frontiers in Tropical Diseases

REVIEW
published: 08 November 2021
doi: 10.3389/fitd.2021.778724

Advances in Preclinical Platforms of *Loa loa* for Filarial Neglected Tropical Disease Drug and Diagnostics Research

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Specialty section:
This article was submitted to Neglected Tropical Diseases, a section of the journal Frontiers in Tropical Diseases

Received: 17 September 2021
Accepted: 19 October 2021
Published: 08 November 2021

Citation:
Wanji S, Chunda VC, Fombad FF, Njouendou AJ, Gandjui NVT, Ritter M, Enyong PA, Mackenzie C, Taylor MJ, Hoerauf A and Turner JD (2021) Advances in Preclinical Platforms of Loa loa for Filarial Neglected Tropical Disease Drug and Diagnostics Research. Front. Trop. Dis. 2:778724. doi: 10.3389/fitd.2021.778724

The tropical disease, loiasis, caused by the filarial parasite, *Loa loa*, has gained prominence in global public health as a cause of excess mortality and a barrier to the elimination of the related prioritized neglected tropical diseases (NTDs), lymphatic filariasis and onchocerciasis, within Central Africa. There are no effective drug cures or vaccines available to treat loiasis safely. Here we review recent advances in loiasis preclinical platform technologies, including novel *in vitro* culturing systems, animal models and innovations in experimental infections of the *L. loa* vector, *Chrysops*, that have facilitated access to all *L. loa* filarial life-cycle stages. We detail applications of these new model systems in anti-filarial drug screening, diagnostic development, immunology, and pathophysiology research. Finally, we provide an overview of how loiasis preclinical platforms may be further utilized in translational medicine applications to support the development of much needed new interventions against filarial NTDs.

Keywords: *Loa*, loiasis, filariasis, onchocerciasis, lymphatic filariasis, neglected tropical disease

INTRODUCTION

Loiasis is a tropical parasitic infection caused by the filarial nematode, *Loa loa*, endemic in forested areas of Central and West Africa. Infective larvae (L3) are transmitted by diurnal bites of tabanid deer flies of the genus, *Chrysops*. L3 infect subcutaneous tissues and undergo morphogenesis *via* two cuticle moults to develop into immature adults, at approximately 50 days post-infection (1). From five months post-infection, male and female adults mature and sexually reproduce, releasing first-stage microscopic larvae, microfilariae (mf), which migrate into the bloodstream (2). Among individuals infected with *L. loa*, clinically distinct sub-groups can be identified with manifestations

ranging from asymptomatic microfilaremia to a hyper-responsive state characterized by more frequent episodes of local angioedema (Calabar swellings) and pronounced eosinophilia in the absence of mf (3). Despite these symptoms, and that over 14 million people currently reside in high-risk areas, loiasis has often been mis-classified as a benign disease (2). Contrary to other filarial infections such as onchocerciasis and lymphatic filariasis (LF), loiasis has not yet been prioritized in the World Health Organization's list of neglected tropical diseases (NTDs) (4). However, chronic infections can lead to renal, cardiac, pulmonary and neurological pathologies and loaisis has recently been identified as a cause of excess death, calling for re-appraisal of its status as a medically important filarial disease (5). Of additional public health relevance, *L. loa* hyper-microfilaraemias are a significant risk factor for the development of severe adverse events (SAEs) in individuals treated with ivermectin during mass drug administration (MDA) filariasis elimination campaigns in sub-Saharan Africa (6–9). A microfilarial load >30,000 mf/ml is a significant risk factor for SAE development and recent modelling predicts incidence of SAEs will occur in 10% of individuals with parasitaemias of 50,000 mf/ml (10). Non-neurological inflammatory AEs can develop with increased frequency below this level, which still have the potential to incapacitate individuals for several days.

Ivermectin-induced acute pathology presents initially as headache, fever and/or haemorrhaging of the palpebral conjunctiva (11) which can progress to more pronounced neurological manifestations, encephalopathy, coma and death. Whilst the aetiology of this pathology was, until recently, ill-defined due to a paucity of tractable animal models, it has been speculated that drug-mediated paralysis and entrapment of mf in brain tissue capillary beds initiates blockage, haemorrhage (as indicated by ocular haemorrhage) and ultimately breakdown of the blood brain barrier (3). In support of this, *L. loa* mf have been evidenced in cerebral-spinal fluid of individuals suffering SAEs (10). A host inflammatory component is associated with adverse events and may exacerbate neurological pathophysiology. Following microfilaricidal treatment, elevation of allergic mediators such as interleukin-5 and eosinophil degranulation proteins are apparent in the circulation of loiasis patients (12, 13). A distinct mechanism hypothesised by Geary and colleagues suggests that rare mutations in CNS drug efflux pumps (multi-drug resistance 1 gene; *mdr-1*) may result in an ivermectin toxicity syndrome (caused by activation of, for instance GABA neuroreceptors present in mammalian CNS), as is apparent in veterinary filariasis treatment (14).

