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Targeting Human Onchocerciasis: Recent Advances Beyond Ivermectin

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1. GENERAL DESCRIPTION OF THE DISEASE

p0010 Human *onchocerciasis*, or *river blindness*, is a debilitating disease that is endemic in 31 countries in sub-Saharan Africa, in three Latin American countries (Brazil, Guatemala, and Venezuela) and in Yemen.[1] The causative agent, *Onchocerca volvulus*, is a filarial nematode (Order: *Filariata*, Family *Onchocercidae*) that is transmitted by a black fly *Simulium damnosum* and other related species. The disease occurs when embryonic nematodes (*microfilariae*) migrate to eye tissues, causing keratitis and the formation of opacity on the cornea and pupil that leads to blindness. Ocular onchocerciasis is the cause of 4% of avoidable blindness cases (WHO data) in sub-Saharan Africa and affects over 37 million people, while over 80 million Africans are at risk of infection by this parasite. This disease affects some of the world's most disadvantaged communities, 99% of which are in remote, rural areas in sub-Saharan Africa. Onchocerciasis is the second leading infectious cause of preventable blindness. Furthermore, it causes an array of serious morbidities, including intense itching, onchocercal skin disease, musculoskeletal pain and general malaise, weight loss, and elephantiasis of the genital.[2] It is further suspected to be a cause of epilepsy,[3] and reports of onchocerciasis-associated dwarfism in Uganda, presumably resulting from microfilarial damage to the pituitary, have been described.[4] Beyond health issues, onchocerciasis leads to grave social and economic consequences that exacerbate poverty and hinder overall development.[5] Patients also experience social stigmatization besides decreased body mass index and work productivity. Onchocerciasis is responsible for 0.962 million disability-adjusted life years (DALYs) annually, while troublesome itching accounts for 40% of DALYs attributable to onchocerciasis.[6]

p0015 Great progress has been made in the efforts to control onchocerciasis in the last 3 decades. This has occurred both in Africa and the Americas thanks to concerted efforts by the Onchocerciasis Control Programme in West Africa (OCP), the African Programme for Onchocerciasis Control (APOC), and the Onchocerciasis Elimination Programme in the Americas (OEPA). The WHO has verified the remarkable successes obtained by OEPA with the elimination of the transmission of the parasite in ~~Columbia~~, Ecuador, and Mexico in 2013, 2014, and 2015, respectively, and declared them free of the disease.[7] These relevant successes suggest that the disease has been or can be eliminated, even with currently available tools, in most areas if the right strategy is used and if the required financial input and support is given. There are still challenges to face and these open up the need

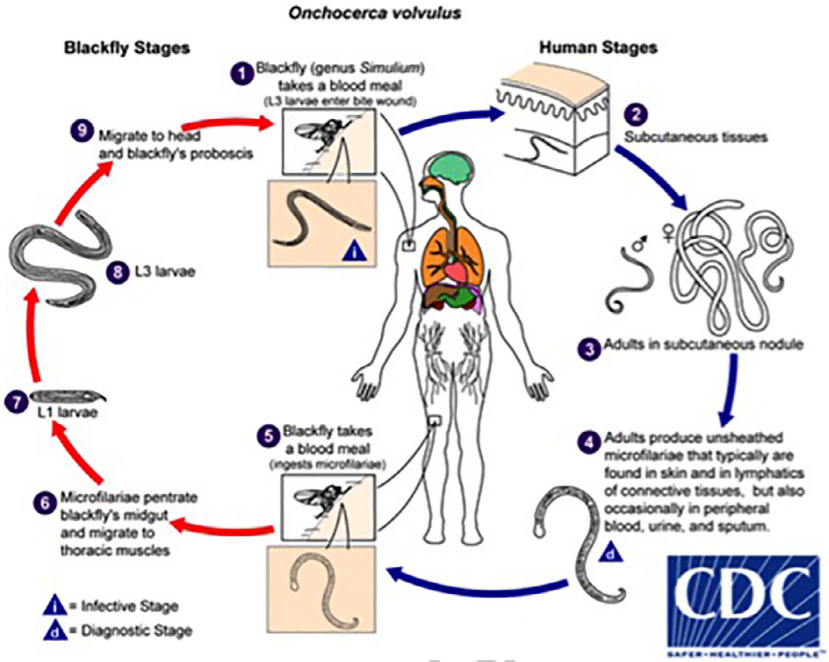
for research to: (a) find the optimum diagnostic tool, especially one that is sufficiently sensitive and specific for *Onchocerca* so as to be used to assess whether onchocercal infection has been eliminated in a locality; (b) discover an acceptable macrofilaricide that can either kill or sterilize the adult worm and therefore help shorten the treatment period; (c) find the best approach for the sustainability of the drug distribution program, which is essential to maintaining pressure on the parasite; and (d) perform research to discover, as early as possible, any resistance to the drug currently in use and the best way to overcome it.

s0015 1.1 The Parasite and Its Life Cycle

p0020 The nematode, *O. volvulus*, is transmitted by small *Simulium* spp. black flies, with mankind its only definitively known reservoir (Fig. 1). An infected black fly deposits one or more *O. volvulus larvae* into the host when it takes a blood meal. These larvae develop into mature adult worms in about a year. They commonly aggregate into fibrous nodules that lie under the skin and usually over bony prominences. The adult female has a size of 30–80 cm and a mean life span of 12–15 years with a reproductive life span of about 9–11 years.[8] When fertilized by a male worm, she releases millions of embryos called *microfilariae*, which themselves live for about 2 years. The *microfilariae* migrate from the nodules, invading the skin, eyes, and some organs from where they are taken up with the bite of a vector. The *microfilariae* develop into infective larvae in the black fly upon migrating from the black fly's midgut, through the hemocoel, to the thoracic muscles. Once there, the *microfilariae* develop into first-stage larvae (L1) and subsequently into third-stage infective larvae (L3). The third-stage infective larvae migrate to the black fly's proboscis and can infect another human when the fly takes a blood meal, thus completing the life cycle of the parasite. Development of the *microfilariae* from the L1 larvae through L3 infective larvae spans a period of 10–12 days.

s0020 1.2 Clinical Presentation

p0025 The clinical manifestation of onchocerciasis occurs 1–3 years after infection. People with severe infections have millions of *microfilariae* in their skin, eyes, lymph nodes, and other organs. In addition to severe itching and possibly secondary infection, a victim often experiences severe dermatitis involving hypo- or hyperpigmentation, and the thickening and wrinkling of the skin. These latter symptom often occurs together with scaling or "*lizard skin*."[9]



f0010 **Fig. 1** *Onchocerca volvulus* life circle. During a blood meal, an infected black fly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ❶. In subcutaneous tissues, the larvae ❷ develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues ❸. Adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. Females measure ~~33–50 cm~~ in length and 270–400 μm in diameter, while males measure 19–42 mm by 130–210 μm. In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220–360 μm by 5–9 μm and unsheathed, have a life span that can reach 2 years. They are occasionally found in peripheral blood, urine, and sputum, but are typically found in the skin and lymphatics of connective tissues ❹. A black fly ingests the microfilariae during a blood meal ❺. After ingestion, the microfilariae migrate from the black fly's midgut through the hemocoel to the thoracic muscles ❻. Once there, the microfilariae develop into first-stage larvae ❼ and subsequently into third-stage infective larvae ❽. The third-stage infective larvae migrate to the black fly's proboscis ❾ and can infect another human Q1 when the fly takes a blood meal ❶. *Reproduced directly from https://www.cdc.gov/dpdx/onchocerciasis/index.html.*

When the *microfilariae* migrate from the skin to enter the eyes, they cause, whether dead or alive, ocular morbidity and visual impairment, including permanent blindness.[10] Loss of vision is caused by acute and chronic ocular diseases of both the anterior segment (sclerosing keratitis and iridocyclitis)

and posterior segments (optic nerve disease, optic atrophy, and choroidoretinitis) of the eye. Blindness that appears later in the disease is the most serious consequence of onchocerciasis. The severity of onchocercal ocular disease varies considerably from one geographical zone to the next. Blindness from onchocerciasis is extensive in hyperendemic populations in the West African savannah, while virtually no, or little, blindness is found in forest villages with a comparable intensity of infection.

p0030 The inefficacy of ivermectin against adult worms means that early diagnosis[11] and an early start to treatment are essential. However, as onchocerciasis is present in underdeveloped countries, important therapy-related decisions must often be made without the benefit of a complete informative scenario. This is what happened to a number of treatment and prevention campaigns that were suspended in some communities after ivermectin was found to harm patients who were also infected with another parasite, commonly known as *Loa loa*, or the African eye worm. *L. loa* is able to cause blindness, quite like *O. volvulus*, and is transmitted via deerflies found in the rainforests of West and Central Africa. Administering ivermectin to patients with high *L. loa* levels (exceeding 30,000 per milliliter of blood) can potentially lead to severe or fatal brain damage. In order to quantify the prevalence of *L. loa* parasites in a patient and determine their eligibility for ivermectin treatment, laboratory technicians traditionally count them manually in blood samples, a technique that is not conducive to use in the field or in mass treatment campaigns. This problem has been cleverly solved by a new iPhone app named *CellScope Loa*, designed by a group of undergraduate students at UC Berkeley and supported by Melinda & Bill Gates Foundation.[12]

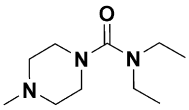
s0025 2. DRUGS AND DRUG COMBINATIONS IN CLINICAL OR PRECLINICAL EVALUATIONS

p0035 In accordance with current *mass drug administration* (MDA) programs, the mainstay chemotherapy against onchocerciasis are combinations of ivermectin (IVM), diethylcarbamazine (DEC) with albendazole (ALB) for lymphatic filariasis and IVM for onchocerciasis.[13] The activity of these drugs is seen in their significant ability to kill *microfilariae* and late-stage embryos in adult female worms. However, these therapies have little effect on the adult worms themselves, and the aim of MDA programs is therefore to break transmission.[1] A thorough description of each of the approved treatments has been provided in the following ~~texts.~~

s0030 2.1 Diethylcarbamazine (DEC)

p0040 Diethylcarbamazine (*N*,*N*-diethyl-4-methyl-1-piperazinecarboxamide, DEC, Fig. 2) was proven to be effective against filarial parasites in the late 1940s.[14]

p0045 Although it was recognized relatively early in its life that DEC can cause adverse reactions in individuals infected with *O. volvulus*, it was the recommended first-line treatment for onchocerciasis for about 40 years until better alternatives, such as ivermectin, were introduced.[15] A treatment course of oral DEC was used for both individual and community treatment,[16] as it presents little intrinsic toxicity. However, large doses may lead to anorexia, nausea, vomiting, headaches, and drowsiness. In onchocerciasis patients however, systemic reactions, which can be attributed to the death of microfilariae and were first described by Mazzotti[15] are observed. The systemic reaction may occur in two phases. In the "*primary*" phase, which is a constant feature, commences within 24 h and manifests itself as a variable combination of increased itching, a rash, headaches, muscle aches, joint pains, gland tenderness, pain and swelling, hypotension, dizziness, tachycardia, fever, and acute respiratory distress. Reaction severity is related to the number of microfilariae killed. A "*secondary*" phase may occur 2–6 days after the initiation of therapy. It commonly presents itself as severe, symmetrical acute polyarthritis predominantly involving the knees, ankles, wrists, the interphalangeal joints, and the shoulders. It is usually accompanied by a recrudescence of fever. Effusions develop, commonly in the knee joints, in which microfilaria *O. volvulus* can be found. Ocular reactions are common in patients with many microfilariae in the eye. These include itching, epiphora, photophobia, injection of the conjunctiva, lid swelling (which may completely shut the eye), limbitis, and acute iridocyclitis.[17] The exact mechanism of DEC action is yet to be elucidated, but is known to involve host components, including the arachidonic acid pathway. Some studies have suggested that DEC has an indirect, host-mediated mode of action as well as antiinflammatory effects.[18] Elsewhere, DEC has been shown to inhibit the cyclooxygenase and lipoxygenase pathways in parasites, resulting in *microfilaria* death, and to result in a sharp decline in *microfilaria* loads and an estimated adulticidal effect of 40% when administered to infected subjects.[19] Moreover, some studies have suggested

