Chapter

Molecular Medicines for Parasitic Diseases

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Abstract

Being the cause for significant amount of morbidities and mortalities, parasitic diseases remain the major challenge for the healthcare community due to the limitations associated with the current chemotherapeutics. Drug discovery/invention can be achieved by collaborative efforts of biotechnologists and pharmacists for identifying potential candidates and successfully turn them into medicine for improving the healthcare system. Although molecular medicine for disease intervention is still in its infancy, however, significant research works and successful trials in short span of time have made it broadly accepted among the scientific community. This chapter identifies different molecular medicine approaches for dealing with parasites that have been coming up on the horizon with the new technological advances in bioinformatics and in the field of *omics*. With the better understanding of the genomics, molecular medicine field has not only raised hopes to deal with parasitic infections but also accelerated the development of personalized medicine. This will provide a targeted approach for identifying the druggable targets and their pathophysiological importance for disease intervention.

Keywords: CRISPR/Cas9 system, monoclonal antibody, immune checkpoint inhibitors, nanomedicine

1. Introduction

Parasitic diseases remain the threat to global healthcare sector, with considerable mortalities and morbidities associated with these diseases. Combating parasitic infection relies mostly on conventional chemotherapeutic approaches; however, an exponential rise in the number of recrudescent cases, lack of vaccines and toxicities issues associated with chemotherapies emphasized the need for the research to develop alternative strategies. It is noteworthy that emergence of drug resistance is not new; microbes have been evolving since ages, by knocking out one or the genes. In context of emerging drug resistance, World Health Organization (WHO) has warned for the upcoming "post-antibiotic era", therefore, molecular medicine has surged. Molecular medicine is the application of gene and/or DNA based information for therapeutic purpose. It involves the study of molecular mechanisms, identification of erroneous genetic and/or molecular pathways and development of molecular intervention with the aim to improve disease management.

This chapter provides an insight into how the anomalies in molecular pathways can be targeted leading to discovery of potential candidates for development of clinical medicine and innovative therapies to improve disease management strategies.

2. Evolution of molecular medicine

The field of molecular medicine evolved over a period of time since the discovery of DNA (in 1953) and recombinant DNA technology. Another major breakthrough in 1975, when it was discovered that DNA can be read base by base through the sequencing technique. Later, in 1985, it was known that DNA can be amplified with PCR, and this was major achievement in the field of molecular diagnostics. This journey gained pace with the advent of automated DNA sequencing in 1987 that served as the background for the human genome project (HGP) in 1990. The journey of HGP started the era of modern molecular medicine with the first successful gene therapy. In 1995, the success of unveiling the DNA sequence from the first model organism (*Hemophilus influenzae*) triggered the endeavor towards the completion of HGP. The completion of HGP (in 2000) and the free access to the human genome sequences provided the ground for the advent of omics era or post-genomic era (or functional genomics) [1].

This led to the use of increasing number of analytical platforms for DNA sequencing termed as the next generation sequencing platforms. Further, metagenomics approaches—omics and/or shot-gun approaches paved the way for the third-generation sequencing, that aimed to reduce the sequencing costs. These advances gained momentum with the computational approaches where synthetic biology has remarkably facilitated the DNA based analysis as well as the development of models for drug testing.

In order to deal with the limitation of the existing chemotherapeutic approaches there remains an urgent need for the conversion of biomedical knowledge into clinical application. Molecular medicine provides the opportunity to fill the gap between the basic research and the clinical application for the diagnosis, prevention and treatment of diseases. It involves the combinatorial application of pharmacology, biomedical and omics technologies for understanding and improving the molecular basis of the disease pathogenesis that will serve in designing disease intervention strategies. Development of molecular drug is a complex process that involves multidisciplinary effort including high throughput screening, chemical synthesis, modification, omics technologies, data mining, structure-based drug designing, phenotypic screening, target and lead identification and validation, etc. The development of molecular medicines involves following steps—first, the identification of target, potential tractability of target (i.e. identifying targets that are more druggable than others, depending upon their chemistry), establish genetic association of target with disease pathophysiology (some targets required for drug action may not necessarily associate with disease genetics) and validation of target (by establishing association of target with the disease development/persistence). Validation of target usually involves different molecular approaches to understand the role of target gene or protein in diseases pathophysiology. Overall, it is an interdisciplinary branch where recent technical advances have served as the milestone in gaining insight into the phenomenon of disease pathogenesis and development of innovative therapeutic measures.