The clinical association between loiasis microfilaraemia and ivermectin SAEs was established more than 20 years ago. For elimination of onchocerciasis, the subsequent impact has been to avoid ivermectin MDA in loiasis co-endemic health districts where levels of *Onchocerca volvulus* are hypo-endemic (less than 20% prevalence). MDA campaigns have ensued in meso- and hyper-endemic onchocerciasis regions but with increased surveillance of post-treatment SAE and protocols for clinical care established in the local health system. Whilst a 'test-and-not-treat' strategy based on a novel point-of-care *L. loa* diagnostic is one potential strategy to tackle elimination of hypo-endemic onchocerciasis in co-endemic regions (15), the cost estimate per test (between $4-8 USD) may be prohibitive to rollout in annual MDA campaigns (16). Further, social science investigations have identified the perceived risk of loiasis adverse events (whether neurological or non-neurological yet interfering with economic activities) as a major factor in persistent non-participation in ivermectin MDA where onchocerciasis remains meso- to hyper-endemic (17). Because ivermectin MDA must be delivered annually, at a coverage of 80%, for periods of 15 years or more to prevent onchocerciasis transmission, elimination may not be feasible with the current strategy in loiasis-endemic Central African foci. Recent modelling suggests onchocerciasis will persist beyond 2045 in *L. loa* co-endemic countries with annual ivermectin MDA (18). Similarly, *L. loa* endemicity would represent a major barrier to future rollout of ivermectin MDA as an endectocide for vector control of malaria (19).

A distinct complication of *L. loa* co-endemicity, disrupting the elimination of LF in Central Africa, is the occurrence of cross-reactivity to current point-of-care rapid diagnostic tests (RDTs) used in mapping of LF endemic regions and decision making to stop MDA (20, 21).

For these reasons, development of new tools to address the treatment of filarial disease in Central Africa are urgently required. Small scale trials have indicated that various extended or intermittent dose regimens of the human anthelmintic, albendazole, may be either partially macrofilaricidal and/or mediate disruption of mf production in loiasis patients (22, 23). An ideal novel short-course therapeutic product profile would include a high degree of selective efficacy against adult stage *L. loa* and/or *O. volvulus* (ideally both), without inducing the ivermectin-like acute microfilaricidal activities which put hyper-microfilaraemic loiasis individuals at risk of SAE. Identification of affordable adjunctive treatments which limit inflammatory adverse events following ivermectin treatment may also improve attitudes to community participation in current MDA. A scalable serological point-of-care rapid diagnostic test for determining *L. loa* clinical status may also become a pre-requisite as part of a test-and-treat algorithm for filariasis elimination in Central Africa.

Research and development of new therapeutics and diagnostics for loiasis have been hampered by a long-standing lack of investment and the inertia created by a paucity of tractable tools to enable facile preclinical research to be undertaken. Recently, renewed investment in filariasis selective macrofilaricide development has been initiated by funders such as The Bill and Melinda Gate Foundation. This has concomitantly spurred research to improve and innovate *L. loa* preclinical platform technologies. In this review, we focus on these most recent advances and applications in loiasis pathophysiology, drug and diagnostic translational medicine.

LOIASIS *IN VITRO* CULTURES

In order to manipulate *L. loa in vitro* accurately (e.g. to examine drug responses) it is first necessary to define culture conditions

which support parasite longevity outside of the body. Optimum culture conditions enabling the successful long-term maintenance of *L. loa* mf or developing larvae has been recently elucidated. Standard mammalian culture media, such as Dulbecco's Modified Eagles Medium (DMEM), supplemented with calf serum (5-15%) is sufficient to maintain *L. loa* mf purified from the venous blood of hyper-microfilaraemic non-human primates (NHP) for periods of between 6-12 days (24) with a fully motile phenotype. Similarly, calf serum supplementation supports full motility of infectious stage L3 larvae in mammalian culture for up to eight days after isolation from the mouthparts of *Chrysops* flies. Completion of the L3-4 moulting process occurs in approximately 20% in these basic cultures. Addition of an immortalised monkey kidney epithelial cell monolayer to serum-supplemented cultures (LLC-MK2 cell line) extends full motility of mf to 22 days. LLC-MK2 co-cultures also increases L3/L4 motility for periods as long as 17 days and concomitantly boosts the moulting success rate to as much as 60%.