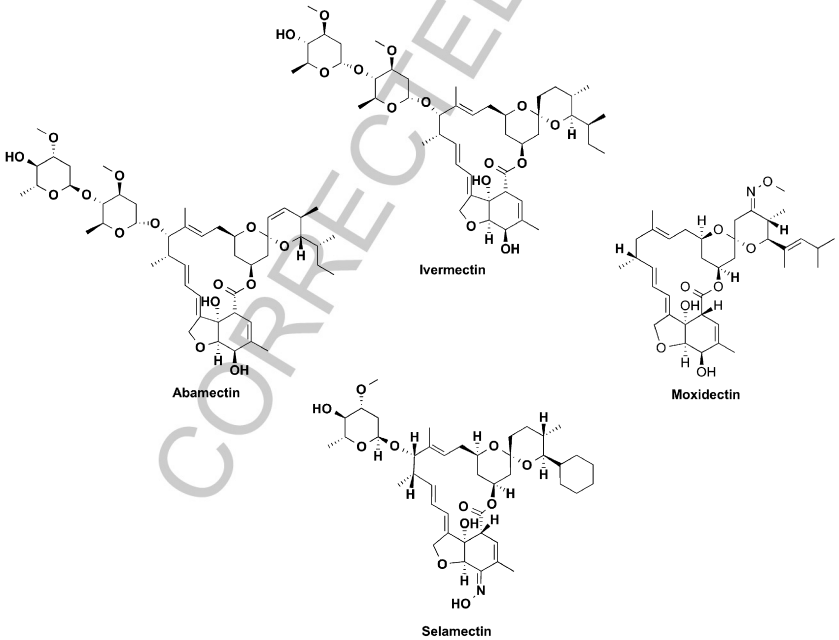


f0015 **Fig. 2** Diethylcarbamazine (*N*,*N*-diethyl-4-methyl-1-piperazinecarboxamide; DEC).

that the drug inhibits the activation of nuclear transcription factor kappa B (NF-κB), a key regulator of proinflammatory genes, such as TNF-α and IL-1β, as well as inducible nitric oxide synthase and cyclooxygenase 2.[20] In addition, DEC alters the host arachidonic acid and nitric oxide metabolic pathways, resulting in the immobilization and sequestration of these parasites via a yet-to-be-elucidated pathway. However, due to its severe adverse effects, DEC is not recommended for MDA programs in onchocerciasis endemic areas where it may induce local inflammation in subjects with ocular *microfilariae*.

s0035 2.2 Ivermectin

p0050 The antiparasitic drug ivermectin (IVM) (Fig. 3) was initially approved in humans in 1987 to orally treat onchocerciasis. Since 1987, the *Mectizan Donation Program* has approved 1.4 billion treatments for the control and elimination of onchocerciasis, and 1.2 billion treatments (administered with albendazole, donated by GlaxoSmithKline) for the control and elimination of lymphatic filariasis (http://www.mectizan.org/resources/2014-annual-highlights). One drawback was that ivermectin does not kill adult worms, which would resume

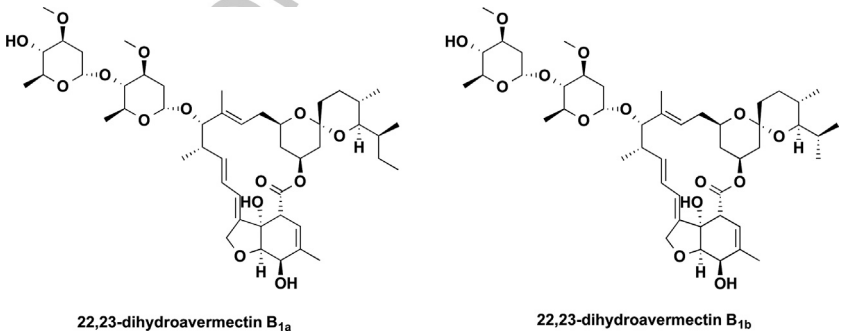


f0020 **Fig. 3** Compounds from the *avermectin* family. Some AVM family members are shown in the picture. All of them are characterized by a 16-carbon macrocyclic lactone core with a bisoleandrisyloxy substituent at C13.

microfilariae production after about 6 months, meaning that around six to eight repeated doses, at 6-month intervals, are thus necessary. Nonetheless, no drug came close to ivermectin's safety and efficacy for river blindness. Ivermectin does not kill adult *O. volvulus*, but a single oral dose (150 mg/kg) given annually suppresses microfilarial production and prevents disease progression.[21] Ivermectin belongs to the avermectins (AVM), which is a group of 16-membered macrocycliclactone compounds discovered in 1967 by the Nobel Prize winner Satoshi Omura of the *Japanese Kitasato Institute*. From fermentation broths of actinomycete cultures and the fungus *Streptomyces avermitilis*, he collected extracts containing macrocyclic lactones, which are responsible for antihelminthic activity, along with ivermectin B1, a component that presents the leading activity.[22] AVM family members include selamectin, abamectin, moxidectin, ivermectin (Fig. 3) among others, all of which differ from the antibacterial and antifungal 16-membered macrocyclic lactones in that they bear a bisoleandrosyloxy substituent at C13.[23]

p0055 Ivermectin is the most commonly employed of the AVM group, as it is a more potent and safer semisynthetic mixture of two AVMs, 22,23-dihydroavermectin-B1a and dihydroavermectin-B1b, at a ratio of 4:1, respectively (Fig. 4).[24]

p0060 Ivermectins potentiate neurotransmission by disrupting glutamate-gated chloride channels, and they also have minor effects on γ-aminobutyric acid (GABA) receptors. They disrupt neurotransmission in nerve and muscle cells, causing neuronal membrane hyperpolarization and inducing paralysis in the somatic muscles, particularly the pharyngeal pump, which kills the parasites. GABA-related channels are commonplace throughout nematodes and insects, whereas GABA receptors and neurons are restricted to the



f0025 **Fig. 4** AVMs. 22,23-Dihydroavermectin-B1a and dihydroavermectin-B1b structures.

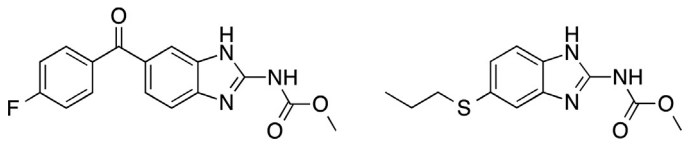
central nervous system in mammals. Ivermectin is therefore very safe for vertebrates, as it cannot cross the blood–brain barrier.[23]

p0065 Attempts have been made to improve ivermectin efficacy in recent years. In *The Lancet*, Nicholas Opoku et al. have presented the results of a multicenter trial, including about 1500 participants that compared the effect over 18 months of single doses of ivermectin and moxidectin on *O. volvulus microfilariae*.[25]

p0070 Skin microfilarial loads (i.e., parasite transmission) are lower after moxidectin treatment than after ivermectin treatment. Moxidectin (Fig. 3) would therefore be expected to reduce parasite transmission between treatment rounds more effectively than ivermectin and thus accelerate progress toward disease elimination. Programmatically speaking, moxidectin has two main advantages: annual treatment could affect onchocerciasis transmission to a similar extent to that of biannual ivermectin treatment, while the effect on transmission is not as dependent on the relationship between treatment time and peak transmission season as that ~~of~~ ivermectin ~~is~~.[26]

s0040 2.3 Benzimidazole Drugs

p0075 The most appealing benzimidazole drug with regard to combating filarial parasites is flubendazole (Fig. 5),[27] which is a benzimidazole approved for the treatment of gastrointestinal nematodes in humans that has been shown to be active also against the pathogens that cause onchocerciasis,[28] and to have essentially 100% efficacy as a macrofilaricide when administered parenterally.[27] However, parenteral administration is a concern as the subcutaneous administration of flubendazole causes intolerable pain, while intramuscular injections induce inflammation.[28] Furthermore, there is a grave shortage of qualified healthcare workers that can safely administer injections in developing countries.[29] Improper sterilization practices and a lack of injection equipment supplies have been reported in various African countries by the WHO.[30] The promise shown by flubendazole as a macrofilaricide and the opportunities that novel drug formulation techniques offer for an oral formulation with high bioavailability have been indicated by Mackenzie and Geary.[27] A partnership



f0030 **Fig. 5** The structure of *flubendazole* (*left*) and *albendazole* (*right*).

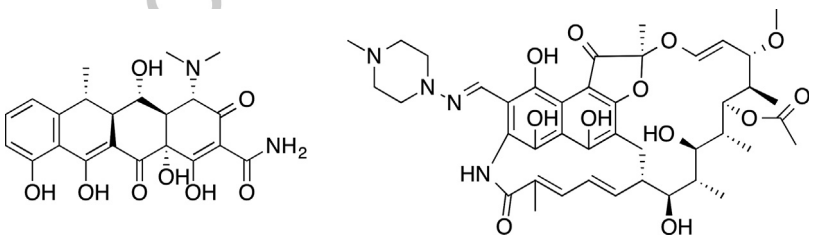
between Michigan State University in the United States, McGill University in Canada, and the not-for-profit organization "*Drugs for Neglected Diseases Initiative*" (DNDi) was formed in 2011 to develop a suitable oral formulation of flubendazole and conduct the relevant pharmacodynamic, pharmacokinetic, and toxicology studies. The work of the partnership was supported by a grant from the BMGF and Johnson&Johnson joined the initiative in 2012. DNDi has since completed its involvement in the project and has transferred all data to Johnson&Johnson. New oral formulations, one that makes use of an aqueous hydroxypropyl-~~b~~-cyclodextrin ~~(CD)~~ suspension and another using an aqueous carboxymethyl cellulose suspension, have been developed, as has a Tween 80-based formulation ~~(TWEEN)~~.[31] The oral bioavailability enhancement of flubendazole has recently been obtained via the development of nanofibrous solid dosage forms.[32] An oral formulation with higher systemic bioavailability than the formulations used for treatment of intestinal nematodes requires additional preclinical toxicology and pharmacotoxicity studies in view of the fact that flubendazole interferes with microtubules and may thus present reproductive toxicity.[27] Benzimidazoles work by interfering with the equilibrium between tubulin subunits, tubulin~~,~~ and microtubules. Unsurprisingly, benzimidazoles can affect host tubulin as well as that of the parasites ~~are typically positive in mammalian cell cytotoxicity assays and cause chromosomal nondisjunction during mitosis~~.[33] However, benzimidazole anthelmintics show a differential preference for binding to nematode tubulin over mammalian tubulin,[34] which is an important factor in the development of a drug against nematodes in mammals. The results of the first preclinical toxicology studies conducted have been published[35] and show that, when flubendazole was administered orally in a bioavailable formulation, it interfered with normal rat embryonic development in a dose-dependent manner, which is deeply and strictly related to the amount of drug in the maternal plasma that consequently reaches the embryo. This fact does not impact negatively on the possibility of proceeding with the development of a new flubendazole formulation which is highly bioavailable and highly effective as a macrofilaricide, as the benefit of developing this drug in terms of control and eradication of filariasis overcomes the disadvantages of having a drug with a potential risk in pregnancy. In fact, the potential new drug could be used under controlled administration for the treatment of individual patients in order to avoid the risk of inadvertently treating pregnant women. However, the development of flubendazole by Johnson&Johnson was discontinued due to ~~nonclinical toxicology data~~ (~~Fig. 5~~).[36]

s0045 2.4 Doxycycline

p0080 The search for new drugs that can enhance elimination by permanently sterilizing and killing the adult worms has identified a 4–6-week course of doxycycline (Fig. 6), which is a slow-killing drug due to its indirect mode of action of killing endosymbiotic bacteria.[37] Doxycycline is the first and, so far, only macrofilaricidal drug against onchocerciasis. More importantly, Mand et al.[38] have observed that doxycycline can be effective in patients without active infection since it demonstrated exceptional antiproliferative activity and led to improved pathology conditions. This suggests that this drug can be used as an effective tool for individual drug treatment in filarial endemic areas.[39] While doxycycline application in field studies has shown interesting macrofilaricidal effects when compared with conventional antifilarial drugs,[40] its universal application has been hampered due to contraindications among pregnant women and children under 9 years of age. This fact, coupled with current reports of IVM resistance in some endemic communities,[23] highlights the need for the development of new and effective antifilarial drugs and/or vaccines if the goal of eliminating *Lymphatic filariasis* (LF) and onchocerciasis is to be achieved by 2020 and 2025, respectively.[19] ~~Long treatment length and contraindications in children and pregnancy are obstacles to implementing doxycycline as a public health strategy.~~[41] Doxycycline elicits a gradual, yet sustained, reduction in microfilaridermia, not because it kills *O. volvulus* microfilariae directly, but because the microfilarial population is not replenished by (sterilized) female worms, with skin microfilariae declining through natural attrition. Adult worms suffer a similar "*slow-kill*" effect. An increased abundance of dead *O. volvulus* worms is observed approximately 2 years after the start of doxycycline therapy.[39]

s0050 2.5 Rifampicin

p0085 Recent studies have also shown that rifampicin exhibits (Fig. 6) macrofilaricidal activity.[42] Clinically relevant dose elevations of rifampicin,



f0035 **Fig. 6** The *doxycycline* (*left*) and *rifampicin* (*right*) structures.

which have recently been determined as safe in humans, can be administered as short courses to filariasis target populations with the potential to reduce anti-Wolbachia curative therapy times to between 1 and 2 weeks.[42]

