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Filariasis research – from basic research to drug development and novel diagnostics, over a decade of research at the Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany

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Filariæ are vector borne parasitic nematodes, endemic in tropical and subtropical regions causing avoidable infections ranging from asymptomatic to stigmatizing and disfiguring disease. The filarial species that are the major focus of our institution's research are *Onchocerca volvulus* causing onchocerciasis (river blindness), *Wuchereria bancrofti* and *Brugia* spp. causing lymphatic filariasis (elephantiasis), *Loa loa* causing loiasis (African eye worm), and *Mansonella* spp. causing mansonellosis. This paper aims to showcase the contribution of our institution and our collaborating partners to filarial research and covers more than two decades of research spanning basic research using the *Litomosoides sigmodontis* animal model to development of drugs and novel diagnostics. Research with the *L. sigmodontis* model has been extensively useful in elucidating protective immune responses against filariæ as well as in identifying the mechanisms of filarial immunomodulation during metabolic, autoimmune and infectious diseases. The Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn (UKB), Bonn, Germany has also been actively involved in translational research in contributing to the identification of new drug targets and pre-clinical drug research with successful and ongoing partnership with sub-Saharan Africa, mainly Ghana (the

Kumasi Centre for Collaborative Research (KCCR)), Cameroon (University of Buea (UB)) and Togo (Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires (LAMICODA)), Asia and industry partners. Further, in the direction of developing novel diagnostics that are sensitive, time, and labour saving, we have developed sensitive qPCRs as well as LAMP assays and are currently working on artificial intelligence based histology analysis for onchocerciasis. The article also highlights our ongoing research and the need for novel animal models and new drug targets.

KEYWORDS

Filaria, onchocerciasis, lymphatic filariasis, Litomosoides sigmodontis, eosinophils, immunomodulation, diagnostics, macrofilaricide

1 Introduction

With extraordinary host manipulative strategies combined with a co-evolutionary history spanning over 5,000 years, parasitic helminths are some of the most successful pathogens. Filariæ, vector borne parasitic nematodes (round worms), endemic in tropical and subtropical regions, comprised of *Onchocerca volvulus* that cause onchocerciasis (river blindness) (1); *Wuchereria bancrofti* and *Brugia* spp., causing lymphatic filariasis (elephantiasis) (2); *Loa loa* causing loiasis (African eye worm) (3); and *Mansonella* spp, causing mansonellosis (4). The majority of these neglected tropical diseases (NTDs) can cause stigmatizing pathologies leading to considerable socio-economic burden to the affected people and, thus, are serious public health problems to endemic countries. The burden of onchocerciasis is estimated at 21 million affected people, including 14.6 million with skin diseases and around 1.15 million people suffering from vision impairment. In 2017, at least 220 million people required preventive chemotherapy against onchocerciasis (1, 5). Lymphatic filariasis is confined to sub-Saharan Africa and South-East Asia with a few foci in Central and South America, including Brazil, Haiti, Dominican Republic and Guyana. It is estimated that 51 million people were infected in 2018, with 863 million people in 47 countries worldwide living in areas that require mass drug administration (MDA). While around 25 million men are estimated to suffer from hydrocele, lymphedema occurs in around 15 million people (2, 6). Loiasis affects over 13 million individuals (7) and around 600 million people are estimated to be at risk of infection with mansonellosis (3, 8). For onchocerciasis annual community-directed treatment with ivermectin, with or without albendazole, is conducted. MDA with a triple combination of ivermectin, albendazole and diethylcarbamazine has been the mainstay of treatment against lymphatic filariasis that is not co-endemic for onchocerciasis or loiasis (9–11). However, this treatment strategy is potentially accompanied by severe adverse events (SAE) in onchocerciasis and loiasis patients on treatment with diethylcarbamazine citrate (DEC) or ivermectin, suboptimal drug efficacy and the lack of a prominent macrofilaricidal efficacy (12–14). No MDAs are performed for loiasis and mansonellosis. For loiasis, individual therapy consists of daily DEC treatment for 21 days, which

cannot be given to patients with high microfilariae loads. In such patients, fatal encephalopathy may occur following DEC or ivermectin treatment, which is the reason that MDAs for lymphatic filariasis or onchocerciasis cannot be performed in areas co-endemic for loiasis.

The WHO targets to end the transmission of onchocerciasis in 12 countries (31%) and eliminate lymphatic filariasis as a public health problem in 58 (81%) endemic countries (defined as infection sustained below transmission assessment survey threshold) by 2030, and has formulated a roadmap to attain this goal, including new alternative treatment strategies and development of macrofilaricidal compounds (15–18). Loiasis and mansonellosis are not included in the list of NTDs so far and are therefore not targeted by the WHO NTD roadmap 2030.

In this context, the present review summarizes the work done at the Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn (UKB), Germany along with its main collaborators in Ghana (the Kumasi Centre for Collaborative Research (KCCR) and the Kwame Nkrumah University of Science and Technology, Kumasi), Cameroon (Research Foundation in Tropical Diseases and Environment (REFOTDE) and the University of Buea (UB)) and Togo (Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires (LAMICODA) and the University of Lomé, Togo) at the forefront of filarial research for the past two decades. The research conducted ranges from basic research and delineating the pathology of these diseases, to development of novel treatment options, pre-clinical research and diagnostics (Figure 1). Of particular emphasis is the support we receive from non-profit organizations like the Bill & Melinda Gates Foundation (BMGF) and Drugs for Neglected Diseases initiative (DNDi), and our collaborations with partners from academia and industry.

2 Basic research

The rodent filarial nematode infection with *Litomosoides sigmodontis* has been a model for filariasis basic and translational research for 70 years. The model, which is available in only 4 labs in

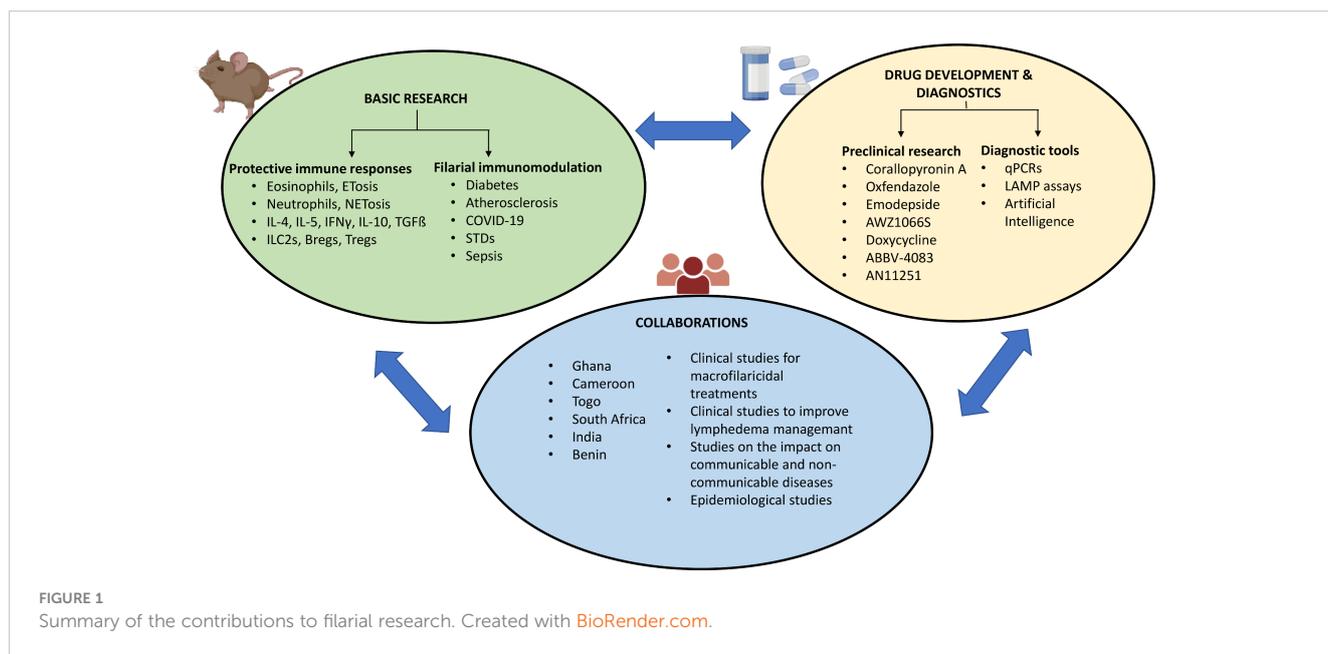


FIGURE 1 Summary of the contributions to filarial research. Created with BioRender.com.

