Spotlights on new publications

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New vaccine candidates II

Intestinal schistosomiasis

Tetraspanins (TSPs), transmembrane 4 superfamily proteins located in the epithelial syncytium of all life cycle stages, are surface proteins involved in the formation and maintenance of cell membrane protein complex, and intercellular signaling, and signal transduction. They also display several functions such as cell adhesion, motility, migration, proliferation, and host-pathogen interactions, i.e., involved in pathogenesis and virulence. In other words, TSPs act as scaffolding proteins allowing attachment of the specific membrane proteins (integrins) to cytoskeleton of schistosome-cells to facilitate adhesion. Accordingly, *Schistosoma* TSPs maintain adult worm tegument integrity through preservation of its plasma membrane structure, turnover of its transient outer surface, and mediate regulation of signaling pathways for migration of the juvenile flukes. Schistosomes possess several TSPs encoding genes with different expression profiles; however, two major TSPs (TSP1 and TSP2) attracted much attention in the last decade. Several studies documented that *in vitro* RNA interference led to a significantly thinner and more vacuolated tegument with subsequent failure of closure of tegumental invaginations. Besides, immunized mice showed high protection against a challenged *S. mansoni* infection.

In 2019, a phase Ia clinical trial was conducted on 72 healthy American adults without history of schistosomiasis using *Sm*TSP2 formulated on Alhydrogel administered with or without the toll-like receptor (TLR)-4 agonist, glucopyranosyl lipid A (AP 10–701). The trial showed its safety, and well-toleration that induced significant antigen-specific IgG responses [Keitel *et al*. Vaccine 2019; 37(43):6500-6509]. In the present compilation, collaborating American-Brazilian researchers (**David J Diemert *et al.***) conducted a randomized controlled phase Ib clinical trial on 60 healthy Brazilian adults living in an endemic area. Since the study investigated *Sm*TSP2 efficacy, three doses (10 μg, 30 μg, and 100 μg) were administered to three groups (20 in each). In each group, 16 participants were randomly administered one of two formulations of *Sm*TSP2, either adjuvanted with Alhydrogel, or Alhydrogel and AP 10-701; while the 4th group received control hepatitis B vaccine. After three intramuscular injections with 2 months interval, all participants were followed for 12 months. Prior and after vaccination, specific IgG and IgG subclass antibody levels were assessed. Results revealed that *Sm*TSP2/Alhydrogel administered with or without AP 10-701 was safe and well-tolerated, and the most common adverse effect was local mild tenderness and pain at the injection site with mild headache. The highest specific IgG antibodies was recorded in participants receiving *Sm*TSP2/Alhydrogel, and AP 10-701 with a significant dose-response relationship, and a peak at two weeks after the last injection. Although IgG subclass levels mirrored those of total IgG, the IgG1 was the most predominant subclass. Interestingly, IgG levels did not fall to the baseline (prior to vaccination) till the end of observation (12 months). Instead, they fell after ~16 months except for 57% of participants who received 100 μg *Sm*TSP2/Alhydrogel, and AP 10-701 and showed higher IgG levels (≥4-fold higher than baseline).

Based on these promising results, the investigators decided to initiate a clinical trial phase 2 in another African endemic area, e.g., Uganda. Compiled from **"A randomized, controlled Phase 1b trial of the Sm-TSP-2 vaccine for intestinal schistosomiasis in healthy Brazilian adults living in an endemic area. PLoS Negl Trop Dis 2023 Mar 30; 17(3):e0011236."**

Lymphatic filariasis

Four main obstacles face development of an efficient potential vaccine to protect against lymphatic filariasis. These included life-cycle complexity, complicated host immune response against filarial nematodes in animals and humans, limited previous evidences proving existence of natural host protective immunity, and finally no appropriate animal models. As previously described in leishmaniasis, reverse vaccinology technique (epitope-based vaccines) replaced conventional strategies utilized for vaccine development.