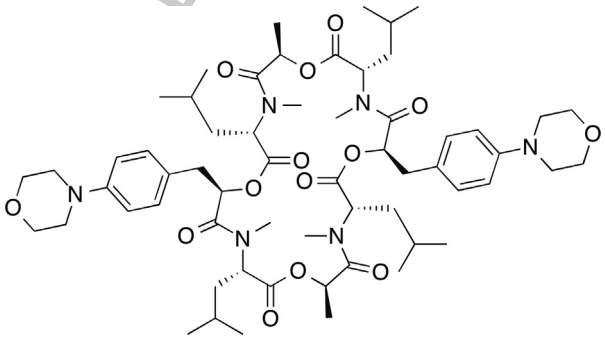
s0055 2.6 Emodepside

p0090 Emodepside (Fig. 7) is a semisynthetic compound whose precursor is a metabolite of the fungus Mycelia sterile. Clinical development is underway in the United Kingdom and preclinical research is underway in Germany.

p0095 Emodepside, which has been approved in combination with *praziquantel* for veterinary use, has efficacy in animal filarial models.[13] The DNDi has initiated a phase 1 study of its safety, tolerability, and pharmacokinetics in healthy volunteers (NCT02661178). Studies in *O. volvulus* and Loa-infected individuals are planned for 2018. Emerging efficacy and safety profiles will determine whether emodepside development will continue and whether it will be developed for Community Direct Treatment/MDA or Test and Treat strategies.

s0060 2.7 Combination Therapy

p0100 The unique activities of each of the above-mentioned drugs have led to them being used in specific combination therapies endemic regions.[19] Recent studies,[43] have shown that a single-dose combination therapy with three currently approved antifilarial drugs (IDA as combination of Ivermectin, Diethylcarbamazine, and Albendazole)[44] is superior to current regimens used in *Lymphatic filariasis* elimination programs. Although it has not yet been tested, IDA may also be useful for treating onchocerciasis. Additional



f0040 **Fig. 7** The *emodepside* structure.

research will be needed to test whether it is safe and effective for treating onchocerciasis, first in individuals and later in endemic populations both with and without coendemic LF. IDA is likely to be more effective for the clearance and suppression of *microfilariae* than ivermectin alone. Compared to drugs that have not yet been tested in humans, IDA provides a potential fast-track and low-cost option that could accelerate the elimination of LF and onchocerciasis in Africa.[43] The risk of general and irreversible ocular diethylcarbamazine-related secondary adverse effects in *O. volvulus*-infected individuals with high microfilaridermia requires that a low microfilariae burden is confirmed in each individual before IDA treatment. A TNT strategy that uses skin snips to quantify microfilaridermia and carefully examines the ocular anterior and posterior segments would be able to exclude individuals at risk of diethylcarbamazine-related SAEs from IDA treatment. Moreover, the development of drug resistance or the induction of severe adverse events following treatment, or contraindications for DEC in areas endemic for onchocerciasis, drives the need for the identification and development of alternative therapeutics, along with sensitive diagnostic tools to assess for efficacy of these alternative treatment strategies.[45]

3. RECENT ADVANCES BEYOND IVERMECTIN

p0105 Although the lack of a safe and effective drug against the adult worm and the emergence of animal parasite strains that are resistant to ivermectin have put many at risk of the devastating effects of onchocerciasis, the *Drug Design* scenario of studies directed toward alternative therapeutic treatments has been very poor over the past 20 years. Unfortunately, this means that onchocerciasis really falls within the definition of "*neglected diseases*." The following sections present and comment upon the results of a bibliography search on events in the field over the past 20 years. In Section 3.3, the authors also describe the scenario of possible new targets for *O. volvulus*, which are not currently being investigated in terms of Drug Design, but as sources of future possibilities.

3.1 Searching for Chemodiversity within Natural Sources

p0110 Organic compounds obtained from mother nature still have extensively uses in the treatment of many diseases.[46] These approved substances and other natural products undergoing clinical trials represent very wide chemical

diversity and continue to demonstrate the importance of compounds from natural sources in modern drug discovery.[47] Traditional medicines make use of natural products and are still of great importance all around the world, in particular where neglected diseases are involved. Such forms of medicine have been practiced for hundreds or even thousands of years and are a valuable repository of human knowledge and possibly sources of future drugs.[48] In this sense, Metuge et al.[49] investigated the acclaimed anti-*onchocerca* activity of the roots/rhizomes of *Cyperus articulatus* in the traditional treatment of onchocerciasis in North Western Cameroon with the purpose of identifying potential filaricidal lead compounds. The essential oil from the roots/rhizomes of *C. articulatus* was found to be active against *O. ochengi* microfilariae and adult worms in a dose-dependent manner in vitro. Although no further steps have yet been reported, these results may prove to be a source of new antifilarial compounds that support the traditional use of *C. articulatus* in the treatment of human onchocerciasis.

p0115 Following a similar approach, Samje et al.[50] screened the chromatographic fractions of crude extracts from *Craterispermum laurinum* and *Morinda lucida*, plants which have traditionally been used to manage *O. ochengi*, against the disease in vitro. Of the 18 extracts screened, the methanolic extracts of the leaves of both plants recorded the highest activities against both the microfilariae and adult worms. The most active chromatographic fraction was obtained from *M. lucida* and had an IC_{50} of 7.8 and 15.63 μg/mL on microfilariae and adult worms, respectively. A phytochemical analysis of the most active fractions revealed the presence of sterols, alkaloids, triterpenes, saponins, and flavonoids, which are sources of potential future chemotypes. *Acacia nilotica* fruits with high tannin contents are used in the northern parts of Cameroon as antifilarial remedies by traditional healers.

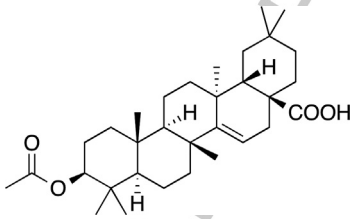
p0120 A further two studies have investigated traditional Cameroonian medicine. In the first, Vildina et al.[51] assayed the hydro-alcoholic fruit extract of plants traditionally used and one of the main constituents in its most active fractions, (+)-catechin-3-*O*-gallate, together with four related proanthocyanidins, for their potential in vitro anthelmintic properties against the free-living model organism *Caenorhabditis elegans* and the filarial cattle parasite *O. ochengi* and found activities in the 1.2–350 μg/mL LC_{50} range. These results are in good accordance with the use of *A. nilotica* against nematode infections by traditional healers, herdsmen, and pastoralists in Cameroon.

p0125 In the second study, Nyasse et al.[52] investigated a number of Cameroonian medicinal plants that are known to be active against *O. volvulus* and *Onchocerca gutturosa*. They screened three compounds, polycarpol,

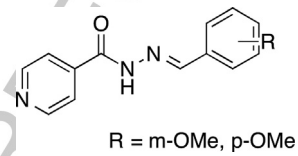
polyveoline, and 3-*O*-acetyl aleuritolic acid (Fig. 8) isolated from *Discoglypremna caloneura* (Euphorbiaceae), *Polyalthia suaveolens* (Annonaceae), and *D. caloneura* (Euphorbiaceae), respectively. Only polycarpol and 3-*O*-acetyl aleuritolic acid exhibited significant inhibitory activity on the vitality of adult male *O. gutturosa* worms and Amocarzine was used as positive control compound.

s0075 3.2 The Repositioning of Closantel: Targeting OvCHT1 Chitinase

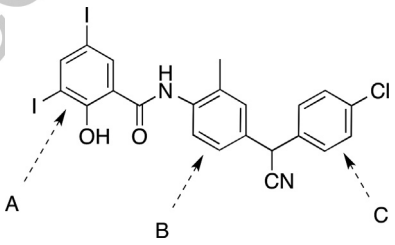
p0130 Besides the studies of Mainsah et al.,[53] which reported in 2016, the activity of Cu(II) isoniazid-derived Schiff bases (Fig. 9) against both micro- and macrofilaria, with IC_{50} values of 5 and 10 μg/mL, respectively, the only other drug design study presenting an appreciable level of complexity, in the last decade, was the repositioning of *closantel* (Fig. 10), a veterinary anthelmintic



f0045 **Fig. 8** 3-*O*-Acetyl aleuritolic acid.



f0050 **Fig. 9** Schiff bases designed by Manisah et al.

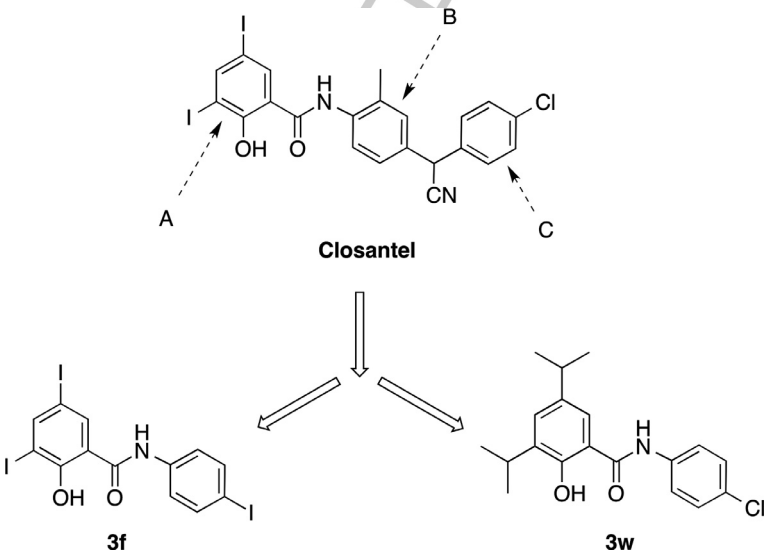


f0055 **Fig. 10** The *closantel* structure (the three rings have been indicated referring to Gloeckner et al.[55]).

with known proton ionophore activity, that was performed by the Janda group at the Scripps Institute in La Jolla (USA)[55] (see Figs. 10 and 11).

p0135 *Neglected diseases* have not been the focus of big pharmaceutical company research because the difficulties in recouping research and development costs do not make them profitable over the long term. However, pharmaceutical companies are recently beginning to view rare and neglected diseases as an opportunity to bring in more revenue as well as to improve public relations.[54] One strategy that can solve the problem is combining a hastening of the drug discovery of ND and the benefits for pharmaceutical companies, thus expanding their market. The strategy of examining new uses for existing approved drugs, so called "*drug repositioning*" or "*drug repurposing*,"[55] works in this direction. One of the benefits of repositioning a drug to a different purpose is the availability of materials and data that can accelerate the entrance of the new molecular entity to the market.

p0140 In 2001, Wu et al.[56] reported the presence of a chitinase, which belongs to the family 18 glycosyl hydrolases and were designated OvCHT1, in *O. volvulus*. Chitin is one of the most widespread amino polysaccharides in nature and constitutes a major structural component of the microfilarial sheath and eggshells of parasitic nematodes. Two enzymes, chitin synthases



f0060 **Fig. 11** Simplification of the dual activity profile *closantel* structure how described by Garner et al.[59] The 3,5-diiodosalicilate analog **3f** retained the OvCHT1 chitinase activity profile loosing the proton ionophore profile characteristic of closantel. The opposite behavior was found for **3w**.

and chitinases, regulate the dynamic biosynthesis and degradation of chitin, which is a crucial process for the growth and development of these organisms. In the *O. volvulus* parasite, OvCHT1 is expressed solely in the infective L3 larvae and is stored within the granules of the cells of the esophageal glands until postinfective development, after which it is secreted and found mostly in the cuticle. Its exact mechanism of action is still under investigation although it has been hypothesized to play roles in the infectivity of the parasite, aiding its escape from the vector and/or participating in early postinfective migration and/or development. Its involvement is such in these processes, which are so critical to the life cycle of the parasite, that OvCHT1 was made a relevant therapeutic target in *O. volvulus*. In 2003, the same group reported OvCHT1 as an antigen that strongly induces both humoral and cellular immune responses in humans.[57] Following such discoveries on OvCH1 roles, Gloeckner et al.[55] conducted a screening against OvCHT1 using the *Johns Hopkins Clinical Compound Library*, which that contains over 1500 existing drugs. Closantel, already approved for veterinary uses, was identified as a potent and specific inhibitor of this filarial chitinase, with an IC_{50} of 1.6 μM. Additional experiments showed that the mode of inhibition for this compound is competitive with a Ki of 468 nM. This compound was also found to be highly specific for filarial family 18 chitinases, compared to those from protozoans and the human chitinase, human chitotriosidase. Notably, closantel was also found to completely inhibit the molting of *O. volvulus* infective L3 stage larvae.