the world, has contributed to major discoveries in the field (19–21). *L. sigmodontis* infective third stage larvae (L3) are transmitted by the bite of the hematophagous tropical rat mite *Ornithonyssus bacoti* to the rodent host. Cotton rats (*Sigmodon hispidus*) are the natural host, but Mongolian gerbils (*Meriones unguiculatus*) and BALB/c mice are also susceptible to the infection (20). L3 migration through the skin and lymphatics leads them to the blood circulation and they eventually reach the pleural cavity within one week post infection (22, 23). In BALB/c mice that are susceptible to infection with *L. sigmodontis*, adult worms develop by 30 days after infection and microfilariae are present in the peripheral blood after about 8 weeks (24). On the other hand, in semi-susceptible C57BL/6 mice, adult worms are cleared shortly after the development into adult worms (~ 45 days after infection) and, therefore, no release of microfilariae occurs (25). Work from our institute and others using the *L. sigmodontis* model has contributed to a better understanding of immune responses during filarial diseases and is presented in the following section. With the help of the *L. sigmodontis* model, we, e.g., were the first to describe suppressive regulatory T cell populations during filarial infection and showed that type 1 as well as type 2 immune responses contribute to protective immune responses (26). In this context, the role of an IFN- γ dependent effector mechanism to the function of neutrophils in murine filariasis was shown for the first time (27, 28)

2.1 Protective immune responses against filariae

2.1.1 Role of neutrophils

Neutrophils are the first line of defence against bacteria and are rapidly recruited to inflamed sites and their role in helminth infections is increasingly being recognized. In addition, endosymbiotic *Wolbachia* bacteria, present in most human pathogenic filariae (except for *L. loa*) and *L. sigmodontis*, are

known to recruit neutrophils. The major roles of neutrophils in controlling the early stages of infection have been demonstrated by Prof. Coralie Martin's laboratory using *Cxcr4*⁺¹⁰¹³ mice, which carry a gain-of-function mutation of the chemokine receptor CXCR4 leading to profound blood neutropenia. In this mouse model, subcutaneous delivery of the infective L3 larvae decreased the success of L3 larvae dramatically. Further, an oxidative burst response and the release of neutrophil extracellular traps (NET) were found to be mechanisms that directly contribute to the anti-parasitic strategies implemented by the host (29).

Early neutrophil recruitment, along with increased expression of S100A8 and S100A9 proteins, has been shown by the Martin laboratory to be associated with transient lung inflammation, lung haemorrhages and granulomas. This study suggests a mechanistic route *via* which L3 larvae reach the lung *via* penetration of pulmonary capillaries, endothelium and then to the pleural cavity (22). Using the *L. sigmodontis* model, the role of the neutrophil protein S100A8/S100A9 on the protective immune responses against filariae was investigated. The heterodimer formed by S100A8/S100A9, called calprotectin, is responsible for the mitigation of inflammation and tissue damage. In S100A8/A9 deficient mice, a significantly reduced worm burden was observed. Further, lack of S100A8/A9 was shown to trigger inflammatory responses in response to L3 larvae, leading to increased levels of chemokines, stronger recruitment of granulocytes and activation of neutrophils, which arguably impaired the migration of L3 larvae and susceptibility towards filarial infection (30). In IFN- γ knock out mice, neutrophils were found to be decreased in the thoracic cavity, thereby impairing the encapsulation process; and also displayed diminished chemotactic and phagocytic activities in comparison to the neutrophils of the control mice (27).

Wolbachia synthesize the muramyl dipeptide (MDP) containing cell wall precursor lipid II that is recognized by nucleotide-binding oligomerization domain-containing protein 2

(NOD2). Ligand binding triggers NF κ B-induced pro-inflammation. Therefore, the role of NOD2 was investigated in the *L. sigmodontis* model. While neutrophil recruitment was impaired in the skin of NOD2 deficient mice on intra-dermal injection of L3 larvae, the number of neutrophils in blood was also reduced. Depletion of neutrophils before *L. sigmodontis* infection increased the recovery of worms in the wild type mice, corroborating the importance of neutrophils in fighting invading L3 larvae. This study highlighted the role of NOD like receptors in first line defence against filarial nematodes (31).

Similarly, the importance of IL-6, a cytokine for neutrophil trafficking, in the immune responses to filariae was investigated. In mice deficient in IL-6, infection with *L. sigmodontis* led to a significantly increased worm burden, which may be due to the observed increased vascular permeability that facilitated larval migration. Analysis of skin samples after exposure to L3 larvae or filarial extract indicated a delayed recruitment of neutrophils and macrophages to the site of infection in the IL-6 deficient mice (32). The occurrence of NETosis in human *Onchocerca volvulus* infection was demonstrated for the first time by Tamarozzi et al. (33). NETosis was induced by *Wolbachia* via the direct ligation of lipoprotein peptidoglycan associated lipoprotein to TLR2/TLR6 on neutrophils.

In a mouse model of ocular onchocerciasis, antigen derived from microfilariae harbouring *Wolbachia* were injected into the corneas of mice. 18 hours after infection, neutrophils were observed to surround the nematodes in close proximity to *Wolbachia*. Incubation of parasite extracts with and without *Wolbachia* with peritoneal neutrophils clearly demonstrated that *Wolbachia* depleted extracts did not induce TNF, CXC chemokines, MIP-2, or KC by neutrophils. Taken together these findings indicate neutrophil activation is a crucial mechanism by which *Wolbachia* contribute to the pathogenesis of ocular onchocerciasis (34, 35). This could also be shown by immunohistology on *O. volvulus* nodules, where the strong presence of neutrophils around the adult worms was abolished in nodules from patients that had received a *Wolbachia*-depleting treatment with doxycycline (36).

2.1.2 Role of Toll like receptor (TLR) signalling in protective responses against filariae

Owing to the fact that most human filarial pathogens harbour *Wolbachia* bacteria, the possibility of an inflammatory response through TLRs was investigated. When *Brugia malayi* and *Onchocerca volvulus* extracts containing *Wolbachia* were treated on human embryonic kidney cells, stimulation was observed in those expressing TLR2, but not TLR3 or TLR4. Further, *Wolbachia* containing extracts could not stimulate cytokine production from TLR2 and TLR6 deficient murine macrophages. This report also showed the involvement of the adaptor molecules MyD88 and TIRAP/Mal (37). In the *L. sigmodontis* model, lack of the central adaptor molecular TRIF was shown to lead to a higher worm burden and reduced thoracic cavity cell numbers 30 days post infection. Further, in the thoracic cavity, increased frequencies of macrophages and lymphocytes and a decreased frequency of eosinophils, CD4 and CD8 cells were found, demonstrating the

importance of TRIF on worm recovery and recruitment of immune cells in C57BL/6 mice (38). In a mouse model of chronic filarial infection, triggering TLR2 has been shown to result in a significantly increased patency, while TLR4 has been shown to dampen the CD4 T cell responses (39). In conclusion, TLRs play an important role in the recognition of *Wolbachia* and protective immune responses against filariae.