NON-HUMAN PRIMATE MODELS OF LOIASIS

The *L. loa* NHP model is one of the best-studied animal models of human filarial infections (25). Indeed, a sylvatic *L. loa* life cycle is evident in monkey species in forested Central Africa (26) indicating the cross-species adaptation of this filaria. Much of the biology of *L. loa* has been elucidated *via* parasitological experiments using NHP. The human strain *L. loa* can be experimentally transmitted to *Mandrillus leucophaeus* (drill), *M. sphinx* (mandrill), *Papio anubis* (baboon), *Erythrocebus patas* (patas monkey) and *Macaca mulatta* (rhesus macaque) (26–29). *Chrysops* naturally infected with *L. loa* have been collected by baited sweep traps in high transmission areas or, alternatively, human landing catch on loiasis individuals to provide a source of infectious stage L3 larvae. Experimental subcutaneous inoculations of 600 L3 are typically utilised to infect NHPs (30–33). The pre-patent interval after experimental infection of susceptible monkeys is about 150 days, irrespective of the species of experimental host involved. In most monkey species, once the infection has become patent, the microfilarial densities increase sharply, reach a peak and then fall within several weeks to very low levels, which then persist throughout the infection (30). The spleen plays a major role in the clearance of mf from the peripheral blood (30–33) Splenectomised monkeys develop very high *L. loa* microfilaraemias which can persist for many months (27, 30). Although drills and monkeys are excellent laboratory hosts for *L. loa*, ethically, drills are under strict restrictions according to the convention on international trade in endangered species (CITES) classification of primates. As such, this species is no longer used for biomedical research. As an alternative, the baboon offers potential to be used as an experimental NHP model for *L. loa* as the parasite behaves in this primate in essentially the same way as it does in the drill (28). The pre-patent interval after experimental infection of splenectomised baboons is 5 months, with microfilaremias accruing for periods up to 18 months (32). Importantly for the study of pathophysiology of microfilaricidal adverse reactions, hyper-microfilaremia (>30,000 mf/mL) can be achieved in the majority (approximately 70%) of infected splenectomised baboons and all infected animals develop eosinophilia significantly exceeding the normal range (32).

MOUSE MODELS OF LOIASIS – CHRONIC ADULT LOIASIS MODELS

Whilst closely emulating the life cycle of human loiasis, throughput of the baboon NHP model is severely constraining for anti-filarial drug research and to identify potential targets for adjunct therapies to limit ivermectin adverse reactions. Availability of *L. loa* susceptible laboratory rodent models, particularly mice, would be a step-change improvement, both because they are a convenient, standardised model with tractable genetic and immunological tools available but also as a less sentient animal substitute to reduce or replace usage of NHP. *L. loa* does not undergo full development in laboratory 'wild-type' immunocompetent mice. *L. loa* infective larvae (inoculations of between 50-200 L3s) administered sub-cutaneously survive only for a week in BALB/c mice, an inbred laboratory strain that is conversely permissive or semi-permissive to serous cavity infections with related filariae, *Litomosoides sigmodontis* and *Brugia* spp. respectively (34–36). When BALB/c mice are immuno-suppressed with hydrocortisone, *L. loa* survival is extended for up to 3 weeks (37, 38). Control of infection in BALB/c mice is associated with a 'type-2' cellular immune response of splenocytes when re-stimulated with *L. loa* L3 antigen, notably with elevated production of interleukins-4, -9 and -13 (39). Confirming a role for both IL-4/IL-13 signalling and IL-5 in the early adaptive immune control of *L. loa* in mice, BALB/c IL-4 receptor and IL-5 combination deficient mice are susceptible to pre-patent adult *L. loa* infections (40). (CCR)-3 knockout mice, deficient in recruitment of eosinophils *via* eotaxins and other eosinophil chemokines, demonstrate extended survival of *L. loa* developing larvae, linking type-2 immune responses with tissue eosinophil recruitment as a mediator of early immunity to loiasis (41). Since the minimum pre-patent period prior to the release of mf in blood is 5 months, Pionnier et al, investigated the long-term parasitological success of *L. loa* infection in a panel of 'severe-combined' lymphopenic immunodeficient mice lacking all adaptive immunity and facets of innate immune responses (42). Moderate levels of pre-patent adult *L. loa* infection were evident in CB.17 SCID mice (a BALB/c congenic background strain) at 3 months post-infection, meanwhile, Non-Obese Diabetic (NOD) SCID mice and BALB/c $RAG2^{-/-}$ mice had cleared the infection at the same time point. Fecund adult *L. loa* infections in the natural parasitic niche were reproducibly evident at 5 months in compound immunodeficient, lymphopenic mouse strains: NOD SCID $\gamma c^{-/-}$ and BALB/c $RAG2^{-/-}\gamma c^{-/-}$, which lack both lymphocytes and the common gamma chain (γc) cytokine signalling pathway. At this time point, in both compound immunodeficient mouse strains, most worms were found in the natural tissue niches of *L. loa* adult stages, namely the subcutaneous and muscle fascia tissues, while some adults were recovered from cardiopulmonary tissues as well as the

pleural and peritoneal cavities. Parasitism of *L. loa* within these organs suggests that larvae might have migrated *via* the lymphatics through the thoracic duct, corroborating a theory that filariae have a unified lymphatic larval phase (43). By Implantation of a defined number of male and female *L. loa* adults from these strains under the skin of either BALB/c RAG2$^{-/-}$ or RAG2$^{-/-}\gamma c^{-/-}$ recipients, it was possible to establish microfilaraemic mice one-month post-implant with high rates of adult worm survival retained in subcutaneous tissues (42).