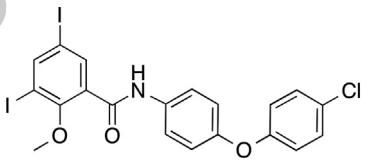
p0145 In 2011, Segura-Cabrera et al.[58] speculated as to closantel's binding mode to OvCHT1 using in silico methodologies. In the absence of a crystallographic structure for OvCHT1, the authors developed a homology model of OvCHT1 using the currently available X-ray structures of human chitinases as templates. Using molecular dynamics simulations and free-energy calculations, they identified the structural features and properties that were responsible for differences in the computationally predicted closantel binding modes. In particular, the authors suggested that the closantel 3,5-diiodosalicylic acid moiety is fundamental for anchoring the molecule to the OvCHT1 biding site, and that it thus plays an important role in closantel specificity (Fig. 10).

p0150 Molting is a key developmental process for parasitic nematodes, and interference with molting is fatal to parasite development. In *O. volvulus*, the L3 to L4 larval molt is of critical significance in the nematode lifecycle, as it represents the transitional stage between the vector and the host and is necessary for active infection of and further development within the human

host. Following the discovery of closantel's OvCHT1 inhibition properties, in 2011, Garner et al.[59] thoroughly investigated the role of the 3,5-diiodosalicylic moiety in closantel's activity toward OvCHT1. As closantel's anthelmintic activity as a veterinary drug is thought to be due to its role as a *proton ionophore*, the authors dedicated affords to discern the impact of chitinase inhibition and mitochondrial uncoupling in the molting of *O. volvulus* L3 larvae. A small series of simplified analogs were synthesized from closantel, and the authors were able to associate the OvCHT1 activity profile to the presence of a 3,5-diiodosalicilamide moiety in the closantel structure. However, when in the salicilamide moiety the iodine substituents were replaced with isopropyl groups, OvCHT1 activity was lost and a potent proton ionophore was found.

p0155 These studies demonstrated that both protonionophore and chitinase inhibition activities are necessary to affect the molting of *O. volvulus* L3 larvae. Starting from these results, Gooyit et al.[60a] focused on scaffold expansion of the aniline moiety of closantel designing a series of analogs.

p0160 In fact, they identified potent bifunctional analogs as well as compounds that were just OvCHT1 inhibitors. These bifunctional compounds were also found to readily bioaccumulate in the model nematode *C. elegans* at concentrations up to >75% of the initial dose. The results clearly established the significance of each biochemical role in modulating the *O. volvulus* L3-to-L4 molt and proved that synergistic activities provided a formidable impact on the molting process. In these series of closantel structure modifications, beside the fact the 3,5-diiodosalicylate moiety is key to closantel's binding specificity by anchoring it within the OvCHT1 active site, the phenolic hydroxyl group was not considered crucial for activity and its removal did not affect activity, as the activity of **3i** (Fig. 12) prove. This suggests that the diidosalicylate moiety most likely interacts with nonspecific hydrophobic contacts within the active site and is therefore amenable to fragment replacement and manipulation. The "*scaffold hopping*" strategy was applied,

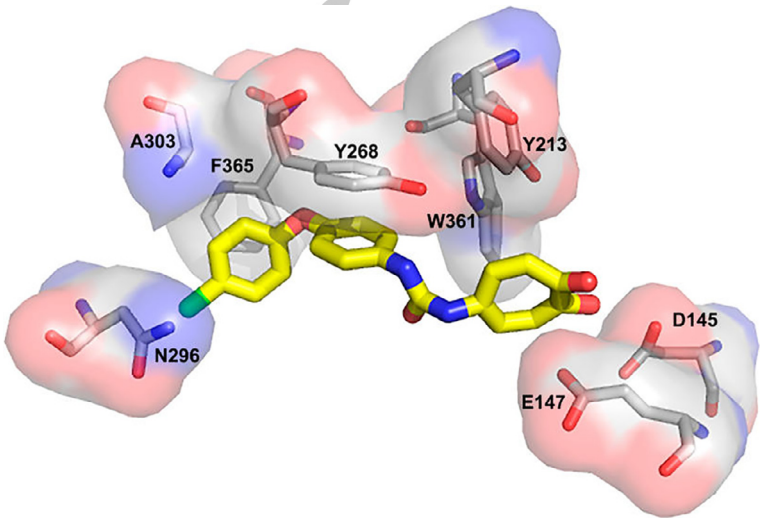


3i (IC_{50} 0.60 ± 0.07 μM OvCHT1)

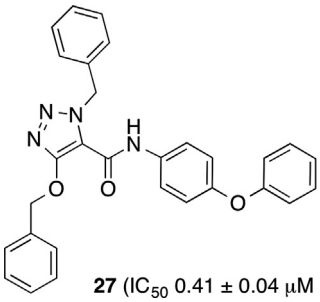
f0065 **Fig. 12** Compound **3i** by Goovit et al. a potent OvCHT1 inhibitor in the nanomolar range ~~(Fig. 13)~~.

on two occasions, to the 3,5-diiodosalycilic moiety in order to explore the biological space of OvCHT1 and generate novel molecular frameworks with improved potency. In the first case, Gooyit et al.[60b] simplified the structure of closantel by replacing the diiodosalicylate moiety, initially, with a phenyl group bridged to a phenoxyphenyl system via an amide bond, and then later with more complex groups (Fig. 13).

p0165 In the second case, applying an effective and consolidated bioisosteric strategy to mimic the acidic moiety by hydrozyazole scaffolds,[61a–h] Pippione et al.[62] replaced the 3,5-diiodosalycilic core with a hydroxytriazole scaffold. During the subsequent fine tuning modulation of the hydroxytriazole scaffold, the role of three possible substitutions on the N-triazole ring was determined. The fact that the phenolic group in Gooyit's analogs can be successfully alkylated without affecting chitinase inhibition meant that *O*-alkylated triazole analogs were also considered with the aim to explore the chemical space around the triazole ring.[63] These studies found that the presence of either N(b) or N(c) alkyl substituents was beneficial to potency according to the size of the alkyl substituent. A combination of these facts, together with the presence of a benzyl substituent on the hydroxyl group, afforded sub-micromolar range compounds. In Fig. 14 is shown the derivative **27**, one of the most potent compound the series based on the hydroxytriazole scaffold.



f0070 **Fig. 13** Lowest energy pose of compound **3a** from Gooyit et al.,[61] docked into OvCHT1 (IC_{50} 0.84 ± 0.01) using the program AutoDock Vina. Color scheme: oxygens in *red*, nitrogens in *blue*, carbons in *yellow* (compound **3a**) or *gray* (OvCHT1).



27 (IC_{50} 0.41 ± 0.04 μM OvCHT1)

f0075 **Fig. 14** In Pippione et al.[62] approach, the hydroxytriazole scaffold was used to successfully replace the 3,5-diiodosalycilic core.

p0170 In 2015, continued efforts to discover OvCHT1 inhibitors led Gooyit et al.,[60c] to identify the β-carboline alkaloid scaffolding as a chitinase inhibitor that is capable of penetrating the worm cuticle. In particular, the authors presented the rich polypharmacology of the β-carboline class of compounds as an approach to abrogate the parasite molting and thus the infection in a human host.

s0080 3.3 Studies on New Therapeutic O. volvus Targets

p0175 Although only a few new chemical entities and drug design programs have been published on onchocerciasis over the last 20 years, a variety of biological targets have been suggested for onchocerciasis therapy. The high-quality genome assembly of *O. volvulus* was recently generated[64] identifying enzymes that are likely to be essential for *O. volvulus* viability as well as generating a list of proteins that could be targeted by repurposed drugs, providing starting points for antionchocerciasis drug development. These studies might be an interesting starting point for future drug design opportunities. A brief introduction to the most interesting of these can be found below.

s0085 *3.3.1 Glutathione S-Transferase (GST)*

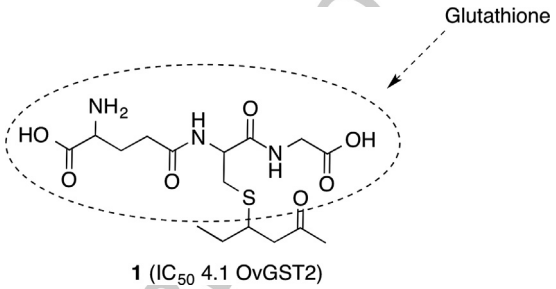
p0180 Adult worm parasites survive an immune response that is directed against them by a variety of mechanisms in their potential 15 years of development in a human host. It has been proposed that *glutathione S-transferase* (GST) enzymes may contribute to the persistence mechanisms that can aid the parasite's survival. Glutathione *S*-transferases are multifunctional proteins that protect a cell from damage from toxic exogenous and endogenous compounds by catalyzing their conjugation to reduced glutathione.

They regulate the oxidative stress response, drug resistance, and modulation of host immune defense mechanisms. Moreover, they also serve as binding proteins for a range of organic substances such as the cytotoxic products of lipid peroxidation, including lipid hydroperoxides and reactive carbonyls. Such compounds would cause membrane damage in the absence of GSTs. Structure-based drug discovery efforts against *O. volvulus* have been focused on GSTs as they have been postulated to protect the parasite against host-mediated lipid peroxidation of the membrane.[65] Three different *O. volvulus* GST enzymes have been identified to date.[66] Ov-GST1 is a prostaglandin D synthase, a glycoprotein which possesses a signal peptide that is cleaved off during the process of maturation. Ov-GST1 was found in the media surrounding adult worms maintained in culture, indicating that this enzyme is released from the worm in vitro. It potentially participates in the modulation of immune responses by contributing to the production of parasite-derived prostanoids and restraining the host's effector responses, thus making it a potential target for chemotherapy and vaccine development. The glutathione *S*-transferase, Ov-GST2, shows significant and unusual differences in sequence and overall structure when compared with other counterparts, particularly those of the human host. This is especially true of helix α-2, an important region for substrate recognition. It has been proposed that the principal role of cytosolic Ov-GST2 is either to neutralize peroxidase activity or to passively bind the cytotoxic lipid peroxidase products that arise from the immune-initiated attack on parasitic membranes via glutathione (GSH) conjugation. A third GST, Ov-GST3 plays a protective role against intracellular and environmental oxygen species,[67] and can be observed in the egg shell of the worm at the morula stage of embryo formation unlike Ov-GST1, thus indicating its extremely defined, stage-specific expression for a short transient period only. In 2015, Metuge et al. thoroughly compared the structural differences of the three enzymatic classes in an attempt to explore the potential inhibitory mechanisms of plant-derived anti-*Onchocerca* compounds.[65] In this paper, a docking study postulated the binding pose inside a plant-derived chemical library. Besides work by Brophy et al.[68] who presented a series of β-carbonyl substituted glutathione conjugates as inhibitors of OvGST2 (Fig. 15), no other attempts to design inhibitors of these targets are present in the literature. In the case of Brophy's work, a series of β-keto SG conjugates were synthesized and assayed toward OvGST2 achieving low micromolar concentrations and selectivity that was greater than 10-fold higher than that of human π-GST (Fig. 15).

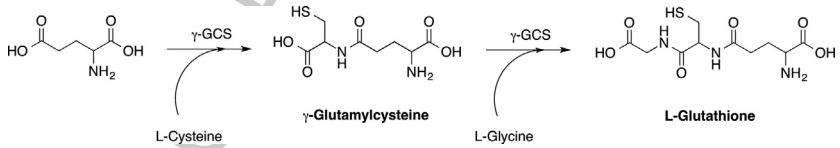
s0090 ### 3.3.2 Glutamylcysteine Synthetase

p0185 The tripeptide glutathione (GSH), which plays an important role in the maintenance of the intracellular thiol redox state and in detoxification processes, is intracellularly regulated by glutathione reductase. The first and rate limiting step in the synthetic pathway is catalyzed by *γ-glutamylcysteine synthetase* (γ-GCS) (Fig. 16).

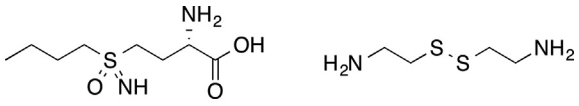
p0190 In a study by Lüersen et al.,[69] γ-GCS was partially purified from the filarial parasite *O. volvulus* and preliminary steady-state kinetics were performed. In order to determine its activity, the Ki value of inhibitors of γ-GCS L-buthionine-*S*,*R*-sulfoximine (BSO) and cystamine (Fig. 17) were determined to be 0.13 and 3.9 μM, respectively. The two inhibitors presented ~~Kis~~ 54-fold and 5.9-fold lower Ki values for the mammalian enzyme, respectively. Furthermore, the cDNA and the *O. volvulus* γ-GCS gene encode a polypeptide of 652 amino acids with 50% and 69% sequence identity to the human and the *C. elegans* counterparts, respectively. Filarial γ-GCS is proposed as a potential drug target (~~Fig. 17~~).



f0080 **Fig. 15** The most potent OvGST2 glutathione adduct of the Brophy's series.



f0085 **Fig. 16** Glutathione biosynthetic pathway.