2.1.3 Role of eosinophils, IL-4, IL-5, ILC2s, IL-10, and regulatory B and T cell populations

Although filariae are known to induce type 2 responses in their host and eosinophils are the essential part of this protective immune response, the exact role of eosinophils in protective immunity against filariae is still poorly understood. Using eosinophil-deficient dblGATA mice, IL-5^{-/-} mice, IL-4R^{-/-} mice and IL-4R^{-/-}/IL-5^{-/-} mice infected with *L. sigmodontis*, it was demonstrated that mice lacking IL-4R/IL-5, and therefore alternatively activated macrophages (AAM) and eosinophils, showed the greatest susceptibility to *L. sigmodontis* infection. All IL-4R/IL-5 knock out mice became microfilaremic, which developed earlier and had increased numbers of microfilariae and adult worms. The adult worms had an extended lifespan, leading to enhanced pathology in the pleural cavity; especially within diaphragm and lung tissue (40, 41). Mice, solely lacking IL-4R (and AAM) had an increased frequency of animals developing microfilaremia, but no significant differences in adult worm burden and microfilariae levels. In the absence of eosinophils (dblGATA mice, IL-5^{-/-} mice), increased microfilariae counts, frequency of animals developing microfilaremia, and adult worm counts with an extended survival were observed (41). In BALB/c mice, that are fully permissive for filarial infection, IL-5 was shown to be essential for protection against *L. sigmodontis* after vaccination with irradiated L3 larvae (42). Moreover, in eotaxin-1 deficient mice infected with *L. sigmodontis*, increased survival of the adult filariae was observed, which was associated with a reduced expression of CD80 and CD86 on eosinophils from eotaxin-1 deficient mice (43). To further characterize the role of eosinophilic granule proteins, mice deficient in eosinophil peroxidase (EPO) or major basic protein (MBP) were infected with *L. sigmodontis*. These mice developed a higher worm burden and increased levels of the anti-inflammatory cytokine IL-10 from thoracic cavity macrophages (44).

Eosinophils have several mechanisms to mediate protective immune responses and our group first described that eosinophils release extracellular DNA traps (EETosis) in response to filariae. Ehrens et al. (45) showed that these DNA traps inhibit the motility of *L. sigmodontis* microfilariae in a DNA dependent manner. The microfilariae induced EETosis was mediated by dectin-1 signalling and the released DNA was of mitochondrial origin (45). A more recent comprehensive review describes not only the role of eosinophils in the pathology of filarial infections, but also discusses eosinophils as drivers of pathology during tropical pulmonary eosinophilia in lymphatic filariasis and in inducing adverse events in DEC treated patients (46). Work from the Martin group showed that in *Meriones unguiculatus* infected with *L. sigmodontis* infection, fibrotic polypoid structures were observed in the pleura and these structures were associated with eosinophils, macrophages and lymphocytes (47).

Given that ILC2s are potent producers of IL-5, which can induce eosinophil responses, ILC2s are potentially also involved in protective immune responses against filariae. To elucidate the role of ILC2 during murine filariasis, susceptible BALB/c and semi-susceptible C57BL/6 mice were infected with *L. sigmodontis*. At 30 days post infection, the C57BL/6 mice showed higher numbers of ILC2s and increased levels of IL-5 and IL-13; indicating a stronger type 2 immune response. Depletion of ILC2s in T and B cell deficient *Rag2*^{-/-} mice indicated that ILC2s are involved in the control of microfilaremia in this mouse model, as more microfilariae were present in the absence of ILC2s (48).

Another immune cell subset involved in protective immune responses is the myeloid derived suppressor cell (MDSC) subset, which have been shown to expand during inflammation and have inhibitory effects on T and B cell responses. MDSCs consist of two distinct sub-populations, polymorphonuclear (PMN)-MDSCs and monocytic (Mo)-MDSCs based on morphology and phenotype. However, we have recently shown that, during *L. sigmodontis* infection, both Mo-MDSCs and PMN-MDSCs accumulate at the site of infection in BALB/c mice and positively correlate with worm burden or microfilariae numbers. However, only Mo-MDSCs were able to suppress the production of IFN- γ and IL-13 by *L. sigmodontis*-specific CD4⁺ T cells and neither of the cellular sub-populations had an effect on IL-5 production (49, 50).

In order to survive, helminths establish an immunomodulated milieu in the host *via* induction of regulatory T cells (Tregs), type 2 responses from Th2 cells, eosinophils, IL-4, IL-5, IL-13, alternatively activated macrophages and anti-inflammatory cytokines like IL-10 and TGF- β (51–54). To study the role of IL-10, which along with TGF- β is involved in the downregulation of T

cell responses, C57BL/6 mice overexpressing IL-10 in CD68+ macrophages were used. *L. sigmodontis* infection in these mice led to higher adult worm numbers and fully patent infections. Thus, macrophage secretion of IL-10 overcomes resistance from *L. sigmodontis* infection (55). Additional proof for the immunosuppressive role of IL-10 came from another study wherein IL-4 knockout resulted in increased susceptibility to *L. sigmodontis* infection in the C57BL/6 background. Double-knockout of IL-4/IL-10 resulted in the reversal of this susceptibility. This finding suggests that IL-10 dependent responses are the major reason for patency and are antagonistic to Th2 responses involved in containing the parasitemia (56).

We also showed that adaptive immune responses involving T, B and NK cell populations are important to prevent development of patent immune responses in the semi-susceptible C57BL/6 mouse strain and that Th17 polarization of the CD4+ T cells plays a crucial role for parasite clearance (57). Figure 2 summarizes the findings on the immune responses against filariae.

Further, the role of IL-10 and TGF- β in mediating hypo-responsiveness to onchocerciasis was studied. PBMCs from individuals with onchocerciasis produced significantly more IL-10, and T cell proliferative hypo-responsiveness in this group could be reversed by the addition of anti-IL-10 and anti-TGF- β antibodies. On the other hand, IL-5 was found to be associated with high cellular *O. volvulus* specific proliferation (26)

Mansonella perstans-specific immune responses were studied for the first-time using worm antigen extracts. The findings of the study showed that during *M. perstans* infection, distinct Th2, regulatory B cell and Treg subsets are induced, resulting in a reduced systemic, innate and adaptive immune response and increased levels of filarial-

