

Spotlights on new publications

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New drug targets - XIX

Plasmodium spp.: Almost all anti-malarial drug resistance is attributed to mutation in genes encoding drug uptake. To overcome this obstacle, allosteric inhibitors were recently investigated. These compounds are able to bind at a site other than the enzyme active site changing its catalytic activity. With such selective and potent inhibition, gene mutation never occurs in the presence of high drug doses since catalytic inhibition does not alter the enzyme active site. Suramin, a drug used in treatment of African sleeping sickness, is a common example of allosteric inhibitors with potent selective catalytic inhibitory activity against rhodesane, the major *T. brucei* cysteine protease. On the other hand, all intracellular protozoa possess functional enzymes for purine synthesis pathway. In contrast, *Plasmodium* spp. lack the enzymes required for salvage pathway of the nucleotide in DNA and RNA synthesis, i.e., pyrimidine. In the present review, **Chao Wang** and her colleagues from Netherland and Brazil drew a figure demonstrating the sex-step process for pyrimidine *de novo* synthesis. Carbamoyl phosphate (CP), formed of bicarbonate, glutamine, and ATP, is synthesized using carbamoyl phosphate synthetase II (CPII). Then, CP is catalyzed to N-carbamoyl-L-aspartate using ATCase. The latter is re-catalyzed by dihydroorotase (DHOase) to dihydroorotate that is oxidized by dihydroorotate dehydrogenase (DHODHase) to form orotate. Orotidine-5'-monophosphate (OMP) is synthesized by combining orotate with 5-phosphoribosyl-1-pyrophosphate (PRPP) using orotate phosphoribosyl transferase (OPRTase). In the final step, uridine monophosphate is produced by OMP decarboxylation using OMP decarboxylase (ODC). Therefore, six enzymes (CPII, ATCase, DHOase, DHODHase, OPRTase, and ODC) are promising anti-malarial drugs in pyrimidine *de novo* synthesis. However, since the reviewers recently identified *PfATCase* crystal structure, they focused their review on discussing studies that dealt with its structures aiming to find a selective pocket in *PfATCase* for development of an allosteric inhibitor. Besides, ATCase attracted much attention in the last decade as a promising drug target in development of novel anti-cancer therapy.

The reviewers first discussed *PfATCase* molecular structure, a 43.3 kDa polypeptide with 375 amino acids.

Three active catalytic sites with two domains in each for binding with substrates L-aspartate, and carbamoyl phosphate, were characterized. It is worth mentioning that any catalytic active site exists in either T or R state. Transition between both states is induced when both substrates are present at their active binding site. However, it was reported that ATCase changed from T state to R state in presence of succinate, an aspartate analog. The reviewers discussed the previously reported ATCase inhibitors. The most potent ATCase inhibitor, commonly used in the last 50 years against *Escherichia coli* and human ATCases was N-(phosphonacetyl)-L-aspartate (PALA). Unfortunately, it showed poor inhibitory activity against *PfATCase in vitro*. Based on the results obtained from a study conducted by Lunev *et al.* (2018), the crystal structure of 2,3-naphthalenediol-*PfATCase* complex was demonstrated. It is worth noting that three reviewers (Wang C, Wrenger C, Groves MR) contributed in this study [Identification of a non-competitive inhibitor of *Plasmodium falciparum* aspartate transcarbamoylase. *Biochem Biophys Res Commun* 2018; 497(3):835–842]. After two years, another group of investigators utilized computational approach to discover an allosteric inhibitor against human ATCase that exhibited significant inhibitory results on cancer cell lines. The reviewers concluded that further understanding of the mechanism of *PfATCase* allosteric inhibition would allow further development of selective potent allosteric inhibitors. Compiled from **“Novel highlight in malarial drug discovery: Aspartate transcarbamoylase. *Front Cell Infect Microbiol* 2022 Mar 4; 12:841833.”**