The present compilation summarized a collaborative work in which **Premnath Madanagopal *et al.*** utilized immunoinformatics approach to design a multi-epitope-based subunit prophylactic vaccine for lymphatic filariasis. Although *W. bancrofti* is the major

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causative filarial nematode of lymphatic filariasis, data availability of *B. malayi* bioinformatics and its 95% homology with *W. bancrofti* encouraged Indian and German investigators to conduct the present compilation. Lymphocytes, either B- or T-cells, natural killer (TC), and helper cells (TH) were screened against *B. malayi* antigens, and passed through several immunological filters to determine the optimal epitopes. The major screened antigens epitopes included larval transcript 2 (ALT2), thioredoxin peroxidase (TPX), venom allergen antigen-like protein 1 (VAH), and glutathione S-transferases (GSTs). Screening also included other epitopes described in previous studies, such as embryonic fatty acid-binding protein (FAB-1), thioredoxin, cuticular glutathione peroxidase, cuticular glycoprotein (gp29), and transglutaminase.

Results revealed a total of 21 epitopes (15 CD8+, and 3 of each $CD4^+$, and B cell epitopes) that were investigated for their antigenicity, immunogenicity, and toxicity. Cross-reactivity analysis was also conducted and revealed that the constructed vaccine did not overlap with the human proteome. Interestingly, presence of conformational B cell epitopes, and cytokine-inducing epitopes validated the use of these epitopes in the constructed vaccine due to specific triggering by humoral and cell-mediated immune response. To construct the vaccine, the selected epitopes with toll-like receptor 4 (TLR4) were ligated using suitable peptide linkers. Notably, previous studies showed that TLR4 improved vaccine protection due to its essential link with protective immunity. Besides, the 50 S ribosomal protein L7/L12 was selected as an adjuvant since it acts as TLR4 agonist.

The investigators conducted a protein-protein docking study demonstrating the strong binding affinity of the vaccine structure with the TLR4 adjuvant that confirmed its ability to elicit efficient host immune response. This was followed by an *in silico* immune simulation to access the vaccine ability to elicit a long-lasting protective immunity. Finally, after optimization of the vaccine sequence, the study cloned its reverse translated sequence in a plasmid construct (pET28a) that was termed Filariaccine. Prior to its validation for clinical trials, the investigators recommended further *in vitro*, and *in vivo* studies to confirm Filariaccine efficacy. Compiled from **"Construction and validation of a multi-epitope *in silico* vaccine model for lymphatic filariasis by targeting *Brugia malayi*: A reverse vaccinology approach. Bull Natl Res Cent 2023 Mar 24; 47(1):47."**

Malaria

Several obstacles were reported challenging development of an appropriate effective malaria vaccine, most of them were related to *Plasmodium* immunobiology. Because it has a highly complexed genome with more than 5000 genes that express separately, *Plasmodium* spp. have the high ability to adapt or modulate both host and vector immune systems. Other obstacles included substantial specificity of antigens expressed from several life-cycle stages, i.e., vaccine candidates for hepatic stages are most likely not potentially efficient for either intra- or extra-erythrocytic stages. In addition, there is shortage of appropriate non-human primates that can be used as experimental models for direct evaluation of prospective vaccine candidates. Although rodents or mice present suitable experimental models, *P. berghei*, *P. chabaudi, P. yoelii*, and *P. vinckei* substantially differ from the main *Plasmodium* spp. infecting human. Experimentally, *Aotus* is the only Old-World genera of non-human primates that can be infected with *P. falciparum* or *P. vivax*, and *Rhesus macaques* is infected with *P. falciparum*. Finally, immunological tools to evaluate the immune response of non-human primates are limited.

In 2017, WHO recommended application of pre-erythrocytic *P. falciparum* [Mosquirix® (RTS, S)] malaria vaccine in clinical trials to protect African children living in endemic areas. In 2019, GlaxoSmithKline, RTS, S manufacturer, provided 10 million doses to be administered in Ghana, Kenya, and Malawi through their national immunization programs. The obtained results indicated its safety and immunogenicity in infants, its protection against initial exposure to *falciparum* malaria (34% efficacy), and in clinically manifested malaria (three immunization doses significantly decreased malarial episodes). To increase its efficacy, several trials were conducted thereafter to enhance its immunogenicity, and the results raised the hypothesis that better identification of epitopes and their corresponding antibodies definitely improved vaccine immunogenicity, and its efficiency. In 2022, University of Oxford in collaboration with the Serum Institute of India manufactured R21/Matrix-M, a pre-erythrocytic malaria vaccine candidate. In the phase 1/2b randomized controlled trial against clinical malaria, results showed promising efficacy and immunogenicity after two years' follow-up in children in Burkina Faso. These studies encouraged **Reysla Maria da Silveira Mariano** and her colleagues from Brazil and Peru to review previous investigated strategies to design and develop a potential malaria vaccine with multiple *Plasmodium* targets, especially those involved in blocking transmission. The reviewers hypothesized that blocking transmission is an important step in malaria elimination, and should be strongly advanced in clinical trials, and must be prioritized. In other words, accurate diagnosis and efficient therapeutic management never eliminate malaria. Instead, and in fact, blocking transmission with or without vector control definitely and significantly decreases *Plasmodium* spread and malaria prevalence all over the world.

