CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

Division of Anti-Infective Products
Clinical Microbiology Review

NDA: 210867 (SDN-001, 003); Original NDA
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APPLICANT:
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C/O Target Health Inc
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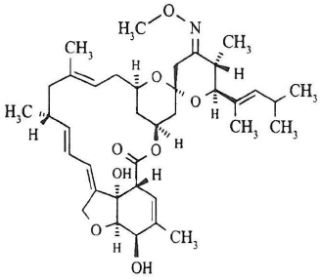
New York, New York 10016

DRUG PRODUCT NAMES:
Proprietary name: None
Non-proprietary name: Moxidectin
Chemical name: (2aE,4E,5'R,6R,6'S, 8E,11 R, 13S, 15S, 17aR,20R,20aR,20bS)-6'-[(E)-1,3-dimethyl-1-butenyl] 5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19- tetramethylspiro [11,15-methano-2H, 13H, 17 H-furo[4,3,2-pq][2,6] benzodioxacyclooctadecin-13,2'-2H]pyran] -4', 17(3'H)-dione 4'-(E)-(0-methyloxime)

STRUCTURAL FORMULA:



Molecular weight: 639.82
Molecular formula: $C_{37}H_{53}NO_8$

DRUG CATEGORY:
Antiparasitic/antheminthic

PROPOSED INDICATION:
Treatment of onchocerciasis due to (b) (4) *Onchocerca volvulus*

PROPOSED DOSAGE FORM, ROUTE OF ADMINISTRATION AND DURATION OF TREATMENT:
Dosage form: Tablets (contains 2 mg of active ingredient)
Route of administration: Oral
Dosage and Duration: 8 mg/day – single dose

DISPENSED:
Rx

RELATED DOCUMENTS:
IND 126876

REMARKS
The nonclinical studies *in vitro*, in animals infected with *Onchocerca* or other filarial species as well as the two clinical studies in subjects with onchocerciasis support the activity of moxidectin against *Onchocerca* parasites. Moxidectin inhibits embryogenesis and release of microfilariae into circulation. The mechanism of action of moxidectin appears to be similar to ivermectin. Studies suggest a potential for development of resistance to moxidectin and cross-resistance with ivermectin. The mechanism of resistance, like ivermectin, may be multi-factorial.

CONCLUSIONS AND RECOMMENDATIONS
From clinical microbiology perspective, this NDA is approvable pending an accepted version of the labeling (for changes to the labeling please see Section 6.3 of this review).

Table of Contents

1. EXECUTIVE SUMMARY

The subject of this NDA is moxidectin for the treatment of onchocerciasis due to (b) (4) *Onchocerca volvulus.*

Mechanism of action

The mechanism by which moxidectin exhibits its effect against *O. volvulus* has not been studied. However, studies with other nematodes suggest that moxidectin, like ivermectin, may exhibit it effect by binding to glutamate-gated chloride ion channels (GluCl), gamma-aminobutyric acid (GABA) receptors and ATP-binding cassette (ABC) transporters leading to an increase in permeability, influx of chloride ions, hyperpolarization, muscle paralysis, reduction in motility and/or excretion of immunomodulatory proteins as well as the fertility of both male and female adult worms. The role of immunomodulatory effects of moxidectin either directly by inducing antibody- and/or cell-mediated adherence or indirectly by inhibiting secretion of proteins, in conferring protection cannot be ruled out.

Activity in vitro

The methods to measure *in vitro* susceptibility of different stages of *Onchocerca* and other filarial parasites, to drugs, are not standardized. Few studies have reported the *in vitro* activity of moxidectin based on motility of a single strain/isolate of the filaria parasites (*O. volvulus, Onchocerca lienalis* and *Brugia malayi*). Sensitivity to the drug appears to vary with the filarial species and the stage of the parasite. Overall, the studies suggest that moxidectin is effective in reducing the motility of the adult worms (*B. malayi*) and the microfilariae (*Onchocerca* species and *B. malayi*) as well as the release of microfilariae from female adult worms. Adult worms appear to be more sensitive than microfilariae of *B. malayi* under the experimental conditions tested.