Parasitic diseases are amongst the common infections in humans caused by protozoan and helminthic parasites. The causative agents, parasites, are diverse ranging from single celled protozoan to worms that be seen with naked eyes. Till the end of nineteenth century, parasitologists were mainly focused on understanding their life cycle; however, the concept took turn when some parasites were found to be associated with several human diseases that led to significant morbidity and mortality. Parasitic diseases are cosmopolitan, that may affect any part of world however, mostly the diseases are common in tropical countries, but tourism and migration can transmit them outside their geographical boundaries. The signs and symptoms of disease may not be obvious, and it may vary from mild abdominal pain to chronic

Diseases	Causative agent (pathogen)	Transmitting agent (vector)	Manifestation	Treatment options
Protozoan parasites				
Leishmaniasis (visceral, cutaneous and mucocutaneous)	*Leishmania* species	Sandfly (*Phlebotomus* & *Lutzomyia* species)	Fever, anemia, splenomegaly, lymphadenopathy; cutaneous forms manifests as skin lesions and ulcers	Liposomal amphotericin B, miltefosine, antimonials; fluconazole, itraconazole
Malaria	*Plasmodium* species	Female mosquito (*Anopheles* species)	Headache, fever, paroxysm, joint pain, anemia, jaundice; neurological symptoms in severe cases	Chloroquine, mefloquine, doxycycline
Chagas disease (American trypanosomiasis)	*Trypanosoma cruzi*	Kissing bugs (Triatominae)	Fever, malaise, enlargement (liver, spleen, lymph nodes), sometimes skin nodules (chagoma); chronic stages affects the brain, heart and digestive system	Benznidazole, nifurtimox
Human African Trypanosomiasis	*Trypanosoma brucei*	Tsetse fly (*Glossina* species)	First stage-intermittent fever, headache, swelling of lymph nodes, joint pain; second stage involves neurological symptoms	Pentamidine, suramin, fexinidazole, nifurtimox, eflornithine
Toxoplasmosis	*Toxoplasma gondii*	Oral route; transmitted by ingestion of parasite oocyst	Headache, fever, fatigue, muscle ache; skin manifestation includes erythema and roseola	Pyrimethamine, sulfadiazine, clindamycin, spiramycin
Trichomoniasis	*Trichomonas vaginalis*	Genital contacts	Pain, itchiness/burning in genitourinary organs, urethritis, prostatitis (in males) while frothy, foul-smelling discharge, vaginitis (in females)	Metronidazole
Giardiasis (beaver fever)	*Giardia* species	Feco-oral transmission by ingestion of cysts	Chronic diarrhea, abdominal cramps, nausea and vomiting	Nitroimdazole, quinacrine, furazolidone, paromomycin
Cryptosporidiosis	*Cryptosporidium* species	Oral transmission by consumption of contaminated water, undercooked food	Diarrhea, abdominal cramps, low-grade fever (in intestinal cryptosporidiosis); inflammation of nasal mucosa, cough, shortness of breath, hypoxemia(respiratory cryptosporidiosis)	Electrolyte replacement by rehydration therapy, nitazoxanide, azithromycin, paromomycin
Amoebiasis	*Entamoeba histolytica*	Feco-oral route	Diarrhea, severe abdominal pain	Amebicidals (metronidazole, tinidazole) and cysticidal agents (iodoquinol)

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Diseases	Causative agent (pathogen)	Transmitting agent (vector)	Manifestation	Treatment options
Helminthic diseases				
Roundworm infection (in murine)	*Nippostrongylus braziliensis*	Skin penetration	Emphysema, loss of alveolar septa, lung hemorrhage	Tetramisole
Ascariasis (Roundworm infection, in human)	*Ascaris lumbricoides*	Feco-oral route	Fever, cough, weight loss, abdominal discomfort, intestinal ulcer accompanied with eosinophilia	Albendazole, mebendazole
Fasciolosis	*Fasciola hepatica*	Oral route, consumption of contaminated food	Acute phase marked by fever, nausea, skin rashes, abdominal pain; chronic phase manifests as jaundice, anemia and intermittent pain	Bromofenofos, triclabendazole, bithionol
Taeniasis	*Taenia* species	Consumption of undercooked pork or beef	Mild (abdominal pain and nausea) to no symptoms	Praziquantel, albendazole, niclosamide, mepacrine
Onchocerciasis (sub-cutaneous filariasis)	*Onchocerca* species	Blackfly (*Simulium* species)	Itchiness and bumps and depigmentation in skin to blindness	Ivermectin, moxidectin
Filariasis (lymphatic and serous cavity)	*Wuchereria bancrofti* and *Brugia* species	Blackflies and mosquitoes	Edema with skin thickening and underlying tissues	Diethylcarbamazine citrate (DEC)
Neural angiostrongyliasis (eosinophilic meningitis)	*Angiostrongylus cantonensis*	Oral route; upon ingestion larvae in undercooked prawn, snails, slugs, frogs	Headache, fever, malaise, nausea, neck stiffness, varying degree of neurological dysfunctions	No specific treatment, supportive care helps reduce the severity of symptoms
Schistosomiasis	*Schistosoma mansonii*	Contact with fresh water contaminated with parasites (released from fresh water snails)	Abdominal pain, diarrhea, fever, cough, bloody stool and/or blood in the urine	Praziquantel, oxamniquine, metrifonate, artesunate, mefloquine
Trichinosis	*Trichinella spiralis*	Consumption of undercooked pork	Nausea, vomiting, fever, diarrhea, facial swelling	Mebendazole, albendazole