Leishmaniasis: This disease is one of the major neglected tropical diseases presenting with different clinical manifestations including visceral, cutaneous and mucocutaneous leishmaniasis. Besides, several related clinical complications are described such as post-Kala-Azar dermal leishmaniasis, American tegumentary leishmaniasis, and HIV-coinfection. Leishmaniasis is endemic in India and Brazil and is caused by more than 20 *Leishmania* spp. Several risk factors are responsible for this high endemicity including socioeconomic conditions, several reservoir hosts (stray dogs are the major reservoir host), environmental conditions (change in temperature and humidity), and multiple vector species (*Phlebotomus*

and *Lutzomyia* spp.). Although several studies were conducted to identify novel potential drug targets in *Leishmania* spp., few anti-leishmanial drugs were developed. In addition, drug resistance due to gene mutations is a complex phenomenon since *Leishmania* spp. have unique highly plastic genome with increasing potentiality for aneuploidy. This simply means that amastigotes and promastigotes undergo post-translational modification as key regulators in several cellular processes, to modulate gene expression in stress conditions.

In the present review, **Rubens Lima do Monte Neto** and his Brazilian colleagues asserted that metalloids antimony was the main effective chemotherapeutic drug against leishmaniasis since more than eight decades. Besides, metal-based drugs were widely used in medicine and cancer chemotherapy due to their kinetic properties that allow their use as covalent binders to other compounds, e.g. enzyme inhibitors, and catalytic drugs. The reviewers discussed advances in drug development of anti-leishmanial metalodrugs (ALMDs). Several metals were investigated as antimony alternative; among them were bismuth, ruthenium, platinum, selenium, silver, and gold. Although the results obtained from several studies revealed significant *in vitro* efficacy of ALMDs against antimony-resistant strains of *L. major*, *L. donovani*, *L. amazonensis*, *L. mexicana* and *L. infantum*, few studies were conducted *in vivo* with structure-activity relationship (SAR) studies. Even so, *in vivo* studies lacked protocol standardization among governmental authority, research centers, and pharmaceutical companies. Standardization included experimental animal models, *Leishmania* species (infective dose and administration route), and therapeutic scheme, i.e., toxicological and drug combination approach. Briefly, the reviewers discussed more than 20 studies, conducted in the last decade, investigating ALMDs efficacy with no advance in drug development.

For this reason, the organization of drugs for neglected diseases initiative (DNDI) initiated a website with the philosophy of "No one should suffer from lack of treatment because their disease can't turn a profit". To standardize different studies, DNDI listed the minimum requirements for lead optimization in drug development for treatment of visceral and cutaneous leishmaniasis, African and American trypanosomiasis, and onchocerciasis, as well as other bacterial and viral tropical disease (<https://dndi.org/diseases/>). The reviewers summarized additional optimum standard requirements in the following seven points. First, compounds designed for efficacy assessment should be based on pharmacophore studies, i.e., target-based approaches should be considered prior to synthesis design. Second, although molecular docking and molecular dynamic studies for ALMDs are difficult, utilizing computational technology might overcome

this challenge. Third, high throughput drug screening should be based on drug pharmacokinetics, not phenotypic screenings. Fourth, since MDs interact with thiol or albumin, i.e., interfering with drug availability, investigators should pay much attention to culture medium composition when they test either MDs against *Leishmania* spp. or their cytotoxicity against mammalian cells. Fifth, intracellular amastigotes should be included for phenotypic screening beside promastigotes and axenic amastigotes. Sixth, to save time and efforts, pilot studies should be performed. Seventh, in visceral leishmaniasis, golden hamsters are the first choice for oral drug administration.

