaris* adult revealed two distinct genomes, an intact germline and a reduced somatic one. A process of DNA elimination was linked with this reduction (~18%), i.e. parts of the germline genome are lost during germ cells differentiation into somatic cells in early embryogenesis. This programmed DNA elimination of germline genes in somatic cells is specifically directed toward increased gene expression of molecules required for high fecundity, e.g. multiple sex chromosomes, and high transcriptomic levels due to gene expression of several regulatory miRNAs, and histone modifying enzymes. The reviewer recommended further gene sequencing of other new *Ascaris* spp. to elucidate the nature of epigenetic inheritance during gametogenesis, and fertilization, aiming to identify potential drug target or vaccine candidate for this unique nematode parasite[101].

CONCLUDING REMARKS

1. The majority of commonly used anti-helminthics target neuromuscular system (nAch receptors and β-tubulin), metabolic pathways (glycolytic enzymes,

Kennedy pathway), tegument development (proteins and receptors), ABC transporters, and ligand-gated ion channels.

2. In addition to histone modifying enzymes, PKs, ES products, and parasite transporters, recent technology evolution allowed identification of novel potential drug targets such as aminoacyl-tRNA synthetase, parasite proteasomes (20S core proteases), microRNAs (miRNAs), and host mechanistic target of rapamycin (mTOR). Regarding the latter, identifying parasitic interactions towards host mTOR for its survival, pathogenesis, and virulence would lead to identification of potential targets.
3. Utilizing HTS, drug repurposing becomes a new promising approach to increase the number of compounds, i.e. FAD-approved drugs, with potent inhibitory activity against a wide range of potential drug targets. It saves time, cost, and efforts, as well as expands success probability *in vitro* and *in vivo* studies.
4. In *Schistosoma* spp., histone-modifying enzymes (HDAC8 and SIR2), PKs [tyrosine kinases (Abl1 and Abl2), polo-like kinase 1 (PLK1), and mitogen activated PK (MAPK or *Sm*p38)], neuromuscular transmitters (nAchRs), and tegumental tetraspanins (TSPs) attracted much attention as potential drug targets.
5. *Schistosoma* specific drug targets included apical tegument proteins (annexins), thioredoxin glutathione reductase (TGR), proteasome complex, and universal stress proteins (USPS).
6. *Echinococcus* spp. are characterized by high genetic diversity with abundance of SNPs in the genes encoding drug targets and metabolic enzymes. Extensive studies on the stem cell system in the germinal layer of hydatid cysts evidenced its potentiality to be targeted by ribonucleotide reductase inhibitors administered with benzimidazole. Similar to *Schistosoma* spp., HDAC8, PKs (MAPK, Polo-like kinase), TSPs, and TGR (major component of ES proteins) attracted much attention.
7. Due to difficulties in cultivation of filarial worms *in vitro*, yeast-based approach (engineered *S. cerevisiae* strains) was developed. Ligand-gated ion channels are the main drug targets for commercially available nematocidal drugs, and novel drugs investigated in clinical trials nowadays.
8. Tissue nematodes are characterized by complexity in genes expression that are highly regulated and finely tuned across life cycle stages. Therefore, research was directed toward specific drug targets including *Wolbachia* endosymbiosis, EcRs, CALU, LAD-2 and T6PP.
9. Since Emodepside, Moxidectin and Oxfendadazole gained much attention in veterinary medicine; recent trials validated their use in clinical trials for treatment of lymphatic filariasis and onchocerciasis.
10. Cytochrome P 450 (CYP450) was validated a novel potential drug target in STDs, i.e. CYP450 inhibitor (pararosaniline) exhibited potent inhibitory activity on hookworm fecundity *in vivo*. Although sperm-coating proteins (SCPs) and transthyretin-like proteins (TLPs) were identified in several nematodes, their role in pathogenesis and virulence was not identified yet. Further studies are recommended to identify their role, crystal structures, binding sites and substrate preference for development of novel therapeutic drugs.

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