Table 1.
Some parasitic diseases, their symptoms and treatment options.

hepatomegaly and eventually death. Some parasitic infections are easily treated while others are not. In the light of the lack of vaccine for parasitic infection, proper prophylactic measures (proper hygiene, prevention of contaminated food, water, preventing consumption of undercooked food, use of bednets, insecticide spraying to prevent vector borne diseases, etc.) and active disease surveillance remains the key for disease elimination. Unfortunately, poor disease management strategies have made parasitic infections a global healthcare challenge. In this article it's only possible to cover some important parasites (**Table 1**), for which research on molecular medicines are underway.

3. Molecular medicinal strategies and parasitic diseases

Parasitic infections (protozoan and helminthic infections) affect more than a quarter world population and cause chronic illness primarily in developing countries of world. These diseases affect the quality of life and treatment costs possess economic burden on families leading to viscous circle of poverty.

Molecular medicine is a broad field that includes insight into the molecular aspect of diseases. Recombinant DNA and cloning technologies are the conventional tools for studying the disease associated molecular profiles. Recent technical advances have paved the way for utilization of several molecular strategies for treating infectious diseases. Molecular medicine aims to understand the molecular basis of disease pathogenesis and allows the utilization of the information in designing specific diagnostic, therapeutic and prophylactic options. Mainly molecular medicine relies on two strategies—targeting genome and targeting signaling pathways, as targeted approach of disease management. Thus, it aims to improve the human health through the understanding of mechanism in human diseases.

3.1 Targeting genome

Apart from conventional approach of gene therapy (replacement of defective gene by exogenous DNA and editing mutated gene), recent technical advances have opened the arena for other strategies of manipulating the gene expression. Gene editing methods have gained limelight that involves the intrinsic molecular repair processes within the cell. The process of break repair in the DNA involves the homology-directed repair (HDR) and/or non-homologous end joining (NHEJ). The key step in gene-editing tool involves the precise introduction of double strand breaks. This process involves the use of engineered meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the recent CRISPR/Cas system [2]. Further, short antisense oligonucleotides potentially serve as tools for abrogating the transcription of target gene. As compared to other genome editing methods CRISPR/Cas system using guide RNA has shown immense potential for future of molecular medicine.

3.1.1 Engineered meganucleases

Although there remains plethora of meganucleases to choose from, however, most commonly used meganucleases include the ZFNs that have DNA binding zinc finger protein domain and nuclease domain. Cys_2-His_2 zinc finger domain is amongst the most abundant types of DNA binding motifs in eukaryotes [3, 4]. The ZFNs work by binding to the DNA and cleaving it, which then undergoes repair by either homologous recombination or error-prone NHEJ [5]. Site-specific cleavage is induced by manipulating the ZFN complex to recognize two sequences that are on either side of target site. Upon identification, cleavage of genome is induced by restriction enzyme (FokI), thus generating double stranded breaks in genome that can be used for editing the region.

TALEN has been introduced as an alternative to ZFNs. These are similar to ZFNs in using restriction enzyme that incorporates with the DNA binding domain, but are of different origin. Similar to ZFNs, these protein structures lead to the double stranded breaks in DNA for genome editing [6]. Unfortunately, there are no evidences for the use of the aforementioned meganucleases for the development of molecular medicine for parasitic diseases.

3.1.2 RNA-guided engineered nucleases (CRISPR-Cas9 system)

After the discovery of clustered regularly interspaced palindromic repeats (CRISPR) (in 1987) as a part of bacterial immune system against invading viruses. This strategy has potential application in editing human chromosome with great accuracy. It is RNA guided gene editing tool that uses Cas9 endonuclease for generating double-stranded breaks at loci of interest, which are then repaired via. HDR (using a template) or NHEJ or error-prone microhomology-mediated end joining (MMEJ) [7]. Thus, leading to mutation (insertion, deletion or substitution) with no or minimal damage to host genome. Since the sequencing of the parasite genome, CRISPR/Cas9 genome editing tool accelerated the molecular research in parasitology.