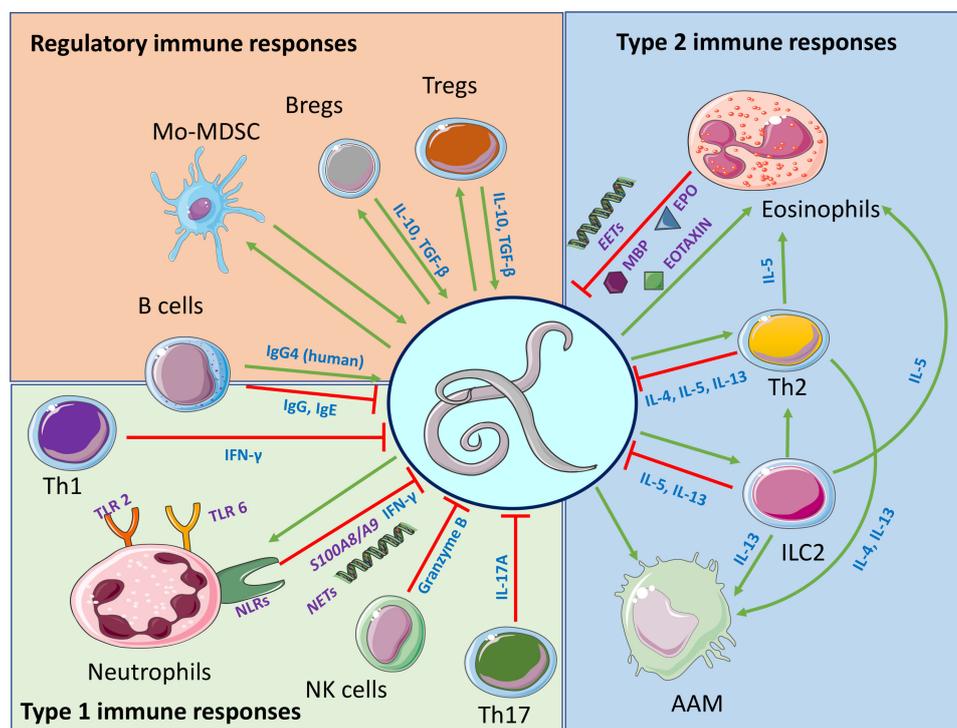


FIGURE 2 Major findings on protective immune responses against filariae and immune responses induced by the filariae. Regulatory, type 1 and type 2 immune responses are highlighted. Interactions among regulatory, type 1 and type 2 immune responses are not highlighted. Created with BioRender.com.

specific IgG4 levels (58). In a study done in Ghanian individuals, an ongoing *W. bancrofti* infection was shown to induce distinct populations of Breg and Treg populations from the peripheral blood mononuclear cells. These regulatory immune populations might contribute to the regulated state of the host immune system and contribute to the survival and fertility of the nematodes (59). However, the regulative state can be imbalanced; reflected in patients with severe pathology. The majority of these patients are characterized by increased Th1 and Th17 responses and constant immune activation (60, 61). Indeed, we recently showed that constant immune activation by *W. bancrofti* (62) can lead to CD4 and CD8 T cell exhaustion in patients suffering from lymphedema (63, 64), showing that imbalanced filarial-driven immune modulation, despite clearing the infection, can cause impaired T cell function; which might contribute to a higher risk for bacterial infections and consequently acute dermatolymphangioadenitis (ADLA) attacks.

2.2 Filarial immunomodulation on metabolic, infectious and autoimmune diseases

As described in the previous chapter, filarial nematodes establish a predominance of Tregs, AAMs, TGF- β and IL-10 etc., which results in the creation of an anti-inflammatory environment that helps parasite survival (51–54). Our group has demonstrated a significantly increased worm load in IFN- γ /IL-5 double-knockout mice indicating synergism between IFN- γ and IL-5 in controlling worm infection in the *L. sigmodontis* model (65). There is also some evidence that there is a “dichotomy” between Tregs and Th2 (+Th17) which is effective during hyperreactive onchocerciasis in humans (66). On one hand this kind of immunomodulation can affect bystander immune responses and can improve the outcome of allergies (67, 68) or autoimmune diseases (69–71), but, on the other hand, efficacy to vaccinations could be reduced due to nematode mediated immunomodulation (72, 73). Further, filarial nematode infections could alter immune responses to unrelated pathogens like bacterial and viral infections. The following section highlights the comprehensive work done to understand how filarial infections could regulate the outcome of a range of metabolic, autoimmune bacterial and viral infections.

2.2.1 Sepsis

Our work with the *L. sigmodontis* animal model has demonstrated that the exacerbated systemic inflammatory immune response characteristic of sepsis could be contained through helminth immunomodulation. The impact of the different life-cycle stages of *L. sigmodontis* on mice injected with sublethal doses of LPS was investigated. Infection with female adult worms from prepatent infections protected mice from LPS-induced sepsis. However, microfilariae worsened LPS induced sepsis through induction of pro-inflammatory cytokines, upregulation of granulocytes, NK cells and monocytes in the peripheral blood (74). Mice chronically infected with *L. sigmodontis* and subsequently challenged with *E. coli* showed significant protection from hypothermia, improved bacterial clearance and survival. These

outcomes were correlated with decreased levels of pro-inflammatory cytokines and chemokines and a diminished inflammatory profile of macrophages. These effects were shown to be mediated in a *Wolbachia* and TLR2 dependent manner (75).

TGF- β is one of the major hallmarks of chronic filarial infection and the dependence of TGF- β on the protective effect of filarial infection on sepsis was investigated. This effect was found to be independent of TGF- β signalling as depletion of TGF- β before challenge with *E. coli* did not lead to changes in pro-inflammatory cytokine or chemokine levels, nor increased bacterial burden and hypothermia. Despite TGF- β depletion, *L. sigmodontis* infected mice demonstrated milder hypothermia, reduced bacterial load and pro-inflammatory cytokine levels (76). In addition, the impact of chronic *L. sigmodontis* infection on T cell responses during the compensatory anti-inflammatory phases following sepsis was studied. Chronic filarial infection did not worsen *E. coli*-induced impairment of adenovirus-induced CD8 T cell cytotoxicity and adenovirus-specific secretion of IFN- γ , suggesting that filarial immunoregulation does not exacerbate T cell paralysis induced by *E. coli* (77).

2.2.2 Diabetes and atherosclerosis

In the backdrop of cross-sectional studies from China, India, Indonesia and aboriginal Australian populations showcasing an inverse association between the prevalence of helminth infections and type 2 diabetes, the idea of helminth-induced immune modulation delaying the onset of type 2 diabetes started gaining popularity (78–81). Our group was the first to test the mechanisms underlying this in an experimental mouse model of diet-induced obesity. Berbudi et al. demonstrated that after diet-induced obesity, infection with the filarial nematode *L. sigmodontis* or administration of crude *L. sigmodontis* adult worm extract (LsAg) resulted in an improved glucose tolerance and an increase in AAM, ILC2s and eosinophils in the visceral adipose tissue (82).

In another study, a novel role for the adipose tissue secreted adipokine adiponectin was demonstrated in orchestrating the beneficial effects of LsAg administration during diet-induced obesity. While stimulation of T cells with adipocyte conditioned media from LsAg treated mice resulted in decreased frequencies of Th1 and Th17 cell populations, this effect was ameliorated when the adipocyte conditioned media from LsAg treated mice was depleted of adiponectin; highlighting a novel role for adiponectin in mediating some of the beneficial effects of helminth infections during obesity and insulin resistance (83). Regarding type 1 diabetes, we showed that infection with *L. sigmodontis* or administration of crude LsAg prevented the development of diabetes in non-obese diabetic mice (NOD) (70). This protective effect was dependent on TGF- β (84). A combination therapy of LsAg and pro-insulin was found to prevent the onset of diabetes in NOD mice even after insulinitis occurred (85, 86). In addition, administration of LsAg to ApoE knockout mice, a mouse model for atherosclerosis, was found to decrease plaque size with both prophylactic and therapeutic treatment regimens. LsAg administration reduced the expression of Th-1 specific gene expression and intraplaque inflammation. The plaque size showed an inverse correlation with the AAM in the plaques (87).