MOUSE MODELS OF LOIASIS – STAGE-SPECIFIC MICROFILARAEMIC LOIASIS MODELS

Due to the long pre-patent period following L3 infection, a more facile approach of infusing purified *L. loa* mf derived from baboon NHP directly into venous blood of mice was evaluated (42). This approach was based on the success of establishing long-term microfilaraemias in a variety of inbred mouse strains and genetic knockouts using the human LF parasite, *B. malayi* (44, 45). After infusion with inoculates of $4x10^4$ purified mf, persistent microfilaraemias could be established in either BALB/c or CB.17 SCID mice over a period of at least eight days. The majority of mf were sequestered in cardiopulmonary circulation (approximately 10% of the inoculate) with a scant peripheral microfilaraemia evident. Both peripheral and cardio-pulmonary microfilaraemias were improved slightly with splenectomy in BALB/c mice although non-splenectomised SCID mice supported the highest parasitaemias, indicative of adaptive immunity regulating density of *L. loa* microfilaraemia. Increasing the *L. loa* inoculate to $1x10^5$ mf per mouse lead to significantly increased cardiopulmonary microfilaraemias in both SCID and BALB/c mice toward a hyper-microfilaraemic density associated with risk of SAE in humans (**Figure 1A**). The bias of *L. loa* mf accumulation in the cardiopulmonary circulation may reflect the anatomical differences between murine and human microvasculature. In addition, because in humans *L. loa* exhibits a diurnal periodicity (46), physiological cues for peripheral circulatory migration *versus* cardiopulmonary sequestration may vary between mice and humans. Human sub-periodic *B. malayi* also demonstrate a tropism for cardiopulmonary circulation when infused into mice (47, 48). In follow on experiments, mf purified from venous blood samples from human volunteers was found to be comparable to those isolated from baboons and was used as a more abundant and ethical source to avoid NHP usage in onward applications of the mouse models (**Figure 1B**).

EXPERIMENTAL GENERATION OF *L. LOA* INFECTIVE LARVAE

The successful development of a range of loiasis *in vivo* models increases opportunity for translational science applications. However, the generation of infectious stage larvae has been limited to dissections of wild-caught *Chrysops* in baited traps or human landing catch, which is laborious and hinders throughput. Intrathoracic injection of mf into blackfly vectors have been successful with *Onchocerca* species (49–51). Recently, it was demonstrated that *L. loa* mf purified from experimentally infected baboons and intrathoracically injected in to wild caught *Chrysops* developed to infective larvae after 14 days of fly rearing with high resultant yields of L3 (52). Validation experiments with these experimentally reared *L. loa* L3 demonstrated they could be cultured to undergo L3-L4 moulting *in vitro* and developed to adult stages in C57BL/6 RAG2$^{-/-}$IL-2$\gamma c^{-/-}$mice.

APPLICATION OF LOIASIS *IN VITRO* CULTURES IN DRUG SCREENING

The microfilaricidal activities of anti-malarial drugs (mefloquine, amodiaquine, artesunate, chloroquine and quinine), anthelmintics (praziquantel, flubendazole and its metabolites), trypanocidal agents (fexinidazole and Scynexis-7158) and the anti-cancer drug, imatinib, have been evaluated against *L. loa* mf in dose titration assays (53). Imatinib is of interest as it has established *in vitro* adulticidal activity against *B. malayi* (54). Further, 'counter-screening' experiments have been run to scrutinise potential off-target effects of the onchocerciasis anti-*Wolbachia* macrofilaricide clinical candidate, ABBV-4083 and lead-optimised quinazoline anti-Wolbachial compounds (55, 56). Rapid inhibition of *L. loa* motility was noted with mefloquine and amodiaquine. These antimalarial compounds achieved 50% inhibition concentrations (IC_{50}) <5 μg/mL within the first 24 hours of exposure. Scynexis-7158 also induced a concentration-dependent reduction in mf motility but more gradually, with an IC_{50} of 10μg/mL after 5 days, whereas imatinib only had minor reductions in mf motility (<50%) over five days in culture, in a concentration-dependent manner. Contrastingly, praziquantel and fexinidazole were completely inactive. Flubendazole and its metabolites as well as the anti-Wolbachials ABBV-4083, CBR417 and CBR490 were also inactive until doses far exceeded the physiological range (55, 56). In a distinct study, 10μM flubendazole or its active metabolite was confirmed inactive against *L. loa* mf after 72 hours exposure (57). The direct activities of anti-malarial or anti-trypanosomidal drugs on *L. loa* mf *in vitro* highlights a potential opportunity for re-purposing development but also flags a possibility of AE risk during the treatment of other tropical diseases in loiasis microfilaraemic individuals. However, in a single open-label randomised trial, standard dose amodiaquine did not impact on *L. loa* mf in circulation up to 90 days post-treatment (58).