Genome editing in malarial parasite (*P. falciparum* and *P. yoelii*) has provided the ground for development of molecular medicine [8, 9]. CRISPR system of genome editing has played crucial role in understanding the drug resistance and pathogen survival thus, genome editing of the parasite genome holds promises to trigger host immune responses while preventing disease pathology. There are several studies for CRISPR/Cas9 analyses in *T. gondii* however, the most remarkable one include the genome-wide screening that identified the genes involved in infection process [10, 11]. This has greatly accelerated the research to understand the parasite metabolic needs for survival and virulence, conversely, shedding light on dealing with drug resistance mechanisms. Similarly, CRISPR/Cas9 system for *T. cruzi* and *T. brucei* has facilitated functional studies of drug targets and/or vaccine candidates [12, 13]. Genetic manipulation has always remained arduous for *Leishmania* however, CRISPR/Cas9 system has proven efficacy for rapid genome editing and understanding gene functions with therapeutic implication [14, 15]. Similarly, genome editing for *T. vaginalis* has been recently introduced with potential *in vivo* toxicity issues which has been dealt by using nucleofusion based transfection [16, 17]. Large-scale functional genomic screening cannot rely on conventional CRISPR/Cas9 approach thus, CRISPRi and CRISPRa approaches have gained significant attention for generating knock-in/knock-down libraries.

Genome editing using CRISPR/Cas9 system in *Schistosoma mansoni* eggs reduced the infection induced granulomas. Similarly, CRISPR/Cas9 mediated deletion of granulin gene from liver fluke (*O. viverrini*) significantly improved the disease symptoms [18]. This is just the start of the use of CRISPR/Cas9 system in parasitic diseases, it has broader potential in designing molecular medicine for disease intervention.

3.1.3 RNA interference (RNAi)

RNA interference is the approach of inhibiting the gene expression or translation by insertion of double-stranded RNA into the cells and/or organism that mediates targeted degradation of its homologous RNA. It can be done *via*. Short interfering RNA (siRNAs), micro RNA (miRNA) and piwi-interacting (piRNA). It mediates gene silencing by forming RNA-induced silencing complex (RISC) that eventually degrades target mRNA. This unique ability of silencing target gene holds hopes for controlling parasitic infections.

T. brucei was the first protozoan parasite where RNAi based targeting of β-tubulin gene, changed parasite morphology [19]. Conversely, *T. cruzi* [20], *L. donovani* [21] and *L. major* [22] lack the essential protein (Ago-1) for suppressing the gene expression. RNAi mediated targeting of cathepsin B reduced the disease progression [23]. RNAi mediated targeting of topoisomerases and farnesyl pyrophosphate synthase have proven efficacy of RNAi based molecular medicine for disease intervention [24, 25]. Later, in 2011, RNAi target sequencing (RIT) identified several potential targets for genome-scale functional analyses which could potentially therapeutic targets [26]. RNA aptamers (synthetic RNA and DNA molecules) binds the target ligands (RNA/DNA) with high-specificity, and have been developed as pharmaceutically active compounds against *T. brucei* [27].

Malarial parasites lack the conventional RNAi pathway however, antisense oligodeoxynucleotides treatment (against parasite topoisomerase) has shown to significantly reduce the parasite multiplication [28]. Further, topoisomerase targeting antisense nanoparticles and chitosan-based nanoformulation have also been used to inhibit *P. falciparum* growth [29, 30]. Recently, synthetic siRNA targeting the β-actinin and cysteine protease served as potential molecular target for *T. vaginalis* infection [31]. Although still scrappy, better understanding of RNAi pathway in protozoan parasites is likely to revolutionize the molecular medicine due to its genome homeostatic potential.