The mammalian cells such as the Vero monkey kidney cells are less sensitive to moxidectin compared to microfilariae and adult worms of *B. malayi*.

Activity in animal models

Studies in rodents experimentally infected with microfilariae of *O. volvulus* and *O. lienalis* suggest that treatment with moxidectin is active against the microfilariae; moxidectin is more effective than ivermectin. *O. volvulus* microfilariae appear to be less sensitive to treatment with moxidectin or ivermectin than *O. lienalis* suggesting differences in sensitivity of microfilariae of the *Onchocerca* species to the drug. Similar observations were made *in vitro*. No studies were available supporting the activity of moxidectin against the adult worms or the infective larvae (L_3) of the *Onchocerca* species in experimentally infected rodents. However, studies in naturally infected calves and horses suggest that moxidectin is active against microfilariae and inhibits/reduces embryogenesis in adult worms. Similar observations were reported against other filarial species in rodents and dogs infected with the L_3 or adult worms.

Drug resistance and cross resistance

Studies *in vitro* and/or in animals infected with nematodes suggest a potential for development of resistance to moxidectin and cross-resistance with ivermectin. The mechanism of resistance to moxidectin, like ivermectin, may be multifactorial that include changes in the target GluCl, GABA receptor, and/or ABC transporter.

The methods to assess drug resistance in subjects with filarial infections are not available. Efforts have been made to assess resistance by measuring microfilarial density, the rate of decline in microfilarial density as well as the rate at which relapse i.e., rebound/re-population of

microfilariae occurs. For example, a study conducted in Cameroon, suggests a possibility of development of resistance to ivermectin; the rebound rate post-treatment was higher early on (at 2 to 8 weeks) in frequently treated population compared to the ivermectin-naïve subjects; however, the rate was the same at Month 6. Also, the number of degenerating microfilariae were lower in the frequently treated group than the ivermectin naive group. Similar studies with moxidectin have not been conducted. The Applicant states that "in both the Phase II and Phase III clinical studies comparing the efficacy of moxidectin to ivermectin, evidence of a population of sub-optimal responders in the ivermectin group was noted while in the moxidectin cohort no such sub-optimal responders were evident." It should be noted that the two clinical studies were in ivermectin naïve population and therefore, the prevalence of resistance to moxidectin due to cross-resistance with ivermectin cannot be ruled out. It is possible that a longer half-life of moxidectin compared to ivermectin may allow for less frequent treatments leading to lower selection pressure and a decreased possibility for the development of resistance. However, the effect of persistence of moxidectin for a prolonged duration due to its longer half-life of moxidectin, compared to ivermectin, on development of resistance is not known.

Clinical Microbiology

The Applicant conducted one Phase II and one Phase III study to support the efficacy of moxidectin for the treatment of onchocerciasis. The studies were conducted in sub-Saharan Africa; over 99% of patients with onchocerciasis are found in this region. The communities selected had not previously participated in ivermectin mass drug administration programs. It appears that no vector control measures were in place.

The parasitological assessments included measurement of microfilariae density in the skin and eye. Skin microfilariae were counted directly in the wells of the microtiter plates containing skin snips after overnight incubation in an isotonic solution. The entire well was examined using an inverted microscope. Appropriate training and quality control measures were in place. Ocular microfilariae count was performed by an ophthalmologist using a slit lamp.

The Phase II and III trials enrolled subjects with ≥1 and ≥10 microfilariae/mg skin, respectively. The primary endpoint was a reduction in skin microfilariae density at Month 18 in the Phase II study and Month 12 in the Phase III study. Reduction in skin microfilaria density is an appropriate endpoint as microfilariae are responsible for the etiology and worsening of dermal and ocular disease symptoms and therefore, considered to be of direct clinical benefit to the patient. Overall, the studies suggest that treatment with moxidectin is more effective than ivermectin in decreasing skin microfilariae density for a longer duration. Also, in the moxidectin treatment group higher proportion of subjects remained microfilariae negative in the skin and the rebound of microfilariae was slower compared to ivermectin treatment group. Slower rebound of skin microfilariae in subjects treated with moxidectin, compared to ivermectin, could be due to the longer half-life of moxidectin. The efficacy of moxidectin and ivermectin in the ocular region appears to be similar. Reduction in microfilariae count in the anterior chamber and cornea of the eye as well as an increase in the proportion of microfilariae negative subjects was observed at Month 6 after treatment; reasons for slower effectiveness of treatment in the eye compared to skin are unclear.