Next, the reviewers discussed the evolutionary advance on recent technology in development of novel drugs. In fact, CRISPR/Cas9 allowed the investigators to easily perform gene knockout expanding knowledge of the parasite biology system leading to identification of more potential drug targets. Besides, the association of Cas9 editing coupled with barcode-sequencing helped the investigators in high-throughput screening, hence better validation of gene essentiality. Utilizing CRISPR/Cas9, the investigators observed three surprising points. First, number of identified mutated genes were raised twice (from 209 to 400 genes). Second, 30% of mutated genes (120/400) were observed potentially essential for *Leishmania* viability, growth, and virulence among all species. Third, genes related to post-translational regulation machinery (PTRM) increased from 12% (25 out of 209) to 67% (268 out of 400). This number will certainly increase after complete establishment of LeishGEM project (<http://leishgem.org>). The project uses CRISPR genome modification tools to generate 9000 *Leishmania* gene deletion mutants to determine essential molecules for *Leishmania* survival and virulence, an amazing tool for easy elucidation of potential drug targets. On the other hand, this dramatic increase in genes related to PTRM stimulated the reviewers to propose potential drug targets. They discussed molecules contributing to protein phosphorylation, i.e., protein kinases, and acetylation, i.e., acetyltransferases and deacetylases [previously reviewed by Abaza (PUJ 2022; 15(1): 22-38)]. It is worth mentioning that PTRM utilizes bromodomains, chromodomains, and zinc fingers to recognize the acetylated lysine residues, methylated lysines and arginine on histone, respectively. Moreover, other modifications in PTRM were observed, e.g., arginine methylation and ubiquitination. Regarding the former, *Leishmania* spp. possess seven protein-arginine methyltransferases (PRMT1-7) that contributed to arginine methylation, essentially required for the parasite infectivity and virulence in stress conditions. The latter modification is the attachment of ubiquitin proteins to substrates essentially involved in several cellular processes, e.g., protein degradation. It is worth noting that protein degradation is an essential step in life cycle stage differentiation.

From the present compilation, four points were concluded; 1) standardized pre-clinical protocols worldwide are necessary for developing novel anti-leishmanial drugs; 2) research centers, governmental authorities and pharmaceutical companies should work in a collaborative network; 3) studies investigating ALMDs should be encouraged; and 4) much attention should be paid to elucidating molecules contributed in Leishmania PTRM. Compiled from “**Anti-leishmanial metallodrugs and the elucidation of new drug targets linked to post-translational modifications machinery: Pitfalls and progress. Mem Inst Oswaldo Cruz 2022 Mar 23; 117:e210403.**”

Chagas’ disease (CD): Similar to leishmaniasis, as one of neglected tropical disease recognized by WHO, with no progress in CD drug discovery in spite of vast advances in the genomic studies conducted in the era of identifying potential drug targets. Although benzimidazole nitroheterocyclic compounds and Nifurtimox are the first two choices of treatment, both require long-term administration (two months) with occurrence of drug resistance in 10–30% of cases, besides having undesirable side effects in 90% of patients. Moreover, one of the major obstacles facing delay progress in developing novel efficient drugs for CD is the shortage of the definitive cure biomarkers. Although PCR efficiently offered an accurate diagnosis of several endemic diseases, however not all Chagas-positive patients are PCR positive. In the present compilation, **Iván Beltran-Hortelano** and his Spanish colleagues demonstrated in a figure several validated drug targets essentially involved in protein degradation, pyrimidine *de novo* synthesis, folate synthesis, sterol synthesis, glycolytic pathway, as well as protection against oxidative damage. The reviewers discussed several aspects related to these targets including function, structural characteristics, and target inhibitors. For each target, the reviewers described in detail its active site and tabulated several inhibitors investigated in the last decade. Therefore, such a review might be a useful guide for future studies.

Cruzain is the major cysteine protease, a cathepsin L-like protease belonging to the papain family. It is expressed during all developmental stages for nutrition, host cell invasion, and immunoevasion. It has seven subsites with conserved residues, four (S4, S3, S2 and S1) on the N-terminal domain, composed of α -helices, and three (S1', S2' and S3') on the C-terminal domain, composed of β -sheets. This structure allows binding of the three subsites with their corresponding subsites of the cleaved substrate, and as well forms a cleft between both domains where cruzain active site is localized. Notably, S2 subunit is specific due to its ability to interact with both basic and hydrophobic groups. Therefore, it is a druggable pocket to develop selective inhibitors. It was reported that *T. cruzi* possesses both

pyrimidine pathways, i.e., amastigotes rely on *de novo* pathway, while other forms rely on salvage pathway.