In comparison to inactivity against *L. loa* mf *in vitro*, when tested against developing L3 in culture, flubendazole and its metabolites achieved gradual IC_{50} threshold reductions in larval motility after 15 days, in a dose-dependent manner (41). This benzimidazole anthelmintic also completely blocked the L3-L4 moult at all doses tested (as low as 50 ng/mL). These pilot *in vitro* *L. loa* studies illustrate the potential of new *L. loa* culture systems

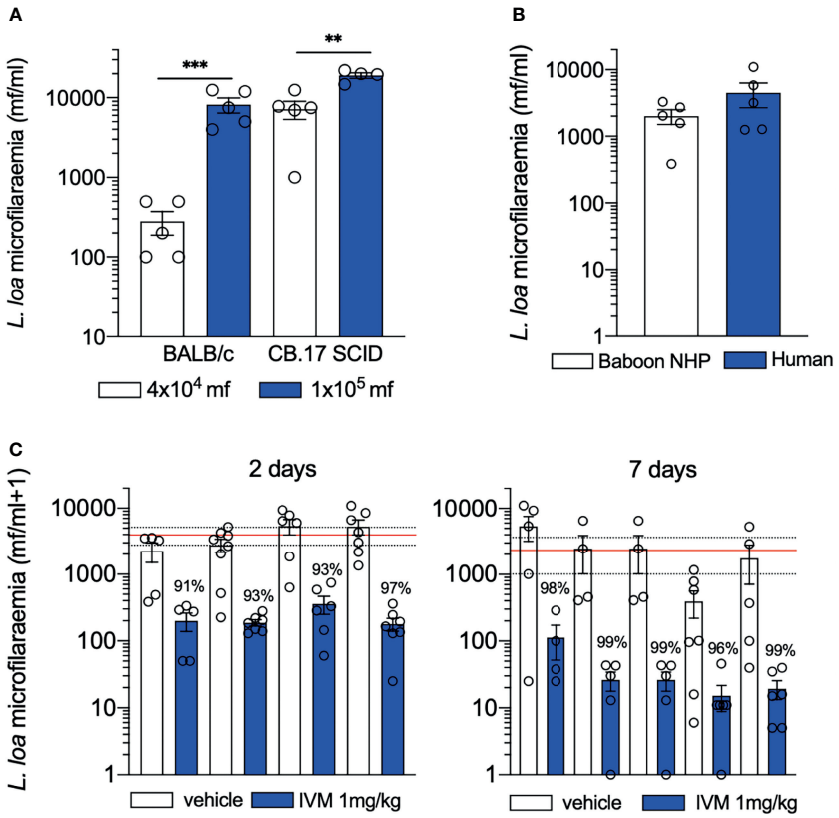

FIGURE 1 | Performance of loiasis microfilaraemic mouse models. **(A)** microfilaraemias ≥10000 mf/ml can be achieved in both BALB/c (immunocompetent) and CB.17 SCID mice by increasing unit of inoculation **(B)** L. loa mf ethically sourced from human volunteers establishes similar microfilaraemias to those derived from NHP **(C)** consistent >90% depletions in L. loa microfilaraemias are mediated following single oral treatment with ivermectin 2 days (left) or 7 days (right) post-treatment in CB.17 SCID mice in multiple independent experiments. Data plotted is mean ± SEM. Lines are global averages of vehicle treated mice (red) and 95% confidence intervals (dashed). Significant differences are indicated ***P < 0.001 and **P < 0.01. Data is previously unpublished **(A, B)** and combination of published (42) and previously unpublished **(C)**.

as pharmacological screens to identify selective efficacies of repurposing or novel small molecule drugs before triaging into animal testing.

APPLICATION OF *L. LOA* MICROFILARAEMIC MOUSE MODELS IN DRUG SCREENING

Because CB.17 SCID mice sustained the highest yields of *L. loa* microfilaraemias, this model was validated as a drug screen. CB.17 SCID mice were infused with $4x10^4$ *L. loa* mf and treated with single-dose oral ivermectin at 1mg/kg, aligning to human standard dose systemic exposures (42). Multiple experiments demonstrated a consistent, rapid >90% clearance of mf two days after single oral dose ivermectin which further increased to approximately 99% mf clearance after seven days (**Figure 1C**). This model has been utilised to evaluate five direct-acting and nine anti-*Wolbachia Onchocerca* macrofilaricide candidates. Oxfendazole, a veterinary benzimidazole anthelmintic with macrofilaricidal activity in *Litomosoides sigmodontis* rodent infection models (59), has undergone phase I trials as a repurposed treatment for human helminthiases (60). Encouragingly, this candidate showed no direct activity against *L. loa* mf (42) and is currently undergoing further clinical development for filarial indications (61). Similarly, an oral-bioavailable formulation of flubendazole developed by Janssen Pharmaceutica as a potential *Onchocerca* macrofilaricide (62) was tested in the *L. loa* microfilaraemic SCID mouse model and found to be inactive (42).