RNAi based silencing of key genes involved in regulating the parasite survival and development have been potential candidates for therapeutics. Several miRNA have been known to regulate the nematode development and survival in the host microenvironment due to their immunoregulatory potential. These have also been crucial players of host-parasite interaction and have been used as diagnostic marker of infection. *Nippostrongylus brasiliensis* (rat parasite) was the first nematode where RNAi was reported [32]. Much evidences for RNAi based knock-down studies have been seen in *Schistosoma* [33, 34], *Brugia* [35], *Trichinella spiralis* [36], *Ascaris suum* [37], *Angiostrongylus cantonensis* [38], *Taenia saginata* [39], *Echinococcus* [40], *H. contortus* [41] and *Onchocerca volvulus* [42]. Unfortunately, RNAi has not achieved the expected success in parasitic nematodes (schistosomes being an exception) [43]. This could be attributed to the lack of several key components of RNAi pathway [44]. Thus, targeted therapy with RNAi based approach (especially miRNA) is still in its infancy, in context of helminthic infections, that needs orchestrated support from the investors as well as the scientific community in order to stand alone as potential candidate for development of molecular medicine.

3.2 Targeting cells and signaling pathways

This area of molecular medicine remains the hottest area of research in the field of parasitic diseases after the World Health Organization (WHO) warning about the risk of post-antibiotic era. The search for novel therapeutic strategies intends to enhance pathogen killing by targeting regulatory molecules/pathways. Better understanding of disease immunobiology and cellular signaling will provide momentum to the identification of the pathways of therapeutic importance. This area of research towards the development of molecular medicine involves the use of genetically engineered antibodies, recombinant proteins, small molecules to alter signaling pathways, targeting the immunometabolic pathways, inflammasomes, etc.

3.2.1 Immunotherapeutic approach

Immunotherapy is use of biological substances (antigen/antibody, immunomodulators administration) to regulate host immune system in order to fulfill prophylactic and/or therapeutic purpose. Immunotherapy aims to trigger the immune power by directly (antigen based or active immunotherapy) or indirectly (antibody based or passive immunotherapy) [45, 46]. This section describes various immunotherapeutic strategies of molecular medicine that have been reported for the parasitic diseases.

3.2.1.1 Recombinant proteins/cytokine therapy

Cytokines are the small molecular weight, chemical messengers that regulates the immune responses in autocrine and paracrine manner. There is plethora of evidences for the involvement of cytokines in determining the pathophysiological consequences. Recombinant protein (cytokine) based therapy aims to trigger T-cell immune responses and induces parasite clearance.

In *T. cruzi* infection, TGF-β (transforming growth factor-β) has been implicated to yield pathological consequences however, treatment with TGF-β receptor kinase, SB-431542, has shown to restrict the entrance of parasite in the cardiomyocytes and disease associated cardiomyopathy [47, 48]. Additionally, cytokine combination therapy with recombinant IFN-γ and TNF-α has anti-parasitic potential [49]. In context of leishmaniasis, Murray et al. first proved the significance of targeting the cytokine, they showed that monoclonal antibody-based treatment targeting the IL-10 receptor (anti-IL-10 receptor) instigated parasite clearance by inducing NO (nitric oxide) production [50]. Likewise, combination of recombinant IFN-γ therapy with conventional chemotherapy yielded promising results in controlling the disease pathology [51]. Not much has been reported about the cytokine-based therapy in other protozoan diseases.

Conversely, in helminthic infection, MAb (monoclonal antibody) based blocking of IL-4 and IL-10 has shown disease improvement by reducing parasitic burden and inducing TH1 immune responses [52]. Likewise, IL-4 based MAb therapy, in schistosomiasis, has shown to inhibit granuloma formation and hepatic fibrosis [53]. Similar findings of marked reduction in granuloma and hepatic fibrosis were reported upon treatment with *Schistosoma* eggs along with recombinant IL-12 treatment [54]. Exogenous IL-25 based therapy has shown to potentially modulate intestinal functions by regulating IL-13 mediated STAT6 signaling in order to favor protective immune responses in intestinal nematode infection by *N. brasiliensis* [55]. Conversely, exogenous treatment with IL-13 and IL-25 triggered ILCs (innate lymphoid cells) responses and conferred protection against helminthic infections [56]. In schistosomiasis, IL-13 inhibitor, sIL-13Ralpha2-Fc has proved therapeutic benefit by preventing tissue fibrosis due to T_H2 dominated inflammatory responses [49] .

The significance of exogenous cytokine therapy has also been underlined in trichiasis, where IL-33 is known to induce thymic stromal lymphopoietin that generates polarized TH2 responses to confer protection against intestinal nematodes [57]. While, IL-25 treatment instigated TH2 responses and restricted infection induced gastrointestinal inflammation [58], MAb based blockade of IL-10 ameliorated disease pathology. There are evidences for the IL-27 mediated suppression of T-cell proliferation thus IL-27 receptor (WSX-1) knock down improved the mucosal immunity [59]. The use of immune triggering cytokines (IFN-γ, IL-12, GM-CSF) and/or blocking immunoregulatory cytokines that possesses pathological consequences holds hopes for the development of molecular medicine. Thus, therapeutic potential of cytokine therapy can be exploited alone and/or in combination with conventional chemotherapy opening up the avenues for improving treatment outcomes.