Dihydroorotate dehydrogenase (DHODH) is a catalytic enzyme in the fourth stage of the six-step process of pyrimidine *de novo* pathway. According to amino acid sequence homology, *TcDHODH* belongs to family 1A with different amino acid sequence from its human orthologue, i.e., a potential drug target. Unfortunately, three studies concluded difficulty in designing selective potent inhibitors due to technical issues in its pharmacophore molecular modelling. Pathogenic trypanosomatids are folate auxotrophs, i.e., are unable to synthesize folate.

Dihydrofolate reductase (DHFR) is a well-known medicinal target commonly used as anticancer, and antibacterial due to its essentiality in DNA biosynthesis. Several studies confirmed that *TcDHFR* has two bifunctional domains, N-terminal DHFR, and C-terminal **thymidylate synthase (TS)**. In contrast, DHFR and TS are separate monofunctional enzymes in almost all eukaryotes. Comparing its structure with human enzymes showed that while *TcDHFR* domain is more hydrophobic than the human domain, the structure of *TcTS* has high homology to human TS. In this concept, DHFR-TS is involved in consecutive reactions in *de novo* synthesis of thymine, and uracil monophosphate (TMP and UMP, respectively). Therefore, inhibition of DHFR or TS prevents DNA biosynthesis with subsequent cell death. Unfortunately, *T. cruzi* possess substitute enzymes (two pteridine reductases, PTRs) that functionally compensate loss of DHFR reduction activity. In fact, this explains failure of anti-folate drugs in CD treatment. Interestingly, only one study demonstrated that *TcPTR1* was expressed only in vector epimastigotes, not in the host stages. In spite of that, DHFR-TS was proposed as a validated drug target more than PTR in *T. cruzi* for two reasons; its pivotal role in nucleotide metabolism and its active site varies among species, i.e., feasible designing selective potent inhibitors.

Sterol 14 α -demethylase (CYP51) is a highly functional conserved protein that belongs to cytochrome P450 superfamily, with essential role in sterol synthesis pathway. Ergosterol is an essential molecule for the membrane stabilization, i.e., permeability and functional regulation of membrane ion channels. This simply means that ergosterol facilitates entry and exit of molecules required for different cellular processes such as viability, survival, growth, and life cycle stages differentiation. It is worth noting that CYP51 is also expressed in organelles’ membranes, e.g. glycosomes, endoplasmic reticulum and mitochondria. In such concept, it is a multi-organelle druggable target with multiple regulatory functions of endogenous sterols in all pathogenic trypanosomatids. It contains an iron-cysteine with a catalytic activity, i.e., once the sterol substrate enters through the membrane, it binds with

CYP51 active site. Structurally, *Tc*CYP51 is composed of 460 residues grouped into 22 α -helices and 12 β -sheets. Generally, a characteristic CYP51 feature is the absence of conformational changes among species in the active site pocket upon ligand binding. Therefore, *Tc*CYP51 is an attractive target for structure-based drug design, and its inhibitors were predominantly investigated in CD treatment in the last few years. An example of these inhibitors are heterocyclic compounds, e.g., imidazole, and triazole.

Since **triosephosphate isomerase (TIM)** is essentially involved in cellular energy production, it is the most extensively investigated drug target in eukaryotes. It reversibly catalyzes the conversion of D-glyceraldehyde 3-phosphate (GAP) \leftrightarrow dihydroxyacetone phosphate (DHAP) in step (5) of the glycolytic pathway (glycolysis). It is composed of two identical subunits with an active site located at its C-terminal end, however each subunit has its own catalytic residues. It was observed that the interface residues between TIM and its substrate are less tightly packed in *Tc*TIM than in the human orthologue, i.e., they are highly accessible and easily inhibited. Although several studies marked *Tc*TIM interface residues as a target site for designing inhibitors, the reviewers claimed shortage of knowledge in understanding the mechanism of action involved between TIM binding sites and its inhibitors.