APPLICATION OF *IN VIVO* LOIASIS MODELS IN PATHOPHYSIOLOGY STUDIES

When baboons with *L. loa* mf densities >8000 mf/ml of blood are treated with ivermectin at a standard dose of 150μg/kg, a significant decrease of microfilaremia in all treated animals, up to 98.4% reduction, is achieved by day 7 (33). Clinical

manifestations are evident 2 days post-treatment mirroring early symptomology observed in human loiasis (7, 8). Body temperature, respiratory rates and pulse rates all increase above the normal range. Other clinical manifestations observed are body rashes and itches, pinkish ears, swollen face, conjunctival haemorrhages, loss of appetite, and diarrhoea. Animals display subdued behaviour 48 hours after ivermectin treatment with fatal SAE apparent in baboons >100,000 mf/ml (33). Autopsy findings in this study revealed a widespread vasculopathy. Petechial haemorrhages were seen in the CNS, the lungs, the conjunctiva, the cardiac tissues, the peritoneum and the omentum. Histopathological examination identified mf in varying degrees of degeneration in small vessels, associated with deposition of fibrin, endothelial changes including damage of blood vessels and the presence of extravascular erythrocytes. There was an increased presence of extravascular eosinophils and mononuclear cells, often in large numbers and associated with mf destruction. Highly vascularized organs like the brain, heart, lungs, and kidneys were observed to have more mf in tissue sections and mf were also present within the peritoneal cavity indicating extravasation following ivermectin treatment (33).

Exploiting the establishment of *L. loa* microfilariaemias in BALB/c immune-intact mice, the inflammatory response post-ivermectin was evaluated. Immune priming of BALB/c mice with subcutaneous inoculations of heat-killed *L. loa* mf 2-weeks prior to infusion with $4x10^4$ *L. loa* mf and coincident treatment augmented the microfilaricidal treatment response from 90% in naive to 97% in immune-challenged mice, providing evidence that prior exposure to *L. loa* mf antigens in immune-competent mice can bolster the already substantial rapid efficacy of ivermectin (42). Elevated systemic inflammatory responses were noted in antigen-experienced mice after receiving ivermectin. Whilst initially both type-1 (IFNγ) type-2 (IL-4, IL-5, CCL11) and regulatory type (IL-10) inflammatory mediators were upregulated post-treatment, by day 7, a switch to a predominant type-2 inflammatory signature was apparent, characterised by maintenance of IL-4 and IL-5, downregulation of IFNγ and IL-10 and significant increases of the eosinophil chemokine, eotaxin (CCL11). Increased eosinophilia was apparent in peripheral circulation, in secondary lymphoid tissue and in the peritoneal cavity of microfilaraemic mice post-ivermectin treatment.

APPLICATIONS OF LOIASIS PRECLINICAL PLATFORMS IN DIAGNOSTICS RESEARCH

Following clinical evidence that *W. bancrofti* antigen-based immunodiagnostic tests cross-react with *L. loa* patients with high microfilaraemias (63), laboratory investigations were initiated to understand the molecular basis of cross-reactivity. Experimental studies using whole blood or sera derived from microfilaraemic baboons or the supernatants of short-term (6h) cultures of *L. loa* mf or L3, determined consistent, strong cross-reactivity with the *W. bancrofti* immuno-chromatographic test (ICT) (64). Follow on culture studies utilising pre-patent or patent male or female 24h cultures derived from $RAG2^{-/-}\gamma c^{-/-}$ mice demonstrated cross-reactivity was evident to the filarial test strip (FTS, a next generation ICT test) (42). Further, whole blood from lymphopenic mice chronically infected with adult stage *L. loa* were FTS positive both before and after the onset of mf production (42). By using a quantitative immunoassay (ELISA), Og4C3, which utilises distinct monoclonal antibodies to those employed in the ICT/FTS rapid diagnostic, cross-reactivity in frozen plasma derived from patently infected $RAG2^{-/-}\gamma c^{-/-}$ mice was also evident. These studies illustrate that a common or several distinct secreted antigen/s from *L. loa* mf, L3, immature adults, mature males and mature females cross-react with current monoclonal antibodies used in available antigen tests for LF mapping and elimination surveillance. Proteomic pull-down experiments have identified a specific 'Av33-like' secreted *L. loa* protein antigen derived from immature adult *L. loa* cultures and from loiasis patient serum that is recognised by the monoclonal antibody, AD12, used in LF ICT/FTS rapid diagnostic tests (65).

CONCLUSIONS: FUTURE USE OF *L. LOA* PRECLINICAL PLATFORMS TOWARD IMPROVED THERAPEUTICS AND DIAGNOSTICS FOR FILARIASIS TREATMENT

In the past decade, a substantial advance has been made in preclinical technologies for the tropical neglected disease, loiasis (**Figure 2**). These have been motivated to a large extent by the pressing public health barrier to filarial NTD elimination within Central Africa caused by this infection, namely: ivermectin SAEs in the treatment of onchocerciasis and cross-reactivity to current LF diagnostics. Important outcomes of applying loiasis preclinical tools have been to confirm that three onchocerciasis macrofilaricide clinical candidates positioned in phase I or II trials (two anti-*Wolbachia* drugs, ABBV-4083 & AWZ1066S, one re-purposed benzimidazole, oxfendazole) (61, 66) do not emulate ivermectin rapid microfilaricidal efficacies *in vitro* or *in vivo*. Application of a baboon NHP model has confirmed experimentally that ivermectin neurological SAEs are associated with pre-treatment hyper-microfilaraemias, multi-organ vasculopathy and CNS inflammation. Finally, use of *L. loa* animal models have elucidated the molecular basis for cross-reactivity with current LF immunodiagnostics.