3.2.1.2 Immune checkpoint therapy

Immune checkpoint molecules are involved in regulating the T-cell activation and functions. The expression of these molecules is enhanced during chronic infections as a result of immune subversion, thus, therapeutically targeting these molecules has shown promising results in cancer and infectious diseases vaccines [60, 61]. Indeed, T-cell dysfunctionality or exhaustion is the key for impaired T-cell responses during chronic infections; exhaustion is marked by loss of IL-2 production, reduced cytotoxicity, impaired production of pro-inflammatory mediators and reduced proliferative ability. The expression of multiple immune checkpoint molecules (PD-1, CTLA-4, LAG-3, Tim-3, TIGIT) remains the hallmark feature of exhausted cells; elevated expressions of these molecules are accompanied with progressive loss of T-cell functionality [62]. Immune checkpoint inhibitors have been novel strategy of reinvigorating the immune cell functions by abrogating the signaling by the immune checkpoint (or co-inhibitory molecules).

A number of immune checkpoint molecules have been reported in leishmaniasis including—LAG-3, Tim-3, CTLA-4, PD-1, etc. that negatively regulates T-cell functionality [63–65]. MAb based blockade of PD-1 and LAG-3 in malaria triggered pro-inflammatory cytokine responses and relieved T-cell inhibition [66]. Likewise, therapeutically targeting LAG-3 and PD-L1 restored CD4+ T-cells functions, restored follicular helper T-cells, plasma cells eventually cleared the blood stage of *Plasmodium* [67].

Unfortunately, this strategy of immune checkpoint therapy has been in its nascent stage for parasitic infections, and has yet not been used for HAT, Chagas disease, gastrointestinal protozoans as well as helminthic infections.

3.2.1.3 Immune cells and stem cell-based therapy

Immune cell manipulation offers another fascinating approach of molecular medicine to fight with parasitic diseases, when other treatment options fail to provide protective immunity [68, 69]. Direct transfer of immune cells has been holding great promises for conferring protection against protozoan, bacterial and viral infections [70]. Adoptive T-cell transfer therapy using tumor-infiltrating lymphocytes is the best example to clinical success of cellular therapy [71].

DC (dendritic cell) based vaccination approach using parasite peptide (KMP-11) elicited TH1 responses, reduced parasite load and induced lymphocyte proliferation in leishmaniasis infection [72]. Similarly, vaccination with DC along with histone H1 elicited pro-inflammatory responses (IFN-γ and IL-12), reduced the IL-10 and IL-4 producing cells and induced polarized TH1 responses [73]. Atypical progenitor cells (IL-7R+ c-kit+ cells) from malaria infected mice are potent fighters against infection, while transplantation of these cells had similar effects in disease recovery [74].

After the success of direct administration of MSCs (mesenchymal stromal cells) and antigen specific T-cells stem cell therapy has recently budded in the field of infectious diseases. MSCs have been shown to be equally important in conferring resistance against *P. berghei* infection, by suppressing IL-10, reducing the regulatory T-cells population and inducing the production of IL-12 [75, 76]. Likewise, autologous transplantation of MSCs and myoblasts has shown to significantly reduce the ventricular dysfunctions [77].

Transplantation of bone marrow mononuclear cells has marked effect on improving the inflammation and fibrosis in Chagas disease [78, 79]. Also, bone marrow transplantation holds promises for improving the quality of life in congestive heart failure due to Chagas disease [80, 81]. Adoptive immunotherapy in toxoplasmosis, by transferring CD8+ T-cells restricted parasite de-encystation; however, it failed to revert the T-cells exhaustion attributing to the short-lives of exhausted cells [82]. Further, MSCs therapy in toxoplasmosis has not been successful however, when used in combination with the spiramycin, pyrimethamine and folinic acid provided therapeutic benefits. Similarly, for coccidiosis, using adoptive transfer strategy, intraepithelial lymphocytes (IELs) and CD4+ T-cells from interferon gamma knock out (*Cryptosporidium parvum*-infected) mice has shown to provide protection against infection in naïve mice [83]. Likewise, adoptive transfer of sporozoites pulsed-DCs upon co-culture with CD4+ and CD8+ T-cells reduced parasite burden [84].