2.2.3 COVID-19

In the wake of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, causing COVID-19, that has infected more than 600 million people worldwide and caused 6.8 million deaths as of 29th January 2023 (88), helminth-mediated immunomodulation on viral infections has been gaining increased interest, especially in the helminth endemic African sub-continent. This is owing to the fact that the prevalence and mortality of COVID-19 on the African sub-continent have been moderate (89). While factors like younger average age and low testing capacities could partially explain the mortality of COVID-19 and low prevalence, the co-endemicity of helminth infections in this part of the world as a potential contributor to this trend cannot be ruled out.

Infections with SARS-CoV-2 lead to a wide spectrum of clinical manifestations ranging from asymptomatic infection to potentially lethal respiratory complications, including hypoxemic respiratory failure and acute respiratory distress syndrome (ARDS) (90). These severe complications of the disease are associated with the hyperactivation of the immune system and the uncontrolled release of cytokines, including IL-2, IL-6, IFN- γ and TNF (91, 92). On the other hand, helminths are known to trigger a modified Th2 response wherein regulatory cell types (Tregs, B cells and anti-inflammatory cytokines like IL-10 and TGF- β) balance the pro-inflammatory components. Considering the important roles of CD4 and CD8 T cell responses in controlling the pathology of SARS-CoV-2 infection (93, 94), we recently investigated the impact of helminth antigen co-stimulation on the activation of SARS-CoV-2-specific T cells from convalescent COVID-19 patients (95). The findings of the study showed that helminth antigens from three major helminth parasites *B. malayi*, *O. volvulus*, and *Ascaris lumbricoides* were able to reduce the frequency of SARS-CoV-2-reactive CD4 T helper cells but not CD8 T cells in the PBMCs of COVID-19 patients. Further, increased IL-10 secretion and decreased levels of IFN- γ and TNF were observed in PBMCs stimulated with helminth antigens (95). Moreover, work in progress in our team demonstrates a robust negative correlation between *Ascaris*-specific antibody expression and hospitalization rate and severe symptoms in COVID-19 patients from Benin. These data suggest that helminth immunomodulation could shift the immune response to SARS-CoV-2 in a manner that SARS-CoV-2-induced hyperactive immune reactions are mitigated similar to our observations in animal models.

2.2.4 Sexually transmitted infections

In another example of helminth mediated immunomodulation, our collaborative work with partners from South Africa, Ghana and Tanzania has demonstrated the influence of helminths on sexually transmitted viral infections (STVIs). Low-middle income countries bear the double burden of parasitic nematode infections as well as STVIs (96, 97). This has led researchers to test the hypothesis that nematode infections, despite not colonizing the female genital tract, cause changes in female fecundity (98) and female genital tract immunity (99). In this respect, Chetty et al. (100) investigated if

nematode infections drive altered immunity to herpes simplex virus-2 (HSV-2) infection. Intriguingly, the findings of the study in the mouse model show that acute infection with *Nippostrongylus brasiliensis* initiates significant expansion of eosinophils in the female genital tract, which in turn leads to increased ulceration of the vaginal epithelium following HSV-2 infection. While our earlier reports have demonstrated a beneficial effect of helminth immunomodulation in sepsis, type 2 diabetes and COVID-19, this study points towards a deterioration of the disease pathogenesis.

Indeed, our collaboration with Dr. Katawa from the University of Lomé in Togo has shown that Togolese women with systemic helminth infections have altered vaginal immunity and an immune milieu that increases sexually transmitted diseases; especially human papillomavirus (101–103). However, the mechanisms behind these observations remain uncertain. Thus, ongoing research using the *L. sigmodontis* mouse model aims to elucidate signalling mechanisms of eosinophils and ILCs in the female reproductive tract, which drive the vaginal type 2 immune signature leading to increased pathology and enhanced risk for STVIs. These observations reiterate the fact that the impact of helminths on other diseases may vary from beneficial to detrimental depending on the specific disease, species of helminth, localization of the helminth stages vs. secondary infection, chronicity of infection, and organs affected.

2.3 Genetics

As has been described in previous sections, infection with filarial nematodes has a spectrum of clinical states with two major poles and a range of responses between the poles. One pole is represented by microfilaremic patients with high parasite numbers and down-regulated cell-mediated responses (28), while the second is represented by patients who typically have few or no parasites, but vigorous specific immune reactions that cause immunopathology, e.g. dermatitis for onchocerciasis or lymphedema/hydrocele for lymphatic filariasis (66, 104, 105). The actual causes of the heterogeneity in infection and disease are not well understood, but have been attributed to differences in inflammatory processes that are mediated by host genetic factors as well as to secondary bacterial infections (105–107)

In onchocerciasis, case-control studies on Single Nucleotide Polymorphisms (SNPs) for IL-13 and IL-10 in onchocerciasis patients identified factors that lead to two different responses. The variant form of the pro-inflammatory cytokine IL-13 was associated with the Sowda form of extreme dermatitis in onchocerciasis. Sowda patients have an exacerbated Th2 response with extremely high IgE levels which leads to the killing of skin microfilariae, but at the expense of severe dermatitis in the infected person. The association of the IL-13 SNP with Sowda is stronger than that for allergy, the benchmark for IL-13 in pathology (106). Similarly, individuals with the variant forms of the immunosuppressive cytokine IL-10 promoter SNPs -1082, -819 and -592 are associated with lower IL-10 expression by immune cells from patients, resulting in lower numbers of circulating microfilariae (108, 109).

Although susceptibility to infection, parasite load, and pathology has been shown to cluster in families (110–114) the genetic architecture underlying lymphatic filariasis is poorly understood. Knowledge of genomic loci linked to clinical disease would help in the understanding of disease etiology which could lead to the development of new diagnostic tests, and prevention and treatment strategies.

In order to identify the first genetic risk factors, we have performed case-control studies of SNPs in TGF- β 1 and VEGF-A. We have shown that reduced TGF- β 1 secretion leads to more inflammatory cells which kill microfilariae but also damage host tissue (26, 115). Because of this strong association of TGF- β 1 with filarial disease, we examined Leu10Pro variant SNPs of TGF- β 1 in Ghanaian patients with lymphatic filariasis. We found that individuals carrying the Leu/Leu genotype have no circulating microfilariae (116).

VEGF-A is a major mediator of vascular permeability and angiogenesis and promotes extravasation of fluid and plasma proteins from the blood vessels that has been associated with Chyluria, a filarial disease manifestation characterized by lymph fluid in the urine (117). We therefore analyzed VEGF-A promoter SNPs for a possible role in the different manifestations in patients with lymphatic filariasis. The C/C genotype at base pair -460 was significantly higher in hydrocele patients than in non-hydrocele patients. Furthermore, plasma levels of VEGF-A were significantly higher in subjects with the C/C genotype than in those with other genotypes. A positive correlation between plasma VEGF-A and stage of hydrocele was observed, suggesting that the C allele at -460 is a genetic risk factor for hydrocele development in lymphatic filariasis (118).

To elucidate the genetic basis and a possible association of genetic markers to hydrocele development in lymphatic filariasis, we performed a candidate gene analysis with 850 *Wuchereria bancrofti*-infected volunteers by genotyping 49 single nucleotide polymorphisms (SNPs) in 32 angiogenesis genes by MassARRAY (119). We found variants in four genes of the angiogenesis pathway significantly associated with hydrocele patients compared to infected individuals without pathology (Endothelin-1 rs5370 and rs1800541; Caveolin-1 rs4730748 and rs926198; Collagen Type 1A1 rs2269336 and matrix-metalloproteinase-2 rs1030868).