Trypanothione reductase (TR) and **iron superoxide dismutase (Fe-SOD)** are two enzymes required for protection against oxidative damage mediated by host immune system. The former (TR) is a glutathione' homologous enzyme that protects human against oxidative stress. It is unique in kinetoplastids regulating and maintaining redox homeostasis during oxidative stress, i.e., production of reactive oxygen species (ROS), through catalyzing the disulphide group of trypanothione. Structurally, it has two subunits, each with four domains, two binding ones [flavine adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide phosphate (NADPH)], as well as central and interface domains. Its active site is formed of residues of the FAD and central domains of one subunit and the interface domain of another subunit. It is considered a potential drug target in all pathogenic trypanosomatids because of its physical, chemical, steric as well as electrostatic properties. Docking studies conducted against *Tc*TR demonstrated that it had a clear pocket for tight binding to inhibitors such as phenothiazine, imipramine, and naphthoquinone. On the other hand, Fe-SOD is a conserved member of metal-containing SODs that protect aerobic pathogens against oxidative damage by production of antioxidants. It is worth mentioning that metal containing SODs have four isoforms, namely iron, copper/zinc, manganese, and nickel; and trypanosomatids possess the first isoform. Its absence in mammals provides a promising opportunity as potential drug target in all

trypanosomatids. Interestingly, a study demonstrated that *Tc*Fe-SOD showed relevant differences from that of the host that could be utilized for designing selective specific inhibitors.

To design a novel strategy in CD treatment with combined therapy, the reviewers recommended multi-targets approaches, instead of depending on a single target. Compiled from "**Examination of multiple *Trypanosoma cruzi* targets in a new drug discovery approach for Chagas disease. Bioorg Med Chem 2022 Mar 15; 58:116577.**"

Free-living amoeba: In contrast to well-known free-living amoeba causing lethal granulomatous amoebic encephalitis (GAE) such as *N. fowleri* and *A. castellanii*, few reports focused on *B. mandrillaris*. It is worth mentioning that GAE has more than 90% fatality rate in USA. However, since GAE caused by *B. mandrillaris* is different from that caused by *N. fowleri*, being preceded by cutaneous lesions, and long incubation time (several weeks), and the disease progress takes a longer time in subacute or chronic infections. Although the clinical process of the disease gives a good opportunity for therapeutic intervention, current treatment of encephalitis due to *B. mandrillaris* is inefficient. In addition, literature for the biochemical pathways necessary for *B. mandrillaris* survival is also insufficient. For these reasons, **Isabella O Phan** and her American colleagues (17 contributors) were encouraged to utilize structure based drug discovery approach to construct the first proteomic draft of *B. mandrillaris*.

The investigators previously developed robust high-throughput screening assays (Rice *et al.*, Antimicrob. Agents Chemother 2020; 64(5):e02233-19), that were utilized in phenotypic screening of axenically cultured *B. mandrillaris* trophozoites. Out of 85 screened anti-parasitic drugs, 59 compounds exhibited 50% inhibitory efficacy at ~ 200 μ M. Interestingly, drugs, e.g., Macrolides (azithromycin, clarithromycin, roxithromycin, spiramycin A), that exhibited potent inhibitory activity against *Naegleria* or *Acanthamoeba* spp. failed to exhibit activity against *Balamuthia* trophozoites.

Proteins of the verified potential targets were identified, and the corresponding human protein sequences were downloaded and checked against *Acanthamoeba* spp. and *N. fowleri* orthologues to identify their assigned functions. Due to absence of a previous proteome study, such approach allowed the investigators to realize that the verified potential drug targets are involved in a specific pathway essential for the survival, viability, and growth. The investigators extracted RNA for mRNA isolation followed by transcriptomic study, and predicted *B. mandrillaris* draft proteome from RNA-sequence reads using a hybrid approach. The predicted proteome was validated

by comparison to proteome identified for *A. castellanii* (Acanthamoebidae, Amebozoa). Results revealed 65% similarity, with an average 44% identity, and 29% with at least 50% identity. Furthermore, they constructed a phylogenetic tree utilizing the non-Amebozoa *Naegleria* spp. as well as other outgroups, i.e., genera of *Dictyostelium*, *Planoprotostelium*, *Polysphondylium*, and *Tieghemostelium*. Results confirmed that proteomes of both, *B. mandrillaris* and *A. castellanii* are closely related.