In helminthic diseases, MSC based therapy have been proven to be efficacious for reducing *Schistosoma japonicum* induced liver injury by using MSCs culture supernatant which inhibited macrophage activation by egg antigen. Macrophages primed with *N. brasiliensis* have been shown to clear the parasitic burden by neutrophil mediated mechanism of macrophage polarization [85] in strongyloidiasis. Filarial infections are associated with increased expression of Foxp3 expressing regulatory T-cells that impairs the CD4+ T-cell immunity. Regulatory T-cells targeted intervention using antibodies against CD25, glucocorticoid-induced TNF receptor family-related gene (GITR), provided cure for filarial infection [86]. In schistosomiasis, basophil depletion strategy has been shown to successfully ameliorate disease pathology and granulomatous lesions [87]. Similarly, *in vivo* DCs depletion has been an efficacious strategy to boost antigen specific T-cells expansion [88]. Antigen pulsed immune cell therapeutics has been extended to *F. hepatica* infection. DCs pulsed with parasite induces T_H1 responses and has been a viable vaccination option that protects against disease associated hepatic damage [89]. Similarly, transfer of *Hymenolepis diminuta* pulsed bone marrow derived DCs cells ameliorated colitis pathology by IL-4 signaling [90]. Therefore, cell based therapeutic strategy serves as potential molecular medicinal approach for parasitic infections.

3.2.1.4 Immunomodulators

Immunomodulators are small molecular inhibitors of signaling pathways that serve as molecular medicine for disease intervention. Imatinib, an Abl/Arg tyrosine kinase inhibitor, induces cytoskeleton remodeling to facilitate leishmanial parasite phagocytosis in the macrophages and reduces disease associated lesions [91]. Another signaling pathway inhibitor, AS-605240 (PI3K gamma inhibitor) has shown to be as efficacious as sodium stibogluconate (SSG) in the treating of *L. mexicana* infection [92]. Similarly, another PI3K inhibitor CAL-101 and IC87114 are known to effectively reduce parasitic burden by reducing the B-cells and regulatory T-cells populations [93, 94]. Another tyrosine kinase inhibitor, ibrutinib has been shown to treat leishmaniasis by triggering T_H1-polarized IFN-γ production [95]. Tellurium based immunomodulator (AS101) has shown to effectively revert t-cell anergy and promote NO production while inhibiting IL-10 signaling in *L. donovani* infection. In Chagas disease, inhibitors of GPCRs provide protection against the disease by preventing the parasite entry and infection [96]. Parasite derived thromboxane A2 signaling induces apoptosis, vasoconstriction and disease associated cardiomyopathy thus use of SQ29548, thromboxane A2 receptor antagonist abrogates the *T. cruzi* infection [97]. Conversely, platelet activating factor and leukotriene B4 induces NO production and effectively controls the parasite [98, 99].

Further, β-adrenergic receptor blockade along with carvedilol has been an effective strategy to improve clinical symptom of Chagas cardiomyopathy [100].

Tyrosine kinase inhibitors (lapatinib) have proven their efficacies in controlling Human African Trypanosomiasis (HAT) pathogenesis by blocking parasite endocytosis [101]. Furthermore, PI3Kγ/mTOR signaling inhibitors as NVP-BEZ235 restricts the *T. brucei* infection [102]. Lectin based therapy using parasite galactose-N-acetyl-D-galactosamine inhibitable lectin (Gallectin) instigates IL-12 production from DCs, T-cell proliferation and IFN-γ production [103].

Rosiglitazone, peroxisome proliferator-activator receptor gamma (PPARγ) agonist, is known to enhance phagocytic clearance of parasitized erythrocytes and reduce parasitic burden in malaria by inhibiting the mitogen-activated protein kinase (MAPK) and NF-κB signaling [104].

In helminthic infection (Strongyloidiasis), anakinra (IL-1β receptor antagonist) potentially improved innate cytokine responses (IL-33 and IL-25) eventually causing parasite expulsion [105]. Therefore, small molecular have shown potential therapeutic benefits in parasitic infection, here is just the tip of huge iceberg, research is underway to explore other molecules.

3.3 Nano-medicinal approach

The application of nanomaterials in the field of medicine for diagnosis and treatment received considerable attention in recent decades for parasitic diseases. The diagnostic potential of nanomaterials has been seen in malaria [106, 107], toxoplasmosis [108], cryptosporiodiosis [109], amebiasis [110] and leishmaniasis [111, 112].