Utilizing four patent databases: International and National (WIPO, patentscope.wipo.int), Latin America (LATIPAT, lp.espacenet.com/), American (USPTO, uspto.gov/patents-application-process), and European

(Espacenet, worldwide.espacenet.com), the reviewers collected a total of 95 registered articles. They included 44, 22, 15 and 14 articles from WIPO, LATIPAT, USPTO, and Espacenet, respectively. According to *Plasmodium* life cycle target, articles were categorized into three targets: pre-erythrocytic, erythrocytic, and sexual stage. Accordingly, a fourth category was created including vaccines that targeted multi-stages. In the following table, 19 registered vaccines investigated in the last four decades are summarized.

Target (No./Year) #	Vaccine component	Notes
Pre-erythrocytic stages		
US5112749A (1992)	Circumsporozoite (CSP) recombinant proteins.	To inhibit liver infections and limit RBCs invasion. **Limitation:** Processes used for attenuation should be precisely adjusted.
EP1544211A1 (2005)	Immunogenic composition of *P. falciparum* sporozoite antigen.	
AU2004309380B2 (2010)	Live *Plasmodium* genetically engineered.	
US20160038580A1 (2015)	Soluble recombinant *P. falciparum* CSP protein.	
AU2013250814B2 (2017)	Live *P. berghei* genetically engineered.	
Erythrocytic stages		
US4957738 (1990)	Synthetic CSP proteins (Protein copolymer).	To protect against malaria episodes *via* inhibition of PPIs between *Plasmodium* and host receptors. **Limitation:** Single *Plasmodium* target.
US20040137512 (2004)	*P. falciparum* serine-repeat antigen (SERA).	
US20080026010 (2008)	Attenuated *Plasmodium* strains lacking nutrient transporters.	
WO2013108272A3 (2013)	Merozoite antigens.	
US20140186402A1 (2014)	Isolated or purified merozoites.	
US20190374629A (2019)	Recombinant *P. falciparum* reticulocyte binding protein homolog-5 (PfRH5)@.	
Sexual stages		
WO2010036293A1 (2010)	Recombinant *P. falciparum* cysteine-rich protein (Pfs48/45).	To produce specific Abs in the host to block *Plasmodium* survival, or Abs in the vector to block transmission.
***CN104736710A (2015)**	*Plasmodium* surface antigen (S47) either Pfs47 or Pvs47.	
***US20150191518A1 (2015)**	*P. falciparum* gliding-associated protein 50.	
DK2763694T3 (2018)	Recombinant *P. falciparum* cysteine-rich protein (Pfs48/45).	
Multi-stages target		
CA2910322A1 (2014)	Recombinant apicomplexans or *Plasmodium* fusion proteins.	To provide additional immune response against several *Plasmodium* targets.
EP2923709A1 (2015)		
EP2992895 (2016)		
WO2017142843 (2017)		

#: Registration number and year of registration; **@:** Reticulocyte binding protein homologs (RHs) are a superfamily of proteins found in *Plasmodium* responsible for cell invasion; ***:** Blocking transmission vaccines; **Abs:** Antibodies; **PPIs:** Protein-protein interactions.