By this transcriptomic study, the investigators were able to clone all *B. mandrillaris* constructs that were sequenced. For further characterization of the predicted proteome, the investigators utilized gene ontology (GO) molecular function term to classify transcriptomic proteins. This was also achieved in comparison to corresponding proteins in *A. castellanii* and *D. discoideum*. Regarding verified drug targets identified in the high-throughput screening, *B. mandrillaris* GO annotations categorized them into three categories, cellular components, proteins for molecular functions and biological process. It was observed that among 284 of proteins possessing kinase activity, 163 (57%) were classified as protein kinases. However, the investigators recommended further kinomic study for accuracy. Finally, the investigators succeeded to determine the sequence of 17 genes encoding verified drug targets, and recommended future structure-guided drug discovery studies to develop novel drugs for treatment of GAE caused by *B. mandrillaris*. Compiled from **"The transcriptome of *Balamuthia mandrillaris* trophozoites for structure-guided drug design. *Sci Rep* 2021 Nov 4; 11(1):21664."**

Trichomoniasis: Due to its association with increased susceptibility to HIV acquisition and transmission, as well as risk for malignancy, trichomoniasis became one of the important goals for global health in both genders. In pregnant women, it may lead to preterm delivery or premature membrane rupture. For autoinfection, prescription of repeated or multiple doses of metronidazole lead to *T. vaginalis* adaptation that increased drug resistance. Carbonic anhydrases (CAs) and metalloenzymes are essential molecules required for several cellular physiological processes such as photosynthesis, CO₂ transport, pH regulation, and biosynthetic reactions. They catalyze reversible CO₂ hydration to bicarbonate and proton. Eight genetic families are known; alpha (α), beta (β), gamma (γ), delta (δ), zeta (ζ), eta (η), theta (θ), and iota (ι) that vary in terms of amino acid sequence, kinetics, and inhibition and activation profiles. The majority of pathogens possess genes encoding β- and/or γ-CA, while only α-CA isoform is identified in humans. Therefore, CAs are potential drug targets as confirmed by development of efficient inhibitors in treatment of several diseases caused by microorganism, e.g., *M. tuberculosis*, *V. cholera*, *Salmonella* spp. and *C. albicans*.

Additional studies demonstrated impairment of *Leishmania* growth and virulence after interference with CA activity. Furthermore, a genomic study revealed two genes encoding β-CA (*TvCA1* and *TvCA2*) with 72% identity. **Linda J Urbański**, and a team from Finland, and Italy hypothesized that identifying *Tvβ-CA* expression, kinetic and structural characterization might open a new avenue for development of a novel drug leading to treatment of trichomoniasis.

In the present compilation, *TvCA1* biochemical characterization and its crystal structure were demonstrated. The investigators expressed *TvCA1* in *Escherichia coli*, purified its recombinant form by affinity chromatography, followed by assessment of its kinetic properties. They also biochemically characterized its crystal structure utilizing X-ray diffraction method. Results revealed that *TvCA1* exhibited significant catalytic efficiency in comparison to those identified in prokaryotes. Besides, *TvCA1* was efficiently inhibited by acetazolamide, a well-known CA inhibitor. Structurally, it was formed of a central β-sheet consisting of 8–10 strands surrounded by several helices, whereas its active site was located in a narrow pocket extending from the protein surface to the catalytic zinc ion. Comparison between active sites in both enzymes (*TvCA1* and human) revealed significant differences in dimensions, i.e., larger, and more accessibility in the latter. Further studies are currently in process aiming to design a *TvCA1* selective inhibitor. Compiled from **"Biochemical and structural characterization of beta-carbonic anhydrase from the parasite *Trichomonas vaginalis*. *J Mol Med (Berl)* 2022 Jan; 100(1):115-124."**