Considering the nanoparticles as treatment option for parasitic diseases, these particles have proven efficacy in targeting the infected macrophages for treatment of VL [113]. Silver alone or in combination with chitosan nanoparticles exhibited anti-toxoplasma effects by exacerbating serum IFN-γ levels and lowering the parasitic burden [114]. Spiramycin loaded chitosan nanoparticles have shown to effectively treat toxoplasmosis [115]. In giardiasis, combination nanotherapy with silver, chitosan and curcumin nanoparticles have been shown to effectively clear the parasites from intestine and stool without any adverse effects [116]. Chitosan as nanosuspension has also shown lethal effects on *Cryptosporidium* oocysts [117]. Similarly, silver nanoparticles have also been shown to effectively reduced oocyst burden by triggering IFN-γ, without any adverse events as seen with standard therapeutic options [118].

The biodegradability and non-immunogenic properties of nanoparticles have made them suitable as delivery agents for drugs and vaccines. Nanoformulation of recombinant *P. falciparum* protein (Pfs25H) served as transmission blocking vaccine for malaria, by abrogating the parasite infectivity to mosquitoes. Similarly, polymeric vaccine using polymer poly(lactide-co-glycoside) acid (PLGA) nanoparticles with malaria antigen, VMP001, and immunostimulatory monophosphoryl A (MPL-A) triggered antigen-specific immune responses against *P. vivax* [119]. Furthermore, iron oxide nanoparticle conjugated with recombinant merozoite surface protein 1 (rMSP1) were efficiently engulfed by macrophages and DCs, that eventually triggered the pro-inflammatory responses [120]. In VL, conjugation of quercetin with gold nanoparticle [121], doxorubicin along with chitosan [122], amphotericin B as chitosan nanocapsule [123] and mannose-chitosan based nanoformulation of rifampicin served as effective delivery system for VL management [124]. Chitosan/poly (vinyl alcohol) based microspheres has also shown to abrogate the *Cryptosporidium* sporozoites attachment to the enterocytes thus served as potential oral chemotherapy for *Cryptosporidium* infection [125].

For helminthic infections, chitosan based albendazole formulation skewed the T-cell responses to T_H1 type and reduced the parasitic burden, which led to parasite clearance in echinococcosis [126, 127] as well as in toxocariasis [128]. Similarly, silver assembled on fungal (*Trichoderma harzianum*) cell wall in the form of nanoformulation improved the anti-fascioliasis potential of triclabendazole [129]. Liposomal nanoformulations (nanoparticles and nanocapsules) have been widely used for enhancing the efficacies and bioavailability of oral drugs for disease intervention. These formulations have gained significance as nanocarriers in helminthic infection due to their ability to diffuse through the intestinal mucosal layers. In schistosomiasis, liposome encapsulated praziquantel has shown significant reductions in parasitic burdens and hepatic granulomas due to increased affinity for parasite phospholipids [130]. Liposome based nanocapsules of praziquantel (PZQ-LNCs) improved the drug efficacy and ameliorated disease pathology [131]. Further, liposomal nanocapsules of miltefosine exerted potential schistosomal effects by ameliorating hepatic histology (reducing the granuloma size, number and inflammation) in single dose [132].

Nanoformulation has also been used for vaccine development and as adjuvants, self-assembling protein nanoparticles (SAPN) have shown to trigger protective antibodies and long-lived memory responses to confer sterile protection against malarial parasites (*P. berghei* and *P. falciparum*) [133]. SAPNs have also been used for delivering the epitopes to induce CD4+ and CD8+ T-cell responses against *Toxoplasma gondii* [134]. Archaea based nanoformulations (archaeosomes) have been used as adjuvant as a part of prophylactic vaccine against *T. cruzi*, instigated humoral as well as cell-mediated immune responses (T_H1 responses) leading to marked reduction in parasitic burdens [135]. Cationic solid lipid nanoparticles have been successfully used as adjuvant as part of prime-boost strategy to reduce the parasitic burdens during VL. The vaccination triggered IFN-γ production, NO production and high levels of immunoglobulins (IgG1 and IgG2a) [136]. Therefore, nanoparticles served as viable, safe and effective vaccine platform as well as development of molecular medicine for cost effective vaccine delivery.

4. Conclusion

In this world where cost of developing medicine for parasitic infections remain the greatest challenge, drug developers are embracing molecular medicine approach that promises to deal with the parasitic infections and improves the chances of successful treatment. Molecular medicine has revolutionized the field of drug discovery/development however, there are significant hurdles in turning the promise into reality. Perhaps, gradually but it is shaping the future of medicine with the help of molecular platforms, better bioinformatics services and better pharmacogenomic analyses has greatly facilitated the scientific community and the stakeholders to come on common platform to fight against the parasitic diseases.

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