Five important issues were concluded and strongly recommended: 1) further search for novel *Plasmodium* potential vaccine candidates with multiple stage targets; 2) combined use of nanotechnology and glycoengineering remains the global delivery system; 3) identifying or development of appropriate adjuvants with high ability to trigger dendritic cells maturation, and cytotoxic T lymphocyte proliferation, and differentiation; 4) search for promising eukaryotic alternative for efficient expression of plasmodial proteins with higher immunoreactivity; 5) much attention should be directed towards post-translational modifications as potential *Plasmodium* key elements. Compiled from "**A review of major patents on potential malaria vaccine targets. Pathogens 2023 Feb 3; 12(2):247.**"

Toxoplasmosis

Compilation No. (1): In 1995, the first commercially-licensed live attenuated protective vaccine (Toxovax®, Wellington, New Zealand) against toxoplasmosis was developed to control abortion in pregnant ewes. It was based on live *T. gondii* (S48 strain) tachyzoites, and it reduced the losses in sheep farming due to congenital toxoplasmosis. Therefore, it was approved in New Zealand, and few countries in Europe in addition to attempts for its exploitation to be used in human immunization. However, it proved to have three main disadvantages making it unsuitable for human use: 1) short-shelf life; 2) ability to restore and/or increase *T. gondii* virulence since it includes live attenuated tachyzoites; 3) possibility to produce unexpected results due to its unknown genetic background. Since then, several studies were conducted to explore different vaccination approaches such as DNA subunit, epitope, protein, and carbohydrate-based vaccines. However, none of them achieved safe and effective protection against toxoplasmosis, even with utilization of nanotechnology. It was observed that DNA subunit vaccines require carrier or adjuvant delivery and provide fewer protective effects. While epitope vaccines formed of small molecules with poor immunogenicity lack the secondary and tertiary structures of the native proteins, protein and carbohydrate-based vaccines may lead to autoimmunity due to high structure similarity to those of the host.

On the other hand, mRNA vaccine strategy yields promising results in addition to several advantages, e.g., safe delivery, low manufacturing cost, accelerated development cycles, and high potency; however, it received less attention. Therefore, a Chinese study (**Dan Li** and his colleagues) explored a new mRNA vaccine candidate and its immunogenicity and immunoprotective effects in comparison to *Toxoplasma* surface antigen 1 (SAG1), a potential surface predominant antigen, and promising vaccine

candidate. The investigators hypothesized that *T. gondii* secretory proteins essentially involved in host-pathogen interactions constitute a main compartment of potential vaccine candidates. A genome wide study was performed to screen *T. gondii* database (http://ToxoDB.org) for secretory proteins that contain transmembrane domains and signal peptides. Utilizing bioinformatics, the screened candidates were analyzed for T- and B-cell epitopes (T/B epitopes). Results revealed that *T. gondii* strain GT1 (TG290 or TGGT1_316290) exhibited higher T/B epitope score with a lower predicted IC50 value than SAG1.

Using lipid nanoparticle (LNP) technology, the investigators constructed a TG290 mRNA-LNP vaccine to be evaluated for its immunogenicity and immunoprotective effect in BALB/c mice through intramuscular injections. Several parameters for humoral and cellular immune responses were assessed. These included specific total IgG and IgG subclass antibodies, cytokine levels, lymphocytes proliferation, cytotoxic T lymphocyte differentiation ($CD4^+$ and $CD8^+$ T lymphocytes activation), dendritic cells (DCs) maturation, and major histocompatibility complexes (MHCI, and MHC2). Additionally, nuclear factor kappa B (NF-kB), interferon regulatory factor 8 (IRF8), and transcription factor 21 (T-Box 21) were also investigated, with calculation of survival time of mice challenged with infection by *T. gondii* RH tachyzoites.

Immunized mice showed that TG290 mRNA-LNP significantly: 1) induced specific humoral immune response (increased total IgG, and IgG1 and IgG2a antibodies); 2) induced cellular immune response (increased IFN-γ, ILs 4, 10, and 12 levels); 3) prevented *T. gondii* invasion into host cell through activation of T-cell-mediated immune responses (increased T lymphocytes proliferation, and cytotoxic T lymphocytes differentiation into $CD4^+$ and $CD8^+$, with subsequent macrophages activation, and perforin-mediated cytolysis, respectively); 4) facilitated DCs maturation that activated innate, and acquired immunity through activation of CD83, and CD86, key regulators of antigen presentation; 5) upregulated MHC-I and MHCII molecules; 6) prolonged mice survival time; 7) overexpressed T-Box 21, NF-kB, and IRF8. It is worth noting that NF-κB and IRF8 pathways are involved in elicitation of IL-12 and IFN-γ expression. Besides, increased splenocytes NF-κB was significantly recorded suggesting induction of IL-12 or IFN-γ expression through NF-kB signal pathway. Additionally, T-Box 21 expression that regulates Th0-specific differentiation with activation of Th1/Th2 exchange, was immunologically evoked by TG290 mRNA-LNP. Compiled from **"A novel *Toxoplasma gondii Tg*GT1_ 316290 mRNA-LNP vaccine elicits protective immune response against toxoplasmosis in mice. Front Microbiol 2023 Mar 21; 14:1145114."**