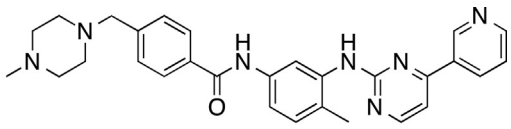
f0090 **Fig. 17** L-Buthionine-*S*,*R*-sulfoximine and cystamine.

s0095 ### 3.3.3 cAMP-Dependent Protein Kinase

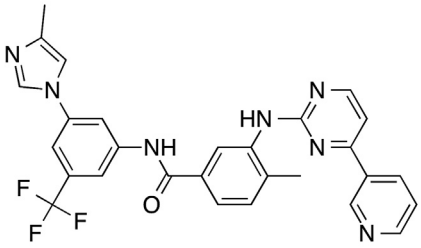
p0195 In the search for new drug target candidates and in order to characterize the molecular events in the signal transduction of *O. volvulus*, Fischer et al.[70] focused on the identification of signaling molecules of this human parasite. Using cDNA with an open reading frame of 1122 bp, they isolated the regulatory subunit of the cAMP-dependent protein kinase (Ov-pka-r) of the pathogenic human nematode *O. volvulus*. The predicted protein displays 84% homology to the corresponding protein in *C. elegans* and 71% to the human homolog. The authors also localized native Ov-PKA-r within the nervous system and sensory organs of adult *O. volvulus* worms and of microfilariae using rabbit antisera raised against the recombinant protein for immunohistology. As the predominant expression in the nervous system and sensory organs, and the unique structural features identify this *O. volvulus* signaling molecule, ~~cAMP-dependent protein kinase (Ov-pka-r) was claimed~~ as a new and interesting target for drug development.

s0100 ### 3.3.4 Abl-Like Kinases

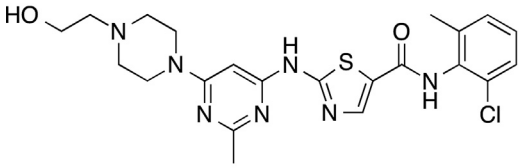
p0200 As the genomes of *O. volvulus* are now available, new drug targets have been identified. Abl, a tyrosine kinase ~~inhibitor~~, that plays an important role in cell proliferation and survival, has been suggested as a potential treatment ~~for a broad range~~ of protozoan infections. O'Connell et al.,[71] have therefore assessed the ability of FDA approved tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib (Fig. 18) to kill each of the mammalian stages (adults, L3 larvae, and microfilariae) of the filarial parasite *Brugia malayi*, the only human filarial parasite for which each of these lifecycle stages is available for in vitro testing. The researchers also assessed the universality of this activity against the other pathogenic filariae (*L. loa*, *Wuchereria bancrofti*, and *O. volvulus*) and used three-dimensional modeling to understand the interactions between the tyrosine kinase inhibitors and the filarial Abl-like proteins. For microfilariae, median inhibitory concentrations (IC_{50} values) on day 6 were 6.06 μM for imatinib, 3.72 μM for dasatinib, and 81.35 μM for nilotinib. For L3 larvae, 11.27, 13.64, and 70.98 μM, respectively, were found. For adult males, 41.6, 3.87, and 68.22 μM, respectively, were observed, while for adult females, the values were 42.89, 9.8, and >100 μM, respectively. Three-dimensional modeling suggests how these tyrosine kinase inhibitors bind and inhibit filarial protein activity. Given the safety of imatinib in humans, the authors then reported that plans were underway for pilot clinical trials to assess its efficacy in patients with filarial infections (Fig. 18).



Imatinib



Nilotinib



Dasatinib

f0095 **Fig. 18** Imatinib, nilotinib, and dasatinib structures.

s0105 3.3.5 S-Adenosylmethionine Decarboxylase

p0205 The activity of *S*-adenosylmethionine decarboxylase (AdoMetDC), a key enzyme in polyamine biosynthesis, is stimulated specifically by *putrescine* in many eukaryotes. The AdoMetDC of the filarial parasite *O. volvulus*, however, is not only stimulated by putrescine, but also by the naturally occurring polyamines *spermidine* and *spermine*. Ndjonka et al.[72] assayed a variety of polyamine analogs as competitive inhibitors toward the stimulatory effect that putrescine has on *O. volvulus* and, for comparison purposes, on *C. elegans* and human AdoMetDC. Such experiments demonstrate that bis(aralkyl)- and bis(alkyl)-substituted polyamine analogs with a 3-7-3 backbone, besides other potential impacts on polyamine metabolism, directly affected the catalytic activity of the key enzyme AdoMetDC, whereby the nematode enzyme was found to be more sensitive than the human enzyme.

s0110 3.3.6 Superoxide Dismutase

p0210 Originally isolated by Tawe et al.,[73] the extracellular superoxide dismutase from *O. volvulus* (OvEC-SOD), which was found in the excretory/secretory

products of adult worms, was the focus of a study by Ajonina-Ekoti et al.[74] Using a combination of site-directed mutagenesis and homology in silico modeling, the authors highlighted the role of Cys-192 in stabilizing the active site channel. Moreover, the authors provided evidence that SOD is a target of the immune response during filarial infections in general. Besides the classical role of superoxide anion reduction in the extracellular space and the control of the redox state in and around the parasite, OvEC-SOD might also participate in the regulation of inflammatory responses, prevent the depletion of filarial-derived nitric oxide, deal with ROS produced by the iron deposits in its gut, and improve the ability of the filarial parasite to resist oxidative injury caused by the host defense.

s0115 ### 3.3.7 Serine Protease Inhibitor

p0215 Ford et al.[75] identified, via an analysis of a molting third-stage larvae-expressed sequence tag dataset, a novel filarial serine protease inhibitor (SPI), named Ov-SPI-1, from the human parasitic nematode *O. volvulus*. Four other members of this family were identified in other filariae. These proteins are related to the low molecular weight SPIs originally isolated from *Ascaris suum* where they are believed to protect the parasite from host intestinal proteases. The two Ov-SPI transcripts are upregulated in the molting larvae and adult stages of the development of the parasite. The authors suggest that Ov-SPI proteins play a vital role in nematode molting by controlling the activity of an endogenous serine protease(s).

s0120 ### 3.3.8 ~~Prolyl 4-Hydrolase~~

p0220 The cuticle of parasitic nematodes consists primarily of a network of collagen molecules. Prolyl 4-hydroxylase is the enzyme responsible for collagen maturation and is therefore central in cuticle biosynthesis and a potentially important chemotherapeutic target. Merriweather et al.[76] characterized and expressed enzymatically active recombinant filarial prolyl 4-hydroxylase. The derived amino acid sequence of Ov-phy-1 encoded a peptide that was very similar to the two *C. elegans* prolyl 4-hydroxylase homologs and to isoform II enzymes of vertebrates. Studies, based on expressed sequence tag analysis and developmental polymerase chain reactions (PCRs), demonstrated that Ov-phy-1 was expressed in L3 and adult parasites. At present, besides the in vitro production of enzymatically active *O. volvulus* prolyl 4-hydroxylase, this technique should also facilitate the identification of specific inhibitors of the parasite enzyme. However, no examples have yet been released.

s0125 ### 3.3.9 Phosphoglycerate Mutase

p0225 Phosphoglycerate mutase catalyzes the isomerization of 3-phosphoglycerate and 2-phosphoglycerate in glycolysis and gluconeogenesis. Two distinct types of PGM exist in nature, one that requires 2,3-bisphosphoglycerate as a cofactor (dPGM), and another that does not (iPGM), as it is structurally distinct and possesses different mechanisms of action. Nematodes possess the iPGM form, whereas mammals have dPGM. Raverdy et al.[77] cloned and expressed iPGM from *O. volvulus* and described the catalytic properties of *O. volvulus*, *B. malayi*, and *C. elegans* iPGM enzymes. The high similarity in catalytic properties shown by the enzymes indicates that a single enzyme inhibitor would probably be effective against all nematode enzymes, which supports the development of iPGM as a promising drug target in parasitic nematodes. In work by Dhamodharan et al.,[78] an analysis of partial genomic and amino acid sequences and the phylogenetic tree of *W. bancrofti* (Wb-iPGM), the major causative agent of human lymphatic filariasis, indicated that this gene, apart from being a potential drug target, could also provide diagnostic, taxonomical, and evolutionary markers. This study is the first report of the characterization of the iPGM gene from *W. bancrofti*. The Wb-iPGM isoform-1 gene encodes an ORF of 515 amino acids and is found to share 96.0% amino acid sequence identity with the iPGM of *O. volvulus*. Serine and all the other 13 amino acid residues involved in the catalytic function of iPGM are highly conserved. Such similarities make such enzymes promising drug target in parasitic nematodes.

s0130 ### 3.3.10 Homologous Transcription Factor (SKN-1)

p0230 Choe et al.[79] have recently and effectively reviewed the role of the detoxification gene regulator, SKN-1, in mediating resistance to anthelmintics in nematodes. The homologous transcription factor, SKN-1, presents in the free-living model nematode *C. elegans*, acts as a master regulator of detoxification and antioxidation genes. Despite similar functions, SKN-1 and NRF2 have important differences in structure and regulatory pathways. Protein alignment and phylogenetic analyses indicate that these differences are shared among many nematodes, making SKN-1 a candidate for specifically targeting nematode detoxification and antioxidation.

s0135 ### 3.3.11 Glyoxalase

p0235 Detoxified enzymically by the glyoxalase system, glyoxal, methylglyoxal, and other physiological α-oxoaldehydes are formed by the lipid peroxidation,

glycation, and degradation of glycolytic intermediates. Sommer et al.[80] investigated the physiological function of glyoxalase I in parasitic organisms. The cDNA for glyoxalase I, from the filarial nematode *O. volvulus* (designated Ov-GloI), was cloned and characterized. Because of the high degree of sequence identity (60%) with human glyoxalase I, for which the X-ray structure is available, it was possible to build a three-dimensional model of Ov-GloI. Critical differences in the residues that line the hydrophobic substrate-binding pocket of Ov-GloI are present, as compared to human glyoxalase I, and should be used as a starting point for the design of inhibitors. Using semiquantitative PCR enzyme-linked immunosorbent assay (ELISA), the authors also showed that Ov-GloI is expressed at elevated levels under conditions of oxidative stress.

s0140 ### 3.3.12 Glyceraldehyde-3-Phosphate Dehydrogenases

p0240 Erttmann et al.[81] focused their attention on the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein family in the *O. volvulus* worm producing, via the screening and cloning of a cDNA library, a clone of 1650 bp. A comparison of the complete amino acid sequence identified this protein as a member of the GAPDH protein family. The recombinantly expressed protein shows GAPDH enzymatic activity and plasminogen-binding capacity. Using immunohistology, Ov-GAPDH was observed in microfilariae, infective larvae, and adult male and female worms, in particular in the musculature of the body wall.

s0145 ### 3.3.13 Cathepsin Proteases

p0245 In a study by Lustigman et al.,[82] the successful use of RNA interference (RNAi) to investigate gene function in the third-stage larvae (L3) of human filarial parasite *O. volvulus* was proposed. In particular, the authors targeted two specific gene products, *O. volvulus* cathepsin L (Ov-CPL) and cathepsin *Z*-like (Ov-CPZ) cysteine proteases, which were proposed to function during *O. volvulus* L3 molting. This study conclusively validates that both enzymes are essential for the molting of *O. volvulus* L3 to fourth-stage larvae.

s0150 ### 3.3.14 N-*Myristoyltransferase*

p0250 *Myristoylation* is a lipid modification involving the addition of a 14-carbon unsaturated fatty acid, myristic acid, to the N-terminal glycine of a subset of proteins. This is a modification that promotes their binding to cell membranes for a variety of biological functions. The process is catalyzed by

N-myristoyltransferase (NMT), an enzyme which has been validated as a drug target in human cancers, and for infectious diseases caused by fungi, viruses, and protozoan parasites. Galvin et al.[83] purified *C. elegans* and *B. malayi* NMTs as active recombinant proteins and carried out kinetic analyses with their essential fatty acid donor, myristoyl-CoA, and peptide substrates. The genetic and chemical studies performed in the study highlighted the importance of myristoylation in the synthesis of functional proteins in nematodes and have shown, for the first time, that NMT is required for viability in parasitic nematodes. The authors suggested that targeting NMT could be a valid approach for the development of chemotherapeutic agents against nematode diseases, including filariasis.

s0155 3.3.15 Onchocerca Decretory Alkaline Phosphatase

p0255 In a study by Cho-Ngwa et al.,[84] an Onchocerca secretory alkaline phosphatase was detected, purified, and characterized. The enzyme was found to be secreted by both *O. ochengi* and *O. volvulus* worms. It was shown to be of *Onchocerca* origin thanks to Western blotting with bovine onchocerciasis sera and its time-dependent release in cultures. The enzyme was competitively inhibited by inorganic phosphate with an apparent inhibition constant (Ki) of 3.33 ± 0.04 mM, whereas l-phenylalanine inhibited it in a mixed way with a Ki of 3.18 ± 0.03 mM. While contributing to the understanding of metabolism in Onchocerca, the present, apparently unique, enzyme, which probably serves in the nutrition of the parasite, could be further characterized as a macrofilaricide target or diagnostic marker in onchocerciasis.