Lymphatic filariasis: For three reasons, **Alexander F. Flynn** and his American colleagues were encouraged to conduct the present compilation. First, out of 71 countries with high endemicity of lymphatic filariasis in the year 2000, only 17 succeeded to eradicate the disease as declared by the WHO report in the year 2020. Second, all drugs currently used, i.e., ivermectin, albendazole, and diethylcarbamazine target the microfilaria with no lethal effects on adults. Besides, triple-drug therapy in mass drug administration programs showed a major disadvantage in using these drugs in areas co-endemic with *L. loa* and/or *O. volvulus*. To avoid this limitation, the investigators focused on searching for a drug target specific for adults. Third, utilizing proteomic analyses of *Brugia malayi* tegument, gut, and reproductive tract, the same group of investigators had previously identified several proteins. They selected only nine proteins with (1) high homology in other filarial adults, (2) possession of an extracellular domain potentially linked with drugs, and (3) a specific function in adults' viability and survival. Among the selected proteins, the investigators observed the essentiality of uridine 5'-diphospho (UDP) glucuronosyltransferase for the adult survival.

They previously noticed that UDP was targeted by probenecid, a drug used in treatment of gout or gouty arthritis (Flynn *et al.*, PLoS Negl Trop Dis 2019; 13). It is worth mentioning that UDP-glucuronosyltransferase is a microsomal metabolic enzyme required for LAD2 biosynthesis, a cell adhesion molecule (CAM) belonging to the immunoglobulin superfamily, IgSF CAM. Members of this superfamily are single-transmembrane proteins that contribute to eukaryote homophilic and heterophilic interactions through their cytoplasmic tail. The latter is characterized by having several binding sites allowing for interaction with different cytoskeleton proteins, e.g., ankyrin, spectrin, and ERM protein family (ezrin, radixin and moesin) as well as phosphorylation sites serving in signal transduction.

In the present work, the investigators obtained *B. malayi* females from experimentally infected jirds. They tried to knock down (i.e., RNA interference, iRNA) seven genes, however they succeeded with only four: LAD2 (a CAM), serpin (an endogenous serine protease inhibitor), tyrosine kinase (one of the typical protein kinases), and reprolysin (a metalloprotease). They failed to knock down genes encoding enfukutin (a molecule necessary for muscle integrity), epidermal growth factor (EGF), and cysteine protease (peptidase). This failure rate was not surprising due to the high challenge in conducting iRNA in helminths.

Except for *BmLAD2*, none of iRNA of other genes resulted in significant changes for worm viability or fecundity. Knock down studies revealed that suppression of *BmLAD2* expression was associated

with significant results in decreasing worm motility over 6 days (80%), and reduction of microfilaria release (93.4%). Moreover, transmission electron microscopy demonstrated complete loss of both microvilli and pseudocoelomic fluid and untying of the mitochondrial cristae in the intestinal epithelium. These results were attributed to disruption of the tight junctions between filarial intestinal cells with subsequent loss of the filarial worms' hydrostatic skeleton in *BmLAD-2* mutants. Utilizing LAD2, the investigators constructed a phylogenetic tree and confirmed that *BmLAD2* amino acid sequence with high similarity with those identified in other nematodes including *B. timori*, *B. pahangi*, *W. bancrofti*, *L. loa*, *O. volvulus*, *C. elegans*, *D. immitis*, *H. contortus*, *A. viteae*, *N. americanus* and *A. duodenale*.

The investigators extended their studies demonstrating absence of anti-*BmLAD-2* IgE in the serum of 30 patients with lymphatic filariasis from an endemic area. Based on the obtained results, LAD-2 was proposed a promising drug target and vaccine candidate without causing adverse reactions. Since the pseudocoelomic fluid has an essential role as a lubricant medium for nutrition exchange and cellular signaling during mitotic cell proliferation, the investigators suggested that disruption of the intestinal tract tight junction *via* suppression of filarial CAM (LAD2) would block cell proliferation with subsequent induction of apoptosis. Compiled from "***Bma-LAD-2, an intestinal cell adhesion protein, as a potential therapeutic target for lymphatic filariasis.*** mBio 2022 Apr 27;e0374221."