Nodulectomy was performed at Month 18 in some of the subjects enrolled in the Phase II study. Based on motility of the excised parasites and histological findings of the onchocercal nodules, the results suggest a trend towards a decrease in the number of live microfilariae and adult worms as well as an increase in degenerative embryos in the moxidectin (8 mg) and ivermectin treated subjects compared to low dose moxidectin treated subjects. These results should be

interpreted with caution as the number of worms in the excised nodules may not reflect parasite burden in the host. Also, the presence of extra-nodular adult worms cannot be ruled out. The pretreatment nodules were not processed for histological evaluation. The formation of new nodules due to the presence of worms at early developmental phase (due to long incubation period) post-treatment, presence of dead or calcified worms in the nodules prior to treatment, or re-infections due to high endemicity cannot be ruled out.

2. INTRODUCTION AND BACKGROUND

The subject of this NDA is moxidectin for the treatment of onchocerciasis due to (b) (4) *Onchocerca volvulus*. Moxidectin was granted orphan drug designation on September 29, 2010. Ivermectin is approved in the US for the treatment of onchocerciasis.

Onchocerciasis is endemic in sub-Saharan Africa, the Arabian Peninsula as well as some foci in the Americas. Over 150 million people are estimated to live in areas at risk of infection; over 99% are in ~30 countries in Sub-Saharan Africa.

2.1. Moxidectin

Moxidectin, a macrocyclic lactone, is a fermentation product of *Streptomyces cyaneogriseus* ssp. *noncyanogenus*; it is marketed in the US as a veterinary endectocide for dogs, cattle, horses, sheep and several other domestic species in numerous dosage forms that include tablets, drench, gel, injectable, topical pour-on and spot-on.

In patients with onchocerciasis, treated with moxidectin 8 mg, the geometric mean (± SD) area under the curve (AUC) was 2714 (± 1535) ng**.**hour/mL. Healthy subjects administered moxidectin, had higher exposures (AUC increased by 24%) compared to patients with onchocerciasis. Following oral administration, moxidectin was absorbed with a peak plasma concentration between 2 and 6 hours. The mean terminal half-life of moxidectin in patients and healthy volunteers following a single dose (8 mg) of moxidectin was 23.3 days (559 hours) and 32.7 days (784 hours), respectively. The plasma protein binding was not determined.