s0160 3.3.16 Blisterase

p0260 Blisterase is a subtilisin-like proprotein convertase of nematodes. The enzyme is named after the blistered cuticle found in *C. elegans* with the bli-4 e937 mutation. The critical role of the enzyme in cuticle production makes it a potential drug target for parasitic nematodes. In a study by Poole et al.,[85] blisterase from the parasitic nematode *O. volvulus* was cloned and expressed. The catalytic domain of the protease exhibits 84% identity with the corresponding domain of its closest homolog, *Caenorhabditis elegans* blisterase. *O. volvulus* blisterase, expressed in insect cells, has maximal activity in 1 mM calcium at neutral pH. The protease is inhibited by EDTA, the suicide substrates decanoyl-RVKR-chloromethylketone, α1-antitrypsin Portland, and by its own propeptide.

s0165 ## 4. METHODOLOGIES FOR ONCHOCERCIASIS DIAGNOSIS

p0265 Accurate filariasis identification in *humans* is essential for the implementation and evaluation of MDA programs to control *onchocerciasis*. Moreover, correct identification is important, especially when other live microfilaria in the skin can produce misidentification and when atypical filarial localizations occurring in the human host make microscopic diagnosis complicated.[86] Determining the infection levels in vector populations is also important for assessing transmission, deciding when drug treatments may be terminated and for monitoring recrudescence.

p0270 ~~Will be describe methodologies~~ for onchocerciasis diagnosis below ~~and pay particular attention of the involvement of new biological assays~~.

s0170 ### 4.1 Clinical Examination

p0275 Individuals which contract onchocerciasis develop subcutaneous nodules containing the adult worm, predominantly over bony prominences. Microfilariae are released from these nodules to the skin and ocular tissues. Diagnosis should be made by taking skin snips from the back, hips, and thighs and visualizing the microfilariae of *O. volvulus*. Eosinophilia and positive serology support the diagnosis.[87] The initial diagnostic methods for determining *O. volvulus* infection are: an examination of the skin for signs of onchodermatitis and the detection of subcutaneous palpable nodules (onchocercomata) by palpation or ultrasonography. However, these methods are time consuming and require qualified individuals that can recognize onchocercomata by palpation and correctly identify the larvae. Although ultrasonography is probably more sensitive than palpation for detecting nodules (especially deeper onchocercomata), it is impractical for programmatic use as it requires special equipment and trained personnel.[88]

s0175 ### 4.2 Microscopic Identification of Microfilariae in Skin Snips

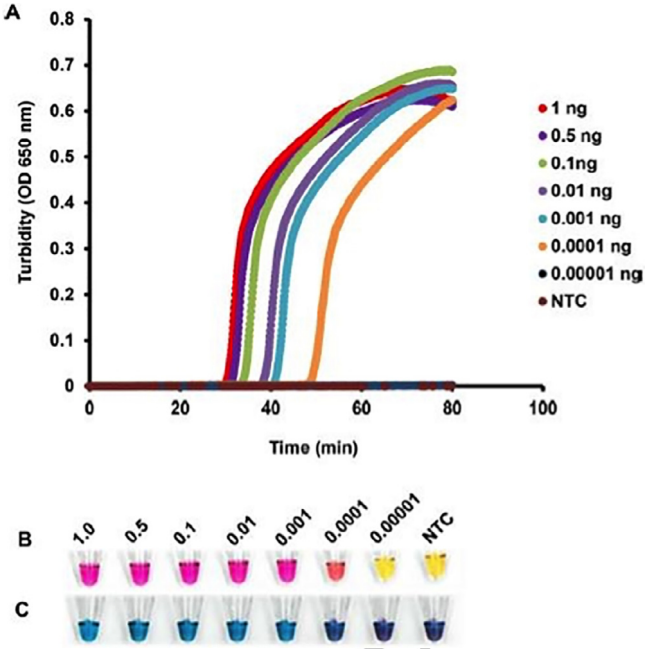
p0280 Skin microfilariae can be identified by microscopic examination of skin snips. Skin snips (superficial biopsies weighing 1–2 mg) are incubated in saline for 30 min and then examined for *microfilariae*. Longer incubation times increase sensitivity, but decrease the practicality of the method. Skin snip microscopy is more sensitive in detecting active infections than clinical examination. Microscopic examination is cheap and easy to perform.

Nevertheless, skin snip examination is not sensitive in the detection of early infections or for the diagnosis of individuals with low microfilaria densities in the skin. Its sensitivity also depends on where skin snips were taken and the stage of the disease. Furthermore, skin snip practice is inconvenient, the instruments used to obtain snips are expensive and, as they are not disposable, particular care must be taken to ensure that blood borne viruses are not transmitted in field surveys. This method is not beneficial in determining when to end ivermectin treatment. A fast, inexpensive and noninvasive diagnostic test, for onchocerciasis, i.e., live adult worms, has therefore been the subject of study in endemic areas to facilitate elimination programs.

s0180 4.3 Nucleic Acid-Based Molecular Assays

p0285 Several studies have reported improvements in the microscopic examination of skin snips, such us the detection of parasite DNA in extracts prepared from skin snips.[89] This method is based on the detection and PCR amplification of an *O. volvulus*-specific repeated sequence, designated 0–150.[89] However, this procedure still entails the collection of a skin snip. Toé et al.[90] demonstrated that the 0–150 PCR assay may be used to detect *O. volvulus* DNA even in skin scrapings collected from infected persons. This assay is not as sensitive as performing the O-l50 assay directly on skin snips. However, the assay overcomes the disadvantages inherent in the collection of skin snips and is less invasive. One limitation of the method is that the existence of a closely related cattle parasite, *O. ochengi*, in Africa complicates the use of this test, and an additional hybridization with a specific *O. volvulus* DNA probe is required to achieve specificity. A new species-specific DNA biomarker, encoding *O. volvulus glutathione S-transferase 1a* (OvGst1a), has been identified that can be used in PCR without geographic restriction.[91]

p0290 PCR is an expensive technique for many countries where onchocerciasis is endemic. For this purpose, loop-mediated isothermal amplification (LAMP), an alternative technique, has recently been developed.[91] LAMP is a one-step, nucleic acid amplification (NINA) technique that quickly amplifies, with minimal instruments, a few copies of target DNA to 10^9 copies in less than 1 h even when large amounts of nontarget DNA are present. LAMP is performed under isothermal conditions (64°C) in a simple heat block or water bath for 80 min. The DNA polymerase is then inactivated, using a Loopamp Realtime Turbidimeter, under heating at 80°C for 2 min. Turbidity data were analyzed using the LA-320c software package, and



f0100 **Fig. 19** Sensitivity of O-150 LAMP. Various dilutions of *O. volvulus* genomic DNA ranging from 0.00001 to 1.0 ng were amplified. (A) LAMP reactions were visualized using turbidity. (B) Colorimetric dyes, neutral red, and (C) colorimetric dyes, hydroxy naphthol blue.[92]

a positive reaction was defined as when the change in turbidity over time reaches a value of 0.1 produced by the precipitation of magnesium pyrophosphate (Fig. 19A).[92]

p0295 For colorimetric LAMP, reactions were carried out at 64°C for 60 min. Neutral red (NR) or hydroxy naphthol blue (HNB) dyes were employed to detect amplification. Samples turn pink if positive or yellow if negative using NR (Fig. 19B). For HNB, samples changed from violet to sky blue when positive (Fig. 19C). LAMP sensibility is between 1 and 0.00001 ng (Fig. 19).[92]

p0300 Poole et al. have described high levels of specificity and sensitivity for various filarial nematode LAMP assays, including *B. malayi*, *L. loa*, *O. volvulus*, and *W. bancrofti*.[93] A recent, technological upgrade for LAMP has been produced that performs the reactions in a portable noninstrumented NINA device that provides a stable heat source.[93]

s0185 4.4 Diethylcarbamazine Patch Test

p0305 Diethylcarbamazine was the drug of choice for onchocerciasis therapy for 30 years. However, treatment with diethylcarbamazine provokes side effects

in patients infected with *O. volvulus* that have been named the *Mazzotti reaction*. These reactions, which occur within a few hours after an oral dose, include, dermal edema, lymphadenopathy, itching, fever, maculopapular eruptions, but also more severe manifestations such as meningism, arthropathy, tachycardia, hypotension, severe prostration, and even death.[94] The mechanism of action is thought one of the inflammatory reactions, particularly involving eosinophils, associated with the exposure and killing of the microfilariae. In order to limit the severity of these reactions, some studies evaluated the use of topical application of diethylcarbamazine patch test to detect early reappearance of *O. volvulus* transmission in a community where control activities have been interrupted. The diethylcarbamazine patch test indirectly detects microfilariae present in the skin by inducing a localized Mazzotti reaction.[88] Diethylcarbamazine cream, in a gauze material, is applied to the skin and 1–2 days after patch application, pruritic papules appear in response to microfilariae death. Multiple patches can be applied to various sites to reduce the possibility of false-negative reactions and can be evaluated in 4–8 h. Some patients with loiasis can give false-positive results, but Ozoh et al.[95] demonstrated that this could be useful in areas where onchocerciasis is coendemic with loiasis. The test is inexpensive and quick for survey work and clinical diagnosis. Finally, the reaction is localized and causes the patient only limited itching.[94]

p0310 A ready-to-use diethylcarbamazine containing patch, called the LTS-2 patch, has recently been developed, and Awadzi et al.[96] have demonstrated that its safety, tolerability, and ability to detect infections are comparable to those of the *diethylcarbamazine patch*.[96]

s0190 4.5 Antibody Diagnostic Tests

p0315 Alternative methods, which are based on the detection of antibodies or antigens in body fluids, have been developed to improve upon the diagnoses of onchocerciasis. Antibody-based diagnostic tests for onchocerciasis are potentially useful as tools for identifying early infections, and also for monitoring the efficacy of control programs. IgG4 antibodies are generally absent in healthy child and adult serum. Some studies have reported an evident IgG4 antibody response in patients with onchocerciasis. Weil et al. evaluated total IgG and IgG4 antibodies to *O. volvulus* adult antigen (OvAg), in serum from patients with *O. volvulus* infections, using an enzyme immune assay and immunoblot analysis.[97] IgG4 antibodies were detected by enzyme immunoassay in onchocerciasis serum samples, but cross-reactive IgG4 antibodies

were present in serum pools from patients infected with other filariae. These results confirm the specificity of IgG4 antibody assays against the IgG antibody for filariasis and extend the observation to include onchocerciasis, but cross-reactivity among filariae limits the utility of this approach. However, another limitation of this approach is that IgG4 antibodies develop relatively slowly in children. Moreover, antibody detection does not distinguish between active and past infections.

p0320 Chandrashekar et al. used measurements of antibodies in *humans* for the detection of filarial antigens in immuno-ELISA assays in 1996.[98] This antibody assay is based on recombinant *O. volvulus* antigens encoded by cDNA clones, OC 3.6 and OC 9.3, which are sensitive and specific for the diagnosis of onchocerciasis in human serum from patients that also present other diseases, such as *lymphatic filariasis*, *loiasis*, *dracunculiasis*, and *schistosomiasis*, that are unreactive with these antigens. OC 3.6 encodes an *O. volvulus* antigen that is similar to the protein product of Ov33 which is believed to be a pepsin inhibitor. OC 9.3 encodes a 15 kDa native antigen, which it is closely related to OV7 that encodes an *O. volvulus* cystatin. Previous studies have shown that these proteins are present in the developing larvae and adult worms of *O. volvulus* and that the antibodies to these antigens can develop long before the onset of microfilariae patency. Antibody detection does not distinguish between active and past infections.[98]

p0325 Bradley et al. developed an ELISA, in 1998, that is able to detect specific *O. volvulus* antibodies using a "*diagnostic cocktail*" of three recombinant antigens, Ov10, Ov11, and Ov29.[99] Ov10 is found in the larval adult stages (L3 and L4) of the worm, while the other two antigens are known to be present in adult female worms.[99] The cocktail showed 96% sensitivity and 100% specificity with sera from microfilariae-positive patients compared with 99% sensitivity and only 59% specificity when using a crude *O. volvulus* extracted based assay.[99] Nde et al.[100] developed recombinant hybrid proteins OvH2 and OvH3. The first OvH2 is composed of Ov20 fused to Ov33, and OvH3 consists of the C-terminus of Ov20 linked to Ov33. Ov20 is a secreted glycoprotein found in the body wall of adult female *O. volvulus*. OvH2 has been shown to have a sensitivity of 98.5% and a specificity of 97.7%, and OvH3 has been shown to have a sensitivity of 98.5% and a specificity of 95.4%, when tested on sera from 132 microfilariae-positive patients.[101]

p0330 The two antigens found in OvH3, Ov20 and Ov33, are also found in infective larvae and microfilariae, meaning that it is possible that the assay is measuring antibody responses to the early stages of the parasite. This indicates

that the assay may have a role to play in the diagnosis of low level and prepatent infections as well as in established infections. The assay may also be valuable in assessing the level of exposure to *O. volvulus* within a community.[101]

p0335 The most developed and advanced serological marker for onchocerciasis exposure is the IgG4 response to the *O. volvulus* marker Ov16 antigen that is expressed by the larval stages (L3 and L4) of the parasite.[102]

p0340 Ov16 IgG4 antibody response is also an accurate marker for active infection in children under 11 years of age. In America, the immunological assay that measures anti-Ov16 IgG4 seroconversion using a ELISA in children has been used widely to demonstrate that transmission has been interrupted. In Africa, the anti-Ov16 marker is increasingly used to confirm the interruption of transmission in foci that receive extensive rounds of MDA.

p0345 A new rapid diagnostic test (RDT) for the detection of IgG4 against Ov16 has been developed as a practical, convenient, and standardized alternative for use in the field (SD BIOLINE Onchocerciasis IgG4 Rapid Test).[103]

s0195 5. CONCLUSIONS

p0350 We have herein conducted a literature search with the purpose of shedding some light onto the drug design scenario in onchocerciasis; a *neglected tropical disease* that has a pressing need for a therapeutic revolution. Despite the existence of a safe drug that can be used for mass treatment, ivermectin, the emergence of ivermectin resistance strengthens the crucial need to identify new drug targets and agents that can effectively treat this disease. Only a few examples of drug design programs directed toward the discovery and design of new molecules for use as possible drug candidates have been seen over the last 18 years. However, knowledge of the biology and physiology of the causative agent, *O. volvulus* filarial nematode, has been enriched, over these years, by linking a variety of enzymes to specific roles in the life of the parasite, both as *microfilariae* and the adult worm. It is possible, that such discoveries could lead to the ~~discovery of drug targets and drug candidates~~ for future onchocerciasis treatment.