Compilation No. (2): One month later, the same group of investigators (**Yizhuo Zhang *et al.***) constructed TG_200 (TGGT1_216200) mRNA-LNP vaccine using the previous methodology mentioned in 'Compilation 1". Similar results were obtained regarding all the investigated parameters. The study showed that TG_200 mRNA encapsulation efficiency in LNPs recorded 95.67%. In addition, due to its administration route (intramuscularly), vaccine efficient expression was investigated in two types of cell lines: mouse myoblast cells, and human embryonic kidney cells that showed no cytotoxicity. Furthermore, to assess TG_200 mRNA adoptive protective effect, survival time was calculated using sera and splenocytes of immunized mice. In comparison to the control group, results revealed significant improvement of mice survival time (13.40±2.32 versus 9.70±1.64 days, respectively). The investigators attributed this improvement to the fact that $CD8^+$ and $CD4^+$ cytotoxic T cells are functional effectors of host resistance to pathogens.

In the present compilation, the investigators discussed limitations of mRNA vaccines including easy degradation *in vivo*, i.e., they have a short intracellular half-life. Besides, due to physicochemical properties, large molecular size, and dense negative charge, crossing cell membrane is almost impossible. Accordingly, a suitable effective delivery system is essentially required to prevent degradation, and facilitate cellular entry. Thereafter, the investigators discussed several appropriate delivery-systems commonly used, and previously reported such as lipids, polymers, protein derivatives, and lipid-like materials. They claimed that LNPs were successfully applied in preventive medicine, e.g., those used to deliver mRNA-1273 in the currently approved vaccine against COVID-19. Compiled from **"Immunization with a novel mRNA vaccine, *Tg*GT1_216200 mRNA-LNP, prolongs survival time in BALB/c mice against acute toxoplasmosis. Front Immunol 2023 Apr 14; 14:1161507."**

Leishmaniasis

Compilation No. (1): In spite of availability of several licensed vaccines against canine leishmaniasis, few potential vaccine candidates were advanced to human clinical trials level. Unfortunately, all are still in the early research and development phase. An ideal leishmanial vaccine should elicit long-lasting protective immunity with effective elicitation of different antigenic T-helper ($CD4^+$), and T-cytotoxic ($CD8^+$) cells, i.e., with helper T-lymphocytes (HTL), and cytotoxic T-lymphocytes (CTL) epitopes. On the other hand, reverse vaccinology technique emerged as a promising field in immunology, and attracted much attention in the last two decades. Utilizing such technology, i.e., use of the pathogen' genomic information to map its epitopes instead of *in vitro* cultures, several vaccines were developed against viruses, e.g., Dengue, and Covid. Nowadays, epitope-based vaccines are a new approach for eliciting a specific immune response, without inducing unfavorable reactions to other antigen epitopes contributing in either immunopathogenic disease process or modifying host immune responses.

An Indian study (**Mainak Bhattacharjee *et al.***) retrieved all proposed potential epitope candidates from several previous immunological *in vitro* and *in vivo* experimental studies as well as *in silico* analyses. Due to their essential role in immunopathogenesis, and virulence, as well as provoking protective immunity, only twelve were selected. They included three highly conserved proteins; translation factor protein (eIF5A), kinetoplastid membrane protein-11 (KMP-11), and *Leishmania*-activated C-kinase antigen (LACK) across all eukaryotes, pathogenic kinetoplastids, and *Leishmania* spp., respectively. Other conserved leishmanial antigens were a surface metalloprotease (leishmanolysin or GP63), two leishmanial membrane proteins (LCR1 and GP46), the hydrophilic acylated surface protein-B (HASP-B), paraflagellar rod (PFR), and beta-tubulin. Three additional molecules were also included, proteophosphoglycans, cysteine proteases, and heat shock proteins. The investigators retrieved the sequences of the selected proteins from UniProt database (https://www.uniprot.org/) that belonged to *L. donovani, L. major, L. maxicana, L. chagasi,* and *L. amazoensis*. Therefore, the investigators hypothesized that designing a polyvalent multi-subunit peptide vaccine from five major *Leishmania* spp. causing visceral, cutaneous, and mucocutaneous leishmaniasis would certainly confer a broad-spectrum cross immunity against all clinical forms of human leishmaniasis.