2.2. Biology of *the* parasite

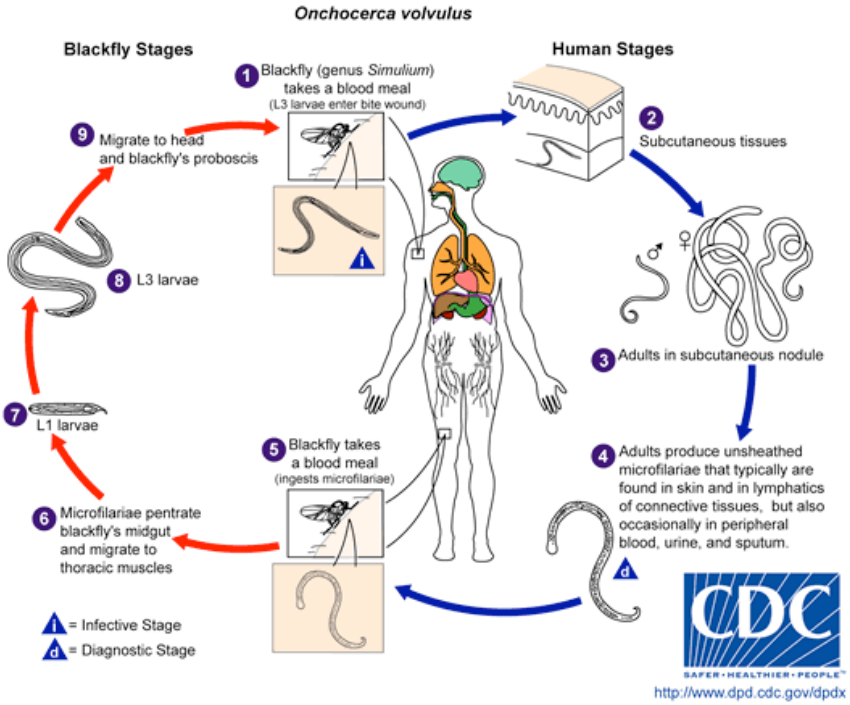
- ***Life cycle***

O. volvulus third stage infective larvae (L_3) are transmitted to humans by the bite of black flies or buffalo gnat (genus *Simulium*) which breed in fast-flowing rivers and streams. The black flies are pool feeders.

The blackfly acquires microfilariae by biting the skin of an infected individual. The microfilariae develop, in the fly over a period of two weeks, to L_3 that migrate to its mouthparts (Figure 1). During the next blood meal, the L_3 are transmitted to humans. Once inside the subcutaneous tissue, larvae form nodules and begin maturing into adult worms; this may take up to one year. The male (15-45 mm in length) and female (230-700 mm, can measure up to a meter in length) adult worms are long, live coiled in mating frequently pairs within the nodules in the skin. The female worms produce microfilariae and this phase is called as the patent phase. The pre-patent period (i.e., before the presence of microfilariae) may vary from 3 to 15 months. The microfilariae numbers may be up to 1000/day for a lifespan (~15 years) of the adult worms, however, they cannot develop further in the body. Microfilariae migrate throughout the body, including the skin, eyes and lymph nodes, where they are accessible to the bite of the blackfly. The saliva probably contains anti-coagulants as well as attractants for the skin dwelling microfilariae. Microfilariae in the skin are responsible for both disease pathology in the individual and continued transmission in the community by the vector.

Death of the microfilariae in the tissues results in an inflammatory immune reaction and causes a variety of pathologies. The major symptoms of onchocerciasis, usually appear between 9 months and 2 years after initial infection.

Figure 1: The life cycle of *Onchocerca volvulus*.



During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate in to 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 to 50 cm in length and 270 to 400 µM in diameter, while males measure 19 to 42 mm by 130 to 210 µm. In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360 µM by 5 to 9 µM and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but are typically found in the skin and in the lymphatics of connective tissues ❹. A blackfly ingests the microfilariae during a blood meal ❺. After ingestion, the microfilariae migrate from the blackfly's midgut through the hemocoel to the thoracic muscles ❻. 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 blackfly's proboscis ❾ and can infect another human when the fly takes a blood meal ❶.

Source: https://www.cdc.gov/dpdx/onchocerciasis/index html

- ***Pathogenesis***

The chronic host response to microfilariae causes clinical manifestations of onchocerciasis that include pruritus, dermatitis, depigmentation, atrophy of the skin, and lymphadenitis. However, visual impairment leading to blindness is the most severe manifestation of the disease; in the eyes, microfilariae can cause inflammation and bleeding, leading to itching, redness, and eventually vision impairment or blindness.

The disease is often not contracted by a single bite, and the most severe symptoms result from years of repeated exposure. Travelers, spending less than three months in endemic areas, are thought to be at little or no risk for the disease; also, for those who have been exposed, symptoms may appear years after leaving the endemic region, as the long-lived adult worms continue to produce microfilariae.

The death of microfilariae is associated with characteristic and wel1-characterized responses of the body known collectively as the Mazzotti reaction. These reactions are most likely due to allergic and inflammatory responses to the breakdown products associated with microfilarial death. The Mazzotti patch test also known as diethyl carbamazine (DEC) patch test involves a topical application of DEC, which produces a local reaction to dying microfilariae at the patch site.