REFERENCES

1. WHO *Wkly. Epidemiol. Rec.* **2017**, 92, 681.
2. Dunn, C.; Callahan, K.; Katabarwa, M.; Richards, F.; Hopkins, D.; Withers, P. C.; Buyon, L. E.; McFarland, D. *PLoS Negl. Trop. Dis.* **2015**, 9.
3. Boussinesq, M.; Pion, S. D. S.; Demanga-Ngangue, A.; Kamgno, J. *Trans. R. Soc. Trop. Med. Hyg.* **2002**, *96*, 537.

4. Duke, B. O. L. *Trans. R. Soc. Trop. Med. Hyg.* **1998**, *92*, 236.
5. Kim, Y. E.; Stolk, W. A.; Tanner, M.; Tediosi, F. *BMJ Glob. Health* **2017**, 2.
6. *Lancet* **2017**, 390, 1260.
7. (a) Rodríguez-Pérez, M. A.; Fernández-Santos, N. A.; Orozco-Algarra, M. E.; Rodríguez-Atanacio, J. A.; Domínguez-Vázquez, A.; Rodríguez-Morales, K. B.; Real-Najarro, O.; Prado-Velasco, F. G.; Cupp, E. W.; Richards, F. O.; Hassan, H. K.; González-Roldán, J. F.; Kuri-Morales, P. A.; Unnasch, T. R. *PLoS Negl. Trop. Dis.* **2015**, *9*, ; (b) Burki, T. *Lancet Infect. Dis.* **2013**, *13*, 922.
8. Boatin, B. A.; Richards, F. O. In *Advances in Parasitology*; Molyneux, D. H. Ed.; Vol. 61. Academic Press, 2006.
9. Murdoch, M. E. *Curr. Opin. Infect. Dis.* **2010**, *23*, 124.
10. Hall, L. R.; Pearlman, E. *Clin. Microbiol. Rev.* **1999**, *12*, 445.
11. Udall, D. N. *Clin. Infect. Dis.* **2007**, *44*, 53.
12. Abbasi, J. *JAMA* **2018**, *319*, 113.
13. Kuesel, A. C. *Int. J. Parasitol. Drugs Drug Resist.* **2016**, *6*, 272.
14. Santiago-Stevenson, D.; Oliver-Gonzales, J.; Hewitt, R. I. *Ann. N. Y. Acad. Sci.* **1948**, *50*, 161.
15. Mazzotti, L. *Am. J. Trop. Med. Hyg.* **1951**, 628. s1–31.
16. Albiez, E. J. *Tropenmed. Parasitol.* **1983**, *34*, 267.
17. Awadzi, K.; Gilles, H. M. *Br. J. Clin. Pharmacol.* **1992**, *34*, 281.
18. Peixoto, C. A.; Silva, B. S. *Eur. J. Pharmacol.* **2014**, *734*, 35.
19. Kwarteng, A.; Ahuno, S. T.; Akoto, F. O. *Infect. Dis. Pover.* **2016**, 5.
20. McGarry, H. F.; Plant, L. D.; Taylor, M. J. *Filaria J.* **2005**, 4.
21. Taylor, H. R.; Greene, B. M. *Am. J. Trop. Med. Hyg.* **1989**, *41*, 460.
22. Burg, R. W.; Miller, B. M.; Baker, E. E.; Birnbaum, J.; Currie, S. A.; Hartman, R.; Kong, Y.-L.; Monaghan, R. L.; Olson, G.; Putter, I.; Tunac, J. B.; Wallick, H.; Stapley, E. O.; Oiwa, R.; Ōmura, S. *Antimicrob. Agents Chemother.* **1979**, *15*, 361.
23. Laing, R.; Gillan, V.; Devaney, E. *Trends Parasitol.* **2017**, *33*, 463.
24. Crump, A. *J. Antibiot.* **2017**, *70*, 495.
25. Opoku, N. O.; Bakajika, D. K.; Kanza, E. M.; Howard, H.; Mambandu, G. L.; Nyathirombo, A.; Nigo, M. M.; Kasonia, K.; Masembe, S. L.; Mumbere, M.; Kataliko, K.; Larbelee, J. P.; Kpawor, M.; Bolay, K. M.; Bolay, F.; Attah, S. K.; Vaillant, M.; Halleux, C. M.; Kuesel, A. C. *Lancet* **2018**, ~~ahead of print~~,
26. Boussinesq, M. *Lancet* **2018**, Published Online January 17.
27. Mackenzie, C. D.; Geary, T. G. *Expert Rev. Anti Infect. Ther.* **2011**, *9*, 497.
28. Dominguez-Vazquez, A.; Taylor, H.; Ruvalcaba-Macias, A. M.; Murphy, R.; Greene, B.; Rivas-Alcala, A. R.; Beltran-Hernandez, F. *Lancet* **1983**, *321*, 139.
29. Naicker, S.; Plange-Rhule, J.; Tutt, R. C.; Eastwood, J. B. *Ethn. Dis.* **2009**, *19*, S1.
30. Dicko, M.; Oni, A. Q.; Ganivet, S.; Kone, S.; Pierre, L.; Jacquet, B. *Bull. World Health Organ.* **2000**, *78*, 163.
31. Ceballos, L.; Mackenzie, C.; Geary, T.; Alvarez, L.; Lanusse, C. *PLoS Negl. Trop. Dis.* **2014**, *8, e2838/1*.
32. Vigh, T.; Démuth, B.; Balogh, A.; Galata, D. L.; Van Assche, I.; Mackie, C.; Vialpando, M.; Van Hove, B.; Psathas, P.; Borbás, E.; Pataki, H.; Boeykens, P.; Marosi, G.; Verreck, G.; Nagy, Z. K. *Drug Dev. Ind. Pharm.* **2017**, *43*, 1126.
33. Delatour, P.; Richard, Y. *Therapie* **1976**, *31*, 505.
34. Lacey, E. *Int. J. Parasitol.* **1988**, *18*, 885.
35. Longo, M.; Zanoncelli, S.; Messina, M.; Scandale, I.; Mackenzie, C.; Geary, T.; Marsh, K.; Lindley, D.; Mazue, G. *Reprod. Toxicol.* **2014**, *49*, 33.
36. Kalmobé, J.; Ndjonka, D.; Boursou, D.; Vildina, J. D.; Liebau, E. *BMC Complement. Altern. Med.* **2017**, 17.

37. Hoerauf, A.; Specht, S.; Marfo-Debrekyei, Y.; Büttner, M.; Debrah, A. Y.; Mand, S.; Batsa, L.; Brattig, N.; Konadu, P.; Bandi, C.; Fimmers, R.; Adjei, O.; Büttner, D. W. *Parasitol. Res.* **2009**, *104*, 437.
38. Mand, S.; Debrah, A. Y.; Klarmann, U.; Batsa, L.; Marfo-Debrekyei, Y.; Kwarteng, A.; Specht, S.; Belda-Domene, A.; Fimmers, R.; Taylor, M.; Adjei, O.; Hoerauf, A. *Clin. Infect. Dis.* **2012**, *55*, 621.
39. Walker, M.; Specht, S.; Churcher, T. S.; Hoerauf, A.; Taylor, M. J.; Basáñez, M. G. *Clin. Infect. Dis.* **2015**, *60*, 1199.
40. Hoerauf, A.; Specht, S.; Marfo-Debrekyei, Y.; Buttner, M.; Debrah, A. Y.; Mand, S.; Batsa, L.; Brattig, N.; Konadu, P.; Bandi, C.; Fimmers, R.; Adjei, O.; Buttner, D. W. *Parasitol. Res.* **2009**, *104*, 437.
41. Wright, J. E.; Werkman, M.; Dunn, J. C.; Anderson, R. M. *Parasit. Vectors* **2018**, 11.
42. Aljayyoussi, G.; Tyrer, H. E.; Ford, L.; Sjoberg, H.; Pionnier, N.; Waterhouse, D.; Davies, J.; Gamble, J.; Metugene, H.; Cook, D. A. N.; Steven, A.; Sharma, R.; Guimaraes, A. F.; Clare, R. H.; Cassidy, A.; Johnston, K. L.; Myhill, L.; Hayward, L.; Wanji, S.; Turner, J. D.; Taylor, M. J.; Ward, S. A. *Sci. Rep.* **2017**, 7.
43. Fischer, P. U.; Weil, G. J.; King, C. L.; Jacobson, J. A. *PLoS Negl. Trop. Dis.* **2017**, *11,* e0005163.
44. Horton, J. *Curr. Opin. Infect. Dis.* **2002**, *15*, 599.
45. Boussinesq, M.; Fobi, G.; Kuesel, A. C. *Int. Health* **2018**, *10*, i40.
46. Chin, Y.-W.; Balunas, M. J.; Chai, H. B.; Kinghorn, A. D. *AAPS J.* **2006**, *8,* E239.
47. Cragg, G. M.; Newman, D. J. *Biochim. Biophys. Acta* **1830**, *2013*, 3670.
48. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. *Molecules* **2016**, *21*, 559.
49. Metuge, J. A.; Nyongbela, K. D.; Mbah, J. A.; Samje, M.; Fotso, G.; Babiaka, S. B.; Cho-Ngwa, F. *BMC Complement. Altern. Med.* **2014**, 14.
50. Samje, M.; Metuge, J.; Mbah, J.; Nguesson, B.; Cho-Ngwa, F. *BMC Complement. Altern. Med.* **2014**, 14.
51. Vildina, J. D.; Kalmobe, J.; Djafsia, B.; Schmidt, T. J.; Liebau, E.; Ndjonka, D. *Molecules* **2017**, 22.
52. Nyasse, B.; Ngantchou, I.; Nono, J. J.; Schneider, B. *Nat. Prod. Res.* **2006**, *20*, 391.
53. Mainsah, E. N.; Ntum, S. J. E.; Samje, M.; Cho-Ngwa, F.; Ndifon, P. T.; Yong, J. N. *Anti-Infect. Agents* **2016**, *14*, 47.
54. Ekins, S.; Williams, A. J.; Krasowski, M. D.; Freundlich, J. S. *Drug Discov. Today* **2011**, *16*, 298.
55. Gloeckner, C.; Garner, A. L.; Mersha, F.; Oksov, Y.; Tricoche, N.; Eubanks, L. M.; Lustigman, S.; Kaufmann, G. F.; Janda, K. D. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 3424.
56. Wu, Y.; Egerton, G.; Underwood, A. P.; Sakuda, S.; Bianco, A. E. *J. Biol. Chem.* **2001**, *276*, 42557.
57. Wu, Y.; Egerton, G.; McCarthy, J. S.; Nutman, T. B.; Bianco, A. E. *Filaria J.* **2003**, *2*, 6.
58. Segura-Cabrera, A.; Bocanegra-García, V.; Lizarazo-Ortega, C.; Guo, X.; Correa-Basurto, J.; Rodríguez-Pérez, M. A. *J. Comput. Aided Mol. Des.* **2011**, *25*, 1107.
59. Garner, A. L.; Gloeckner, C.; Tricoche, N.; Zakhari, J. S.; Samje, M.; Cho-Ngwa, F.; Lustigman, S.; Janda, K. D. *J. Med. Chem.* **2011**, *54*, 3963.
60. (a) Gooyit, M.; Tricoche, N.; Lustigman, S.; Janda, K. D. *J. Med. Chem.* **2014**, *57*, 5792; (b) Gooyit, M.; Harris, T. L.; Tricoche, N.; Javor, S.; Lustigman, S.; Janda, K. D. ACS Infect. Dis. **2015**, *1*, 198; (c) Gooyit, M.; Tricoche, N.; Javor, S.; Lustigman, S.; Janda, K. D. *ACS Med. Chem. Lett.* **2015**, *6*, 339.
61. (a) Lolli, M. L.; Giorgis, M.; Tosco, P.; Foti, A.; Fruttero, R.; Gasco, A. *Eur. J. Med. Chem.* **2012**, *49*, 102; (b) Bonomo, S.; Tosco, P.; Giorgis, M.; Lolli, M.; Fruttero, R. *J. Mol. Model.* **2013**, *19*, 1099; (c) Pippione, A. C.; Federico, A.; Ducime, A.; Sainas, S.; Boschi, D.; Barge, A.; Lupino, E.; Piccinini, M.; Kubbutat, M.;