A similar analysis was also done to assess SNPs associated with filarial lymphedema development, a cross-sectional study of unrelated Ghanaian volunteers genotyped for SNPs in 285 lymphedema patients and 682 infected patients without pathology. In all 131 SNPs in 64 genes were genotyped by MassARRAY (manuscript in preparation). Genetic associations were identified for seven SNPs in five genes: vascular endothelial growth factor receptor-3 (VEGFR3; rs75614493), nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor alpha (NF- κ B α ; rs696), insulin like growth factor-1 (rs7136446), two SNPs in matrix metalloproteinase 2 (rs1030868 and rs2241145), and two SNPs in carcinoembryonic antigen-related cell adhesion molecule -1 (rs8110904 and rs8111171) (119). Interaction analyses revealed an interaction of these genes within the angiogenesis pathway.

A genome wide association study is currently being analyzed on a Ghanaian cohort of 1933 filarial lymphedema cases and 2474

endemic controls that were genotyped for 654,027 SNPs and genetic variations using the Illumina's HumanOmniExpress Bead Chips. With imputation, the total number of genetic variations detected will total 2.5 million SNPs and genetic variations. With the larger sample size and broader genetic coverage, finding SNPs associated with disease etiology with high significance should be identified. Identification of such genetic markers of filarial pathology could aid in ameliorating pathology other than invasive, dangerous, expensive surgery. Knowing genetic markers for filarial lymphedema and hydrocele could provide a way to identify persons at risk before pathology is seen and might become the basis for development of a rapid screening test that, in the case of filarial lymphedema which develops early in life, might be applied to school-aged children.

3 Drug development

Besides contributing to significant advances in basic science, the institute and its collaborating partners has also been actively involved in translational research in contributing to the identification of new drug targets and pre-clinical drug research (Table 1). Detailed reviews on this topic are available (139–141). As the purpose of this review is to provide a comprehensive overview of the overall contribution of the institution to helminth research, this section covers only selected aspects of our work on drug development. These contributions aim to overcome drawbacks of the standard drugs used for MDA - ivermectin, albendazole and DEC. Ivermectin and DEC are microfilaricidal and inhibit the release of microfilariae by the adult filariae for up to 6-12 months (142), but have no prominent macrofilaricidal efficacy. Albendazole has limited macrofilaricidal adult worm sterilising efficacy in some filarial species, but as a broad-spectrum anthelmintic drug it rather targets other intestinal nematodes and cestodes (143, 144).

3.1 Doxycycline, the first macrofilaricidal drug

Chemotherapy against onchocerciasis and lymphatic filariasis in the past was only discussed within the framework of MDAs which despite their benefits also came with a lot of disadvantages. In this regard, the use of doxycycline against *Wolbachia* endosymbionts turned a new and promising chapter in the direction of novel treatment options. Doxycycline came to the forefront of filariasis research when earlier studies in animal models showed effectiveness of the antibiotic tetracycline against *Wolbachia* of the filarial nematode *L. sigmodontis* leading to inhibition of embryogenesis, female worm sterility and subsequent clearance of microfilariae (145, 146). This effect of tetracycline was also confirmed in infections with *O. ochengi* (147), *B. pahangi* and *D. immitis* (148). Doxycycline is a second-generation tetracycline antibiotic and its use in first clinical trials at a dose of 100mg daily for 6 weeks resulted in *Wolbachia* depletion from *O. volvulus*, adult worm killing, and embryogenesis inhibition that resulted in amicrofilaridermia (149). A double-blind randomised field trial of 200mg doxycycline per day for 8 weeks

TABLE 1 Summary of drug candidates and their current clinical status.

Drug	Target	Phase	Expected treatment time	Clinical trials identifier
Flubentylosin/ABBV-4083 (120–122),	Anti-wolbachial	Phase-II in onchocerciasis patients ongoing	7-14 days	NCT04913610
Emodepside (123, 124)	Direct acting	Phase-II in onchocerciasis patients ongoing	7-14 days	NCT05180461
Oxfendazole (125)	Direct acting	Phase-II for onchocerciasis, loiasis and mansoniellosis in preparation	5 days	NCT04713787
High-dose Rifampicin (126)	Anti-wolbachial	Study in onchocerciasis patients scheduled	7-14 days	–
AWZ1066S (127)	Anti-wolbachial	Phase-I study ongoing	7-14 days?	NCT05084560
Corallopyronin A (128–133)	Anti-wolbachial	Phase-I study in preparation	7-14 days	–
DNDI-6166 (134, 135)	Direct acting	Late preclinic	7 days	–
AN11251 (136, 137)	Anti-wolbachial	Pre-clinical – on hold	7-14 days?	–
CBR417/CBR490 (138)	Anti-wolbachial	Pre-clinical – on hold	7 days?	–

strongly reduced the adult worm burden in lymphatic filariasis (as measured by ultrasonography) and resulted in microfilaremia at 8–14 months in the doxycycline group (150). Further testing of this antibiotic in bancroftian filariasis patients also proved its effectiveness in *Wolbachia* depletion as a dose of 200mg for six weeks resulted in 96% loss of *Wolbachia* with a 99% reduction of microfilaremia (151). In addition, a single dose of ivermectin after doxycycline treatment led to a fast and complete elimination of microfilariae, since microfilariae eliminated by ivermectin are not replenished due to embryogenesis interruption by doxycycline (151). In lymphedemic patients after 4 months of doxycycline treatment, a single dose of 150–200 µg/kg ivermectin and 400 mg albendazole led to a significant reduction in the mean stage of lymphedema in the doxycycline-treated patients compared to placebo patients (152). As a result of *Wolbachia* depletion, embryogenesis is blocked leading to a gradual decrease of microfilariae according to its half-life. Ivermectin acts in a complementary manner to target the fully developed microfilariae. Although doxycycline alone resulted in *Wolbachia* depletion and microfilariae reduction, additional ivermectin treatment advances the killing of microfilariae and doxycycline impeded the reappearance of microfilariae. Combination of 200mg of doxycycline regimens for 6 weeks followed by a single dose of ivermectin and albendazole four months after doxycycline led to a macrofilaricidal effect of 89% after 2 years and significant improvements in lymphedema already after one year (152). When doxycycline treatment duration was reduced to 4 weeks, there was still a macrofilaricidal activity of 83% after 24 months (153). However, a 3-week course of doxycycline followed by ivermectin and albendazole 4 months later did not affect the adult worm burden, although it led to ablation of microfilariae (154). In addition, doxycycline, arguably through its effects on lymphatic dilation was shown to be effective against lymphatic pathology in a

study cohort from India (155). However, recent findings from a multicentre clinical trial on lymphedema management indicate that doxycycline has no additional benefit in reducing the progression of lymphedema pathology if strict hygiene measurements are maintained.

In *B. malayi*-infected persons, a daily 6 week treatment with doxycycline alone or in combination with DEC and albendazole was found to decrease microfilaremia as well as reduce adverse reactions following antifilarial treatment (156). A report on doxycycline-induced changes in gene expression has provided new hints regarding the symbiotic relationship between *Wolbachia* and *B. malayi*. The genes in *B. malayi* essential for reproduction, growth and development were found to be down-regulated on treatment with doxycycline. The changes in the genome are consistent with the effect of doxycycline on filarial embryogenesis (157).