Studies in mice show that bacteria of the genus *Wolbachia* live in a symbiotic relationship inside *O. volvulus* worms; the bacteria provide heme for the parasite, as nematodes lack the genes necessary for heme synthesis that is essential for parasite growth. Also, these bacteria are thought to be necessary for female worm fertility. *Wolbachia* is thought to contribute to onchocerciasis pathogenesis; the release of bacteria upon death of the microfilariae contributes to the severity of the inflammatory response.[1]

Diagnosis

The diagnostic techniques used for onchocerciasis are

- *Skin biopsies ("snips") or examination of excised nodules*: Visualizing microfilariae in a skin sample is the standard diagnostic test and is highly specific, but has poor sensitivity during the early stages of infection (pre-patent phase) before significant numbers of microfilariae have been produced. Several samples should be examined.

  The microfilariae (tail nuclei do not extend to the tip of the tail which is pointed) should be distinguished from the microfilariae of another filarial nematode, *Mansonella streptocerca* (single row of nuclei extend to the tip of the tail, tail is curved 'shepherd's crook'), that causes skin disease.
- *Polymerase chain reaction*: Polymerase chain reaction (PCR) of the skin snips has been used for research purposes, however, not cleared by the FDA.
- *Serological assays*: Antibodies specific for the parasite can be found in the blood, but may not indicate active infection. Assays for *O. volvulus* antibodies include the Ov16 card test (to detect IgG4 targeting Ov16 antigen), ELISA to detect antibodies against cocktails of recombinant antigens (e.g., Ov20 and Ov33 or Ov7, Ov11 and Ov16), dot blot assay using native adult parasite antigens, OV luciferase immunoprecipitation system (LIPS). However, none of these tests are cleared by the FDA.

Treatment options

Ivermectin, a macrocyclic lactone, approved for the treatment of onchocerciasis, is active against the microfilariae, relieves the severe skin itching, and stops the damaging effects on the eye caused by the disease. It is both microfilaricidal and embryostatic:

- It causes a rapid decrease of microfilarial density in the skin, starting a few hours following treatment.
- It interferes with the release of microfilariae from adult female worms.

It does not affect the adult worm and therefore, does not cure infection. Ivermectin treatment cannot be used in regions that are co-endemic for another filarial parasite, *Loa*, due to the observation of serious adverse events.

Antibiotics that target the bacteria *Wolbachia* aim to kill the adult worms that rely on this endosymbiont. For example, doxycycline alone, or added to ivermectin, has been found to significantly reduce female worm viability and the number of microfilariae in the skin within 6 weeks of treatment. Short courses of antibiotics (rifampin and azithromycin) were not effective in reducing the number of worms, but these antibiotics may be a useful addition to other treatment regimens.

[1] Saint Andre AV, Blackwell NM, Hall LR, Hoerauf A, Brattig NW, Volkmann L, Taylor MJ, Ford L, Hise AG, Lass JH, Diaconu E, and Pearlman E. The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness *Science* (2002) 295: 1892-1895.

3. NONCLINICAL MICROBIOLOGY STUDIES

3.1. Mechanism of action

Moxidectin, like ivermectin, is a macrocyclic lactone. However, moxidectin and ivermectin are a member of different macrocyclic lactone family, milbemycin and avermectin, respectively. The absence of the hydrophilic C13 glycosyl side-chain contributes to the higher lipophilicity of the milbemycins.

Ivermectin is known to bind to glutamate-gated chloride ion channels (GluCl), gamma-aminobutyric acid (GABA) receptors and ATP-binding cassette (ABC) transporters of the nematodes and increase permeability leading to influx of chloride ions, hyperpolarization, muscle paralysis, interference with parasite pharyngeal pumping, and paralysis/death of the parasites (*for details see ivermectin labeling*). Some of the studies supporting mechanism of action of moxidectin and its similarities with ivermectin are summarized below.