Contreras, J.-M.; et al. *MedChemComm* **2017**, *8*, 1850; (d) Pippione, A. C.; Giraudo, A.; Bonanni, D.; Carnovale, I. M.; Marini, E.; Cena, C.; Costale, A.; Zonari, D.; Pors, K.; Sadiq, M.; et al. *Eur. J. Med. Chem.* **2017**, *139*(Suppl. C), 936; (e) Sainas, S.; Pippione, A. C.; Giorgis, M.; Lupino, E.; Goyal, P.; Ramondetti, C.; Buccinnà, B.; Piccinini, M.; Braga, R. C.; Andrade, C. H.; et al. *Eur. J. Med. Chem.* **2017**, *129*, 287; (f) Pippione, A. C.; Carnovale, I. M.; Bonanni, D.; Sini, M.; Goyal, P.; Marini, E.; Pors, K.; Adinolfi, S.; Zonari, D.; Festuccia, C.; et al. *Eur. J. Med. Chem.* **2018**, *150*, 930; (g) Pippione, A. C.; Sainas, S.; Federico, A.; Lupino, E.; Piccinini, M.; Kubbutat, M.; Contreras, J.-M.; Morice, C.; Barge, A.; Ducime, A.; et al. *MedChemComm* **2018**, *9*, 963; (h) Sainas, S.; Pippione, A. C.; Lupino, E.; Giorgis, M.; Circosta, P.; Gaidano, V.; Goyal, P.; Bonanni, D.; Rolando, B.; Cignetti, A.; et al. *J. Med. Chem.* **2018**, *61*, 6034.

62. Pippione, A. C.; Dosio, F.; Ducime, A.; Federico, A.; Martina, K.; Sainas, S.; Frølund, B.; Gooyit, M.; Janda, K. D.; Boschi, D.; Lolli, M. L. *Med. Chem. Commun.* **2015**, *6*, 1285.
63. Lolli, M.; Narramore, S.; Fishwick, C. W.; Pors, K. Refining the Chemical Toolbox to be Fit for Educational and Practical Purpose for Drug Discovery in the 21st Century. *Drug Discov. Today* **2015**, *20*, 1018.
64. Cotton, J. A.; Bennuru, S.; Grote, A.; Harsha, B.; Tracey, A.; Beech, R.; Doyle, S. R.; Dunn, M.; Hotopp, J. C. D.; Holroyd, N.; Kikuchi, T.; Lambert, O.; Mhashilkar, A.; Mutowo, P.; Nursimulu, N.; Ribeiro, J. M. C.; Rogers, M. B.; Stanley, E.; Swapna, L. S.; Tsai, I. J.; Unnasch, T. R.; Voronin, D.; Parkinson, J.; Nutman, T. B.; Ghedin, E.; Berriman, M.; Lustigman, S. *Nat. Microbiol.* **2016**, *2*, 16216.
65. Metuge, J. A.; Ntie-Kang, F.; Ngwa, V. F.; Babiaka, S. B.; Samje, M.; Cho-Ngwa, F. *Med. Chem. Res.* **2015**, *24*, 2127.
66. Perbandt, M.; Hoeppner, J.; Burmeister, C.; Lueersen, K.; Betzel, C.; Liebau, E. *J. Mol. Biol.* **2008**, *377*, 501.
67. Liebau, E.; Hoeppner, J.; Muehlmeister, M.; Burmeister, C.; Lueersen, K.; Perbandt, M.; Schmetz, C.; Buettner, D.; Brattig, N. *FEBS J.* **2008**, *275*, 3438.
68. (a) Campbell, A. M.; Van Eldik, R.; Liebau, E.; Barrett, J.; Brophy, P. M.; Teesdale-Spittle, P.; Wang, M. F. *Chem. Biol. Interact.* **2001**, 133, 240; (b) Brophy, P. M.; Campbell, A. M.; Van Eldik, A. J.; Teesdale-Spittle, P. H.; Liebau, E.; Wang, M. F. *Bioorg. Med. Chem. Lett.* **2000**, 10, 979.
69. Lüersen, K.; Müller, S.; Hussein, A.; Liebau, E.; Walter, R. D. *Mol. Biochem. Parasitol.* **2000**, *111*, 243.
70. Fischer, P.; Djoha, S.; Büttner, D. W.; Zipfel, P. F. *Mol. Biochem. Parasitol.* **2003**, *128*, 33.
71. O'Connell, E. M.; Bennuru, S.; Steel, C.; Dolan, M. A.; Nutman, T. B. *J. Infect. Dis.* **2015**, *212*, 684.
72. Ndjonka, D.; Zou, Y.; Bi, X.; Woster, P.; Walter, R. D.; Lüersen, K. *Biol. Chem.* **2003**, *384*, 1195.
73. Tawe, W.; Walter, R. D.; Henkle-Dührsen, K. *Exp. Parasitol.* **2000**, *94*, 172.
74. Ajonina-Ekoti, I.; Ndjonka, D.; Tanyi, M. K.; Wilbertz, M.; Younis, A. E.; Boursou, D.; Kurosinski, M. A.; Eberle, R.; Lüersen, K.; Perbandt, M.; Breloer, M.; Brattig, N. W.; Liebau, E. *Acta Trop.* **2012**, *124*, 15.
75. Ford, L.; Guiliano, D. B.; Oksov, Y.; Debnath, A. K.; Liu, J.; Williams, S. A.; Blaxter, M. L.; Lustigman, S. *J. Biol. Chem.* **2005**, *280*, 40845.
76. Merriweather, A.; Guenzler, V.; Brenner, M.; Unnasch, T. R. *Mol. Biochem. Parasitol.* **2001**, *116*, 185.
77. Raverdy, S.; Zhang, Y.; Foster, J.; Carlow, C. K. S. *Mol. Biochem. Parasitol.* **2007**, *156*, 210.
78. Dhamodharan, R.; Hoti, S. L.; Sankari, T. *Infect. Genet. Evol.* **2012**, *12*, 957.

79. Choe, K. P.; Leung, C. K.; Miyamoto, M. M. *Drug Metab. Rev.* **2012**, *44*, 209.
80. Sommer, A.; Fischer, P.; Krause, K.; Boettcher, K.; Brophy, P. M.; Walter, R. D.; Liebau, E. *Biochem. J.* **2001**, *353*, 445.
81. Erttmann, K. D.; Kleensang, A.; Schneider, E.; Hammerschmidt, S.; Büttner, D. W.; Gallin, M. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2005**, *1741*, 85.
82. Lustigman, S.; Zhang, J.; Liu, J.; Oksov, Y.; Hashmi, S. *Mol. Biochem. Parasitol.* **2004**, *138*, 165.
83. Galvin, B. D.; Li, Z.; Villemaine, E.; Poole, C. B.; Chapman, M. S.; Pollastri, M. P.; Wyatt, P. G.; Carlow, C. K. S. *PLoS Negl. Trop. Dis.* **2014**, 8.
84. Cho-Ngwa, F.; Mbua, E. N.; Nchamukong, K. G.; Titanji, V. P. K. *Mol. Biochem. Parasitol.* **2007**, *156*, 136.
85. Poole, C. B.; Jin, J.; McReynolds, L. A. *J. Biol. Chem.* **2003**, *278*, 36183.
86. Ta, T. H.; Moya, L.; Nguema, J.; Aparicio, P.; Miguel-Oteo, M.; Cenzual, G.; Canorea, I.; Lanza, M.; Benito, A.; Crainey, J. L.; Rubio, J. M. *Acta Trop.* **2018**, *180*, 12.
87. McBride, A.; Walker, S. L. *Medicine* **2018**, *46*, 10.
88. Vlaminck, J.; Fischer, P. U.; Weil, G. J. *Trends Parasitol.* **2015**, *31*, 571.
89. Zimmerman, P. A.; Katholi, C. R.; Wooten, M. C.; Lang-Unnasch, N.; Unnasch, T. R. *Mol. Biol. Evol.* **1994**, *11*, 384.
90. Toé, L.; Boatin, B. A.; Adjami, A.; Back, C.; Merriweather, A.; Unnasch, T. R. *J. Infect. Dis.* **1998**, *178*, 282.
91. Alhassan, A.; Li, Z.; Poole, C. B.; Carlow, C. K. S. *Trends Parasitol.* **2015**, *31*, 391.
92. Alhassan, A.; Osei-Atweneboana, M. Y.; Kyeremeh, K. F.; Poole, C. B.; Li, Z.; Tettevi, E.; Tanner, N. A.; Carlow, C. K. S. *Mol. Biochem. Parasitol.* **2016**, *210*, 10.
93. Poole, C. B.; Li, Z.; Alhassan, A.; Guelig, D.; Diesburg, S.; Tanner, N. A.; Zhang, Y.; Evans, T. C., Jr.; LaBarre, P.; Wanji, S.; Burton, R. A.; Carlow, C. K. S. *PLoS One* **2017**, 12.
94. Stingl, P.; Ross, M.; Gibson, D. W.; Ribas, J.; Connor, D. H. *Trans. R. Soc. Trop. Med. Hyg.* **1984**, *78*, 254.
95. Ozoh, G.; Boussinesq, M.; Bissek, A. C. Z. K.; Kobangue, L.; Kombila, M.; Mbina, J. R. M.; Enyong, P.; Noma, M.; Sékétéli, A.; Fobi, G. *Trop. Med. Int. Health* **2007**, *12*, 123.
96. Awadzi, K.; Opoku, N. O.; Attah, S. K.; Lazdins-Helds, J. K.; Kuesel, A. C. *Parasit. Vectors* **2015**, *8*, 515.
97. Weil, G. J.; Ogunrinade, A. F.; Chandrashekar, R.; Kale, O. O. *J Infect Dis* **1990**, *161*, 549.
98. Chandrashekar, R.; Ogunrinade, A. F.; Weil, G. J. *Trop. Med. Int. Health* **1996**, *1*, 575.
99. Bradley, J. E.; Atogho, B. M.; Elson, L.; Stewart, G. R.; Boussinesq, M. *Am. J. Trop. Med. Hyg.* **1998**, *59*, 877.
100. Nde, P. N.; Pogonka, T.; Bradley, J. E.; Titanji, V. P.; Lucius, R. *Am. J. Trop. Med. Hyg.* **2002**, *66*, 566.
101. Andrews, J.; Bligh, W. J.; Chiodini, P. L.; Bradley, J. E.; Nde, P. N.; Lucius, R. *J. Clin. Pathol.* **2008**, *61*, 347.
102. Golden, A.; Faulx, D.; Kalnoky, M.; Stevens, E.; Yokobe, L.; Peck, R.; Karabou, P.; Banla, M.; Rao, R.; Adade, K.; Gantin, R. G.; Komlan, K.; Soboslay, P. T.; de los Santos, T.; Domingo, G. J. *Parasit. Vectors* **2016**, *9*, 338.
103. Weil, G. J.; Steel, C.; Liftis, F.; Li, B.-W.; Mearns, G.; Lobos, E.; Nutman, T. B. *J. Infect. Dis.* **2000**, *182*, 1796.

Non-Print Items

Abstract

Onchocerciasis, also called *river blindness*, is a *neglected tropical disease* which is in desperate need of a therapeutic revolution. This debilitating disease, endemic in 31 countries in sub-Saharan Africa, Yemen, and Latin America, is a leading cause of blindness in the developing world. The infection is caused by the filarial parasitic nematode *Onchocerca volvulus* that is transmitted to humans by the black fly *Simulium* spp. Its pathology, whose symptoms are onchodermatitis, musculoskeletal pain, and various stages of blindness, is a result of the death of the microfilariae in the skin and eyes. This review covers the drug design and early detection fields of this pathology and pays particular attention to the period after the introduction of *ivermectin*, which is the only drug available for mass treatment. The emergence of ivermectin resistance justifies the crucial need to identify new drug targets and agents that can effectively treat *onchocerciasis*.

Keywords: Neglected tropical diseases, Onchocerciasis, *Onchocerca volvulus*, Ivermectin, Chitinase, Scaffold hopping