All selected antigens were subjected to *in silico* analysis for prediction of their major histocompatibility complex (MHC)-binding epitopes. Interestingly, epitopes identification was performed using two independent servers simultaneously. Accordingly, screened epitopes identified and predicted by both servers with significantly higher MHC-binding affinities were utilized to construct the multiple-epitope based vaccine with appropriate linkers, and natural adjuvant. Prior to vaccine construction, all identified epitopes were *in silico* evaluated for their antigenicity profile to determine their cytokine-inducing abilities. Epitopes that induced IFN-γ, IL-4, and IL-10 production were selected to design ideal chimeric peptide vaccine. For natural adjuvant, IL-12 was used since it is one of the most effective adjuvants used in several previous immunogenic reports for *Leishmania* vaccine in animal models. It increased vaccine immunogenicity without causing host adverse side effects. Finally, *in silico* studies were performed to validate the three-dimension vaccine construct, and molecular docking simulation, as well as to demonstrate the protective elicitation of cellular and humoral immune responses. In spite of the promising *in silico* results, the investigators recommended further *in vitro* and *in vivo* studies to guarantee efficient elicitation of protective and long-lasting immunity without adverse toxicity effects. Compiled from **"*In silico* designing of a novel polyvalent multi-subunit peptide vaccine leveraging cross-immunity against human visceral and cutaneous leishmaniasis: An immunoinformatics-based approach. J Mol Model 2023 Mar 16; 29(4):99."**

Compilation No. (2): Greek investigators (**Maritsa Margaroni** and her colleagues) similarly used the reverse vaccinology approach to design a multiepitope vaccine against leishmaniasis (LeishChim). Utilizing proteomics of three different *Leishmania* spp., they selected *L. infantum* antigens for identification of their predictive HTL and CTL epitopes that were fused to construct a multi-epitope chimeric protein for use as a leishmanial protective vaccine. The five selected antigens included three hypothetical proteins (LinJ.34.0420, LinJ.28.0780, LinJ.20.0600), as well as serine/threonine kinase-like proteins, and DNA-directed RNA polymerase-like protein. Criteria for the selected proteins included: 1) the three hypothetical proteins were previously suggested potential vaccine candidates for their significant role in either growth and survival, or host invasion; 2) serine/threonine kinase-like proteins, e.g., GSK-3, MAPKs, and Aurora kinase were also involved in survival and replication as previously proposed in several publications; 3) DNA-directed RNA polymerase-like protein had never been investigated as potential vaccine candidate.

In silico analyses demonstrated LeishChim thermodynamic stability, safety, and antigenicity in addition to being non-allergenic. Additionally, molecular docking and molecular dynamics simulations indicated LeishChim strong binding to both MHC I and II molecules. Encapsulation with mono-phosphoryl lipid A into poly-lactic-co-glycolic acid (PLGA) nanoparticles, elicited significant specific cellular immune responses when administered to BALB/c mice. It was observed that PLGA, an ideal candidate for delivery system and efficient adjuvant co-encapsulation, showed several advantages including vaccine protection from degradation by enzymes, and controlling antigen release. Development of memory $CD4^+$ T cells, as well as IFNγ- and TNFα-producing $CD4^+$ and $CD8^+$ T cells, confirmed LeishChim validation as a potential protective vaccine to efficiently and strongly induce both T and B cell immune responses. Compiled from **"Immunoinformatics approach to design a multi-epitope nanovaccine against *Leishmania* parasite: Elicitation of cellular immune responses. Vaccines (Basel) 2023 Jan 30; 11(2):304."**