3.1.1. Glutamate-gated chloride channel

The molecular interaction between ivermectin and GluCl channel proteins was characterized by X-ray crystallography using *Caenorhabditis elegans*, a gastro-intestinal nematode. No crystallographic studies of moxidectin interacting with GluCl channel proteins have been reported. However, *in silico* docking studies, suggest that the core macrocyclic lactone-protein interactions should be retained (Prichard *et al*., 2012[2]). Compared to ivermectin, moxidectin was reported to be a more potent agonist of GluCl of *Cooperia oncophora* (a gastro-intestinal nematode), expressed in *Xenopus laevis* oocytes (Njue *et al*., 2004[3]).

The GluCls are members of the cys-loop ligand-gated ion channel family found in nematodes and some arthropods, but not in vertebrates; however, GluCl are related to mammalian glycine receptors and are activated by L-glutamate but not aspartate, GABA, glycine, histamine or other amino acid or candidate neurotransmitter (Wolstenheim and Rodgers, 2005[4];Wolstenheim, 2012[5]). GluCls are known to have a wide range of functions in invertebrate nervous systems, that include the control and modulation of locomotion, the regulation of feeding, and the mediation of sensory inputs. It is thought that multiple forms of the GluCl channels, present in nematodes, may differ in their sensitivity to the drug.

GluCls are encoded by several genes (*avr-14, avr-15, glc-1, -2, -3, -4, -5 and -6* genes), which are highly conserved in nematodes (Wolstenheim and Rogers, 2005[4]; Li *et al*., 2014[6]). For *Brugia malayi* (a filarial nematode - causative agent for lymphatic filariasis), immunolocalization

[2] Prichard R, Menez C, and Lespine A. Moxidectin and the avermectins: Consanguinity but not identity. *Int J Parasitol: Drugs and Drug Resistance* (2012) 2: 134-153.

[3] Njue AI, Hayashi J, Kinne L, Feng X-P, and Prichard RK. Mutations in the extracellular domains of glutamate-gated chloride channel α3 and β subunits from ivermectin-resistant *Cooperia oncophora* affect agonist sensitivity. *J Neurochem* (2004) 89: 1137-1147.

[4] Wolstenheim AJ and Rogers AT. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitol* (2005) 131: S85-S95.

[5] Wolstenheim AJ. Glutamate-gated chloride channels. *J Biol Chemistry* (2012) 287 (48): 40232-40238.

[6] Li BW, Rush AC, and Weil GJ. High level expression of a glutamate-gated chloride channel gene in reproductive tissues of *Brugia malayi* may explain the sterilizing effect of ivermectin on filarial worms. *Int J Parasitol: Drugs and Drug Resistance* (2014) 4: 71-76.

and *in situ* hybridization studies showed the expression of the *Bma-avr-14* subunits was associated with the excretory/secretory pore of the microfilariae (Moreno *et al*., 2010[7]) and the reproductive tissues of adult male and female worms (Li *et al*., 2014[6]). In female worms, strong expression signals were detected in the ovary, developing embryos and lateral hypodermal chords, and moderate expression in the uterus wall adjacent to stretched microfilariae. In male adult worms, strong expression was observed in spermatogonia, the wall of the vas deferens and lateral chords, but not in mature spermatozoa. In addition, *avr-14* was highly expressed in somatic muscles adjacent to the terminal end of the vas deferens which contains mature sperms (Table 1). Overall, the study suggests that *avr-14* is highly expressed in *B. malayi* developing embryos and reproductive tissues suggesting the involvement of GluCl in gamete production and embryogenesis in filarial worms.

Table 1: *BmAVR-14* expression patterns by *in situ* hybridization.[a]

subunit	Female reproductive system								lateral chord	Male reproductive system				
	Uteral-epithelium	Oocytes early	Oocytes later	Morulae early	Morulae later	Pretzel early	Pretzel later	Stretched MF		Spermatogonia	Spermatocytes	Spermatids	Spermatozoa	Vas deferens
BmAVR-14A	3	3	3	2	2	1	1	1	1	2	1	0	0	2
BmAVR-14B	3	3	3	2	2	1	2	1	2	3	1	0	0	2

[a] Signal intensity was scored as follows: 1, weak; 2, moderate; 3, strong.

3.1.2. Gamma aminobutyric acid-gated receptors

Moxidectin, like ivermectin, acts on the