Current limitations of loiasis preclinical platforms are the requirements for wild-caught *Chrysops* for infectious larvae generation. Should a method be established to maintain the *Chrysops* life-cycle in the laboratory, this would resolve a final barrier in establishing a full laboratory life-cycle. In the interim, it may be possible to cryopreserve and ship large batches of *L. loa* L3 to expand experimental usage, including *in vitro* screening and experimental infections of mice, as has been founded for *O. volvulus* (67).

Now that tools for loiasis preclinical research are established and scalable, at least within the context of specialist laboratories within Central Africa (proximal to sources of natural infection and

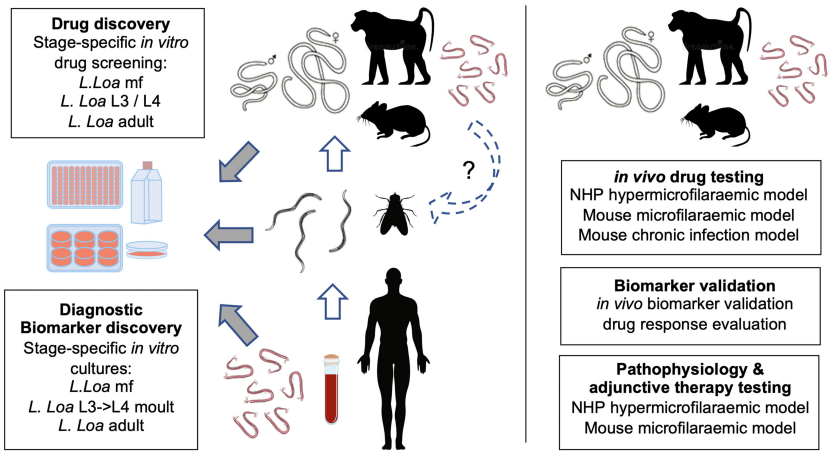

FIGURE 2 | Summary of loiasis preclinical platforms and applications in translational research. Left: generation of all life cycle stages is achievable by blood sampling human patients, purification of L. loa mf, experimental injections in Chrysops, isolation of infectious stage larvae and chronic infections of either compound lymphopenic mice or splenectomised baboons. Derived L. loa mf, L3 or adults can be utilised in long-term *in vitro* cultures for drug or diagnostic biomarker discovery. It is proposed that mf from chronic mouse infections can be used to passage into Chrysops to establish a full laboratory life cycle (dashed loop). Right: *in vivo* infection models can be utilised to determine selective macrofilaricidal efficacy, validate candidate biomarkers and to research the aetiology of ivermectin adverse events including targetable pathways for adjunct therapeutics.

vectors), it is opportune to consider how these platforms may be applied in the future to advance filariasis therapeutics and diagnostics research (**Box 1**). The success in establishing a chronic mouse model of loiasis offers a new tool to define the true extent of albendazole efficacy with chronic dose regimens, utilising human bio-equivalent 400-800mg/day dosages defined in mice (68). Comparatively, this model can also be utilised to determine the minimum sufficient drug exposures of clinical *Onchocerca* macrofilaricide candidates, oxfendazole and emodepside, mediating significant curative activity against *L. loa*. By modelling pharmacokinetic (PK) metrics of drug exposures achieved in mice aligned with tolerated doses in humans (derived from available phase I trial data) an effective dose prediction can be determined which will be an important decision-making tool for proceeding into clinical testing for loiasis-specific indications (**Figure 3**). Simultaneously, establishing a complete curative drug regimen in the loiasis mouse model will be important to evaluate the utility of LF cross-reactive immunodiagnostics as serological biomarkers of adult living *L. loa* and prognostic determinants of macrofilaricidal activity. Biobanking of serum and urine from drug-cured and vehicle control mice could similarly be exploited in future for omics-based discovery of living adult worm biomarkers. For emodepside, a macrofilaricidal regimen with selectivity over acute killing of mf in circulation remains to be resolved for the treatment loiasis or onchocerciasis in *L. loa* co-endemic regions. For this, the SCID microfilaraemic mouse model can be employed to address whether human predicted efficacious doses mediate ivermectin-like rapid microfilaricidal activity.