Chagas' disease

It was recorded that ~30% of patients with chronic Chagas' disease develop cardiomyopathy leading to lethal heart failure in Latin America. Besides, associated mega-organ syndromes effecting esophagus and colon causes high morbidity due to direct or indirect smooth muscles damage. Several factors influence development of chronic symptoms; however, complex interplay of host genetic factors and parasite virulence was hypothesized. Several studies were conducted to protect risky populations against Chagas' diseases using live attenuated vaccines by engineered mutations approach. This approach is based on either mono- or bi-allelic deletion of a single

gene copy in several *T. cruzi* strains. Although mutant attenuated vaccines significantly reduced parasitemia and tissue inflammation with subsequent decrease of mortality and morbidity rates, they failed to induce demonstrable host cellular immunity. However, results of previous studies showed that: 1) mono-allelic *dhfr-ts* gene deletion mutants induced long-term protection; 2) mutants with deletion of 3 out of 4 genes encoding enoyl-coenzyme A hydratases was effective in mice protection after challenge infection; 3) bi-allelic mutants in gp72, a flagellar antigen, showed protection in a few mice after lethal challenge with highly virulent strain; 4) immunization with attenuated mutants lacking one of two gene copies encoding calmodulin-ubiquitin significantly reduced parasitemia on subsequent challenge with wild-type parasites; 5) LYT1-mutant parasites attenuated mice infectivity, and reduced parasitemia, splenic parasite indexes, and tissue parasite burden when challenged with virulent parasites. Notably, LYT1, a *T. cruzi* exclusive gene encoding virulence factor, is expressed into two isoforms: kLYT1 and mLYT1. While the former exhibits a regulatory role during the epimastigote → metacyclic trypomastigote transition, mLYT1 acts as a pore-forming protein for host cell invasion. These results encouraged **Bijay Kumar Jha** with his American and Brazilian colleagues to investigate the efficacy of immunization with cyclophilin 19 (Cyp19) mutant parasites. The present compilation hypothesized that since this enzyme catalyzes cis-trans peptidyl-prolyl isomerization of proteins (PPIases), it is essentially required for several biological cellular functions, e.g., acting as a protein chaperone (protein folding, and re-folding of aggregated proteins), ROS production, gene expression, and miRNA regulation. Additionally, Cyp19 is the only PPIase expressed and released extracellularly by all *T. cruzi* stages confirming its essential engagement in each stage to modify different host target proteins. Meanwhile, Cyp19 was also expressed in winged bugs leading to inactivation of their anti-parasitic peptides with subsequent stage transformation.

Using double allelic homologous recombination method, the investigators generated a mutant strain with Cyp19 depleted expression *via* removal of 2 of 3 encoding genes. Mutant epimastigotes showed a diminished growth rate *in vitro*, and differentiated rapidly into non-replicative metacyclic trypomastigotes without reaching the peak density recorded in wild-type parasites. When inoculated in immunocompromised mice, metacyclic forms entered cells forming few amastigotes-like bodies that failed to replicate over prolonged observation up to several weeks. Due to Cyp19 deletion, there was absence of maturation and expression of several proteins essentially required for intracellular replication, cell cycle machinery, and differentiation. Overall, Cyp15 mutant parasites failed to develop clinical disease or death up to one year of observation. Interestingly, while parasite-specific PCR analysis detected DNA in the cardiac tissue of inoculated mice after 15 w post-infection, RT-PCR analysis for mRNA expression failed to detect mRNA expression in these tissues. Both molecular analyses indicated prolonged persistence of Cyp19 mutant parasites in tissues without replication, i.e., absence of eventual tissue damage and development of acute disease. Moreover, repeated inoculation of Cyp19-mutant strain into immunocompetent mice succeeded to increase anti-*T. cruzi* B-cell and proinflammatory cytokine responses that protected immunized mice against challenge infection with virulent wild-type strain. A single immunization dose demonstrated 100% efficacy in preventing death when challenged with a wild-type parasite 6 months after inoculation. Further studies were recommended to investigate its efficacy for inducing cross-protection against infection by other *T. cruzi* strains. Compiled from **"Immunization with a *Trypanosoma cruzi* cyclophilin-19 deletion mutant protects against acute Chagas disease in mice. NPJ Vaccines 2023 Apr 25;8(1):63."**