In case of onchocerciasis, a 200mg daily 6 week doxycycline regimen resulted in a gradual *Wolbachia* decline starting from month 2 and inhibition of embryogenesis observed by 6 months and a microfilariae decline at 11 months after the start of treatment. In the same trial a dosage of doxycycline 200mg for 4 weeks resulted also in *Wolbachia* depletion and female worm sterility (158). In another trial 100mg doxycycline for 6 weeks was used in patients with persistent microfilaridermia despite many rounds of IVM which led also in this special collective to sterility and enhanced killing of the adult worms (SCOTT) (159). Further studies also validated and confirmed the effects of doxycycline such as macrofilaricidal activity, female worm sterility, and *Wolbachia* depletion during *O. volvulus* infection (160–162). Doxycycline was also found to be safe in areas that are co-endemic for loiasis as *L. loa* is a *Wolbachia* lacking nematode. Because of that, and also because anti-wolbachial efficacy affects only adult worm vitality and embryogenesis but does not have a direct effect on microfilariae, it does not induce the serious adverse events observed after

microfilaricidal treatment with DEC or IVM (163–166). Based on these compelling evidences, WHO has recommended doxycycline as individual therapy against filariasis (1).

The drawback of doxycycline treatment is that it is contraindicated in pregnant and breast-feeding women as well as in children under the age of eight (167). Although a 6-week course of doxycycline has been administered successfully and with high compliance to a whole community of 12,000 people, it is only recommended for individual therapy because it requires daily treatments over 4–6 weeks. Individual treatment with doxycycline is being used as an end game strategy to clear remaining onchocerciasis in regions like Brazil and Republic of Venezuela (1). Its use as mass drug administration for onchocerciasis or lymphatic filariasis is not considered, due to the 4–6 week daily treatment requirement. Further there is also a risk of potential emergence of antimicrobial resistance if doxycycline ends up being used in MDA like Ivermectin.

3.2 New, short course anti-wolbachials under development

Considering the long treatment regimen of 4 to 6 weeks necessary for doxycycline and the contraindications for pregnant and breastfeeding women and children, there is an urgent need for short course macrofilaricidal adult worm killing drugs. Several candidates are currently at the early clinical or late pre-clinical stage (140). These drug candidates include emodepside, flubentylosin/ABBV-4083 (both currently tested in phase 2 clinical studies in onchocerciasis patients), oxfendazole and AWZ1066S (completed and ongoing phase 1 clinical study, respectively) (Table 1). Detailed information on these compounds is available in the article by Ehrens et al. (140) and an overview of their efficacy in the *L. sigmodontis* rodent model is given in Table 2. Next to the testing of those drug candidates in the pre-clinical *L. sigmodontis* model as well as supporting the clinical studies, our institute along with our partner institutions is developing corallopyronin A (CorA) as a novel candidate for the treatment of filariasis (139). In the *L. sigmodontis* model, treatment with CorA for 2 weeks has been shown to clear *Wolbachia* endosymbionts *in vivo* leading to a macrofilaricidal effect. In addition, combining CorA with the drug albendazole led to the shortening of CorA treatment to 7 days as well as the death of adult filariae (128). CorA is a natural product from *Coralloccoccus coralloides* (129) and acts *via* non-competitively inhibiting the DNA dependent RNA polymerase of the bacteria by targeting the switch region (130–133). It has been shown to be potent against gram-positive bacteria including rifampicin-resistant *Staphylococcus aureus* (169). Encouraging results from safety and toxicity testing indicate that CorA is safe and non-toxic up to a dose of 500–1000 mg/kg in rats and dogs. Final GLP-tox studies funded by the EU Horizon 2020 HELP consortium are underway, and a phase 1 trial is in filed in 2024 supported by the German Center for Infection Research (DZIF). The standard Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) studies for CorA has been completed and the results of the study show that CorA has an

acceptable *in vitro* early ADMET profile which is supported by the *in vivo* experiments in mice, mongolian gerbils and rats (170). In addition, CorA has also demonstrated excellent efficacy against other gram-positive and some gram-negative pathogens, e.g., *Chlamydia trachomatis*, *Orientia tsutsugamushi* and *Staphylococcus aureus*. Secondary indications are being developed for these organisms (140, 169).

4 Novel methods

In order to facilitate faster development of drugs against the various developmental stages of the filarial parasite, novel assays that have increased sensitivity, are more objective, or are easy to implement are needed for clinical trials, basic research, and drug development. Additionally, *in vitro* cultures are needed of the human parasitic filarial nematodes to de-risk drug candidates selected using animal filarial nematode models.

For clinical trials, we have developed a sensitive qPCR tool for the quantification of *Wolbachia* from few *O. volvulus* microfilariae in skin biopsies. The ability to monitor the effect of anti-wolbachial drug in few microfilariae is essential because: (i) the level of microfilaridermia is decreasing as MDA coverage is being attained, and (ii) effective anti-wolbachial drugs lead to a reduction in skin microfilariae. The method allows the analysis of anti-wolbachial drug efficacy from as few as 2 microfilariae and can be used to predict the effect in adult nematodes at less than 6 months post treatment without excising onchocercmata, which are limited resource per patient; and the removal of onchocercmata can directly affect end point readouts – removal of onchocercmata also removes adult nematodes, resulting in a concomitant reduction in microfilariae independent from treatment (171). The method is being validated using prospective samples from the flubentylosin phase 2 trial.

The institute, together with its academic collaborating partners and Capgemini is developing artificial intelligence (AI) to evaluate nodules from onchocerciasis patients. Until now, histological analysis was indispensable to assess the effect of a treatment on the adult worm. However, this time-consuming process and the lack of experts worldwide slow clinical trials required to register new drug regimens. With funding from the Bill & Melinda Gates Foundation, the establishment of AI is expected to drastically reduce the time required for the analysis of nodules, replace subjective analysis with objective analysis that can be established by other groups, and provide a constant algorithm of decision making in nodule assessment, an important request by regulatory authorities who have not yet approved antifilarial drugs with adulticidal efficacy. Similar to gold standard microscopic analysis, AI requires the presence of nodules, which prevents the diagnosis of early infection with the infective stage of *O. volvulus*. For this, improved serological diagnostic tests are required.

For new drug screening, an *in vitro* system has been developed for the maintenance of *O. volvulus* L3 larvae, which can develop to the adult stage. This system may provide a platform for the investigation of parameters like mating behaviour and early stage nodulogenesis; which are additional targets for macrofilaricidal

TABLE 2 Efficacy of drug candidates in the *Litomosoides sigmodontis* rodent model.