Building on initial findings in both NHP and mouse microfilaraemic mouse models that ivermectin triggers type-2 inflammation, vasculopathy and a myeloid, eosinophil-rich cell recruitment, the availability of murine reagents and genetic modifications to manipulate facets of this inflammatory pathway can be exploited to define causal inflammatory components driving adverse reactions. Central to this research will be the development of quantitative clinical measures of ivermectin adverse events in mice, such as heart rate, oxygen saturation, core temperature and gross motor activity changes, which can be simultaneously captured

BOX 1 | Future directions in loiasis preclinical research.

drug development

- dose optimization of albendazole as a loiasis indication
- dose prediction of oxfendazole and emodepside as loiasis curative indications
- assessment of emodepside selectivity as a loiasis macrofilaricide *vs* microfilaricide

diagnostics development

- can LF immunodiagnostic cross-reactivity be exploited to measure macrofilaricidal efficacy?
- novel loiasis-specific biomarker discovery

aetiology of ivermectin AE and adjunctive therapeutic development

- role of host inflammatory responses in development of ivermectin adverse events
- assessment of pharmacological and biological interventions that modify host inflammation

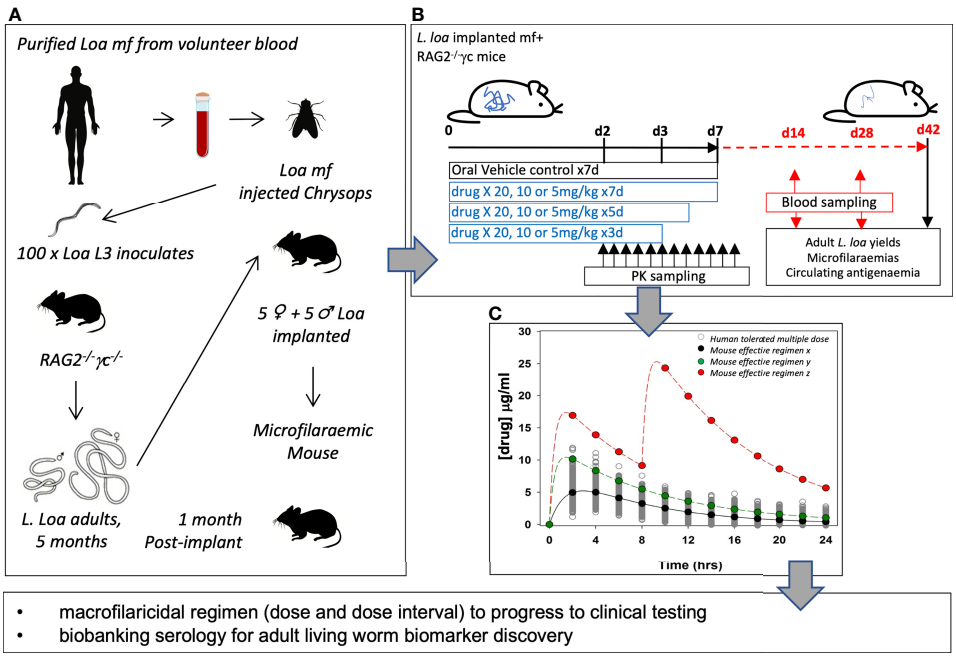

FIGURE 3 | Application of a chronic loiasis mouse model in candidate macrofilaricide drug evaluation. **(A)** strategy for developing microfilaraemic mice with defined implanted number of male and female *L. loa*. **(B)** schematic of drug screen to comprehensively evaluate macrofilaricidal activity of candidate drugs. **(C)** PK modelling of effective macrofilaricidal drug exposures to predict human efficacious dose.

with remote telemetry devices. A detailed knowledge of underlying inflammatory circuits in loiasis microfilaraemic mice following ivermectin treatment can then be considered for pharmacological or biological interventions aimed at disrupting these pathways and thus ameliorating febrile/allergic-type inflammatory adverse events. Selective secondary testing of any highly promising adjunctive intervention in the hypermicrofilaraemic NHP model would evaluate whether it might be possible to mitigate against SAE development or prolongment. Affordable, effective adjunctive treatments may possibly increase acceptability of ivermectin-based MDA campaigns if clinically validated. *In lieu* of safe macrofilaridical treatments for onchocerciasis and/or loaisis, this strategy might be operationalised to achieve filariasis elimination targets set for 2030.

AUTHOR CONTRIBUTIONS

SW: writing, reviewing, and funding. VC: data collection and writing. FF: writing and reviewing. AN: writing and reviewing. NG: writing and reviewing. MR: reviewing and funding. PE: reviewing. CM: reviewing and funding. MT: reviewing and funding. AH: reviewing and funding. JT: data analysis, writing, reviewing, and funding. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by: Bill and Melinda Gates Foundation Grand Challenges Explorations Grant (OPP1119043) to JT, SW, and MT; Wellcome Trust Institutional Translational Partnership Award to JT and SW (219576/Z/19/Z); Merck & Co *via* the Mectizan Donation Programme (MDP) to SW and CM; German Research Foundation (DFG) (Grant DFG HO 2009/10-1, HO 2009/14-1, RI 3036/1-1) within the "German-African Cooperation Projects in infectiology" (grants awarded to AH, MR, and SW). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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