Drug	Oral treatment after adult filariae developed	Efficacy
Flubentylolisin/ABBV-4083 (122) (anti-wolbachial)	Jirds (MF+): 14 days – QD 50-100mg/kg	<i>Wolbachia</i> depletion >99.8%; block in embryogenesis, thus, clearance of microfilaremia,
	Mouse: 5 days – QD 75mg/kg	<i>Wolbachia</i> depletion >99.9%
Emodepside (124, 168) (direct-acting)	<i>Mastomys coucha</i> : 5 days – QD 100mg/kg	Adult worm burden reduction 100%; <i>in vitro</i> efficacious against all filarial life-cycle stages
Oxfendazole (125) (direct-acting)	Jirds (MF+): 10 days – BID 12.5mg/kg	Adult worm burden reduction 99%; inhibition of filarial embryogenesis followed by indirect clearance of microfilaremia
	Mouse: 5 days – BID 12.5mg/kg	Adult worm burden reduction 100%; no direct microfilaricidal efficacy
High-dose rifampicin (136) (anti-wolbachial)	Mouse: 10 days – QD 35mg/kg	<i>Wolbachia</i> depletion >99.5%
AWZ1066S (127) (anti-wolbachial)	Jirds (MF+): 7 days – BID 50mg/kg	<i>Wolbachia</i> depletion >99%; clearance of microfilaremia, probably due to block in embryogenesis
Corallopyronin A (128) (anti-wolbachial)	Jirds (MF+): 14 days – BID 30mg/kg i.p.	<i>Wolbachia</i> depletion >99%; block in embryogenesis, thus, clearance of microfilaremia, reduction of adult worm burden
	Jirds (MF+): 7 days – BID 10mg/kg i.p. + albendazole	Block in embryogenesis, thus, clearance of microfilaremia; adult worm burden reduction 95%
DNDI-6166 (direct-acting)	Not published	
AN11251 (136) (anti-wolbachial)	Mouse: 10 days – BID 200mg/kg	<i>Wolbachia</i> depletion >99.9%
CBR417 (138) (anti-wolbachial)	Mouse: Single dose – 200mg/kg	<i>Wolbachia</i> depletion >99.9%
CBR490 (138) (anti-wolbachial)	Mouse: Single dose – 200mg/kg	<i>Wolbachia</i> depletion >99.8%

*not performed by IMMIP and partners; MF+ = microfilariae-positive animals were used; QD = single dose per day; BID = twice-daily dose, i.p. = intraperitoneal.

drug screening (172). In addition, *in vitro* culture systems for the filariae *M. perstans*, *L. loa* and *O. volvulus* were established by the group of our long-time partner Prof. Wanji from the University of Buea in Cameroon. These systems will provide platforms for the investigation of larvae, adult worms and mating behaviour as well as drug screening (173–178).

In settings where there is a low prevalence of filariasis, e.g., after successful MDA, rapid and sensitive tests are needed to support serological tests (for those infections that have them) to determine the correct timepoint to stop MDA (179). With our partners, loop mediated isothermal amplification (LAMP) assays for detecting filarial nematodes have been developed and evaluated as an alternative to microscopy. For the detection of loiasis, a LAMP assay of parasite DNA has been developed for the detection in infected *Chrysops* spp. The method has been validated under experimental and natural field conditions and is superior to the current gold standard, microscopy (178). This study demonstrated LAMP as a potential tool for surveillance with more sensitivity than microscopy for the detection of experimental and natural *L. loa* infections in *Chrysops* vectors. LAMP assays have also been developed for the detection of other filarial nematode species (180–182): *B. malayi*, *O. volvulus* and *W.*

bancrofti. All the assays have been shown to offer high levels of sensitivity and specificity that are better than microscopy. As *M. perstans* can also modulate the immune system as described in the preceding sections, a LAMP diagnostic test has been developed for the detection of *M. perstans*. The *M. perstans* assay detects up to 0.1 pg, equivalent of 1/1000th of a microfilaria. The assay performed well on infected human and *Culicoides* vector samples. Thus, it will prove useful for mapping and surveillance of these less-studied filarial infections (181). Although real-time PCR also provides high sensitivity and specificity, the advantages LAMP assays offer are: (i) technical ease of use without expensive equipment, e.g. a thermocycler, and (ii) rapid visual detection when using a colorimetric readout.

5 Ongoing research

Besides our ongoing basic research, drug development and diagnostic research, along with our collaboration partners we are also involved in trials that are carried out to improve the morbidity management for patients suffering from lymphedema or hydroceles due to lymphatic filariasis. In two trials it was shown that

doxycycline 200mg for 6 weeks led to halt of progression or even improvement of the lymphedema (152, 183). To confirm these results in other settings and by other research groups, 5 clinical trials were carried out in collaboration with the Taskforce of Global Health (TFGH), Atlanta, USA (184). The IMMIP conducted two of the five trials with the partners in Ghana (<https://doi.org/10.1186/ISRCTN14042737>) and Tanzania (<https://doi.org/10.1186/ISRCTN65756724>) as part of the Tackling the obstacles to fight filariasis and podoconiosis (TAKeOFF) consortium. TAKeOFF is one out of five research networks, funded by the German Federal Ministry of Education and Research (BMBF) for health innovations in sub-Saharan Africa. The three trials supported by the TFGH, funded by the USAID, were carried out in Sri Lanka (NCT02929134), Mali (NCT02927496) and India (NCT02929121). The results of these five trials will help to improve the WHO guidelines for the morbidity management of lymphedema patients. Additionally, we are involved in studying podoconiosis, which is a non-filarial lymphedema of lower limbs considered as one of the most neglected diseases affecting the poorest of people (185). Podoconiosis is a geochemical disease endemic in 32 nations of Africa, Latin America and South East Asia; afflicting 4 million individuals. Contact with volcanic red soil, genetic heritability, high altitude, seasonal rainfall and subsistent farming are the known risk factors and research with our collaboration partners is expected to bring a better understanding of the pathogenesis, novel treatment strategies and point-of-care diagnosis (185). A clinical trial with the same design as the trials mentioned above was carried out by the TAKeOFF consortium in Cameroon with the aim to also improve the morbidity management and to test if doxycycline has a beneficial effect for patients suffering from podoconiosis (<https://doi.org/10.1186/ISRCTN11881662>).

6 Conclusion

A number of exciting developments ranging from novel antifilarial drug candidates and diagnostic tool development to deciphering basic disease mechanisms are exploding in the filarial research field. Our tight network of collaborations throughout the world with filarial researchers in Ghana, Cameroon and Tanzania have contributed immensely to our work. These collaborations are still expanding to other regions like Togo, Benin and South Africa. Further development of novel animal models to study the complete life cycle of human pathogenic filariae and novel drugs with macrofilaricidal capacity will go a long way in achieving the aim of elimination of transmission of onchocerciasis and lymphatic filariasis as a public health problem.

Author contributions

Preparation of the original draft: IK, MR, KP, UK-S, SW, TA, MH, AH. All authors contributed to the article and approved the submitted version.

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Conflict of interest

KP, SS and AH hold patents for the indication of filariasis of Corallopyronin A. US 9168244 B2, Compounds for use in the treatment of filariasis Therapeutic treatment of filariasis/dirofilariasis in humans/animals Inventors: KP, AH, Gabriele Maria Koenig, SS, Andrea Schiefer, Till Friedrich Schaeberle, Alexander Schmitz, Stefan Kehraus granted 2015-10-27, duration: 2032-05-04, US 9687470 B2, Compounds for use in the treatment of filariasis Prevention of filariasis/dirofilariasis in dogs and humans Inventors: KP, AH, Gabriele Maria Koenig, SS, Andrea Schiefer, Till Friedrich Schaeberle, Alexander Schmitz, Stefan Kehraus granted 2017-06-27, duration: 2032-05-04. EP 2704708 B1, Compounds for use in the treatment of filariasis Prevention and treatment of filariasis/dirofilariasis in humans/animals Inventors: KP, AH, Gabriele Maria Koenig, SS, Andrea Schiefer, Till Friedrich Schaeberle, Alexander Schmitz, Stefan Kehraus 2017-10-11 and validated in: DE, GB, NL, CH, IT, ES, FR and HR, duration: 2032-05-04. Patent-Pending: A European application EP 20 172 409.3 "Solid and liquisolid formulations of Corallopyronin A" has been filed with the EPO on 30.4.2020. A PCT application PCT/EP2021/061310 with the same title, claiming priority to the EP 20 172 409.3, has been filed for patent on 29.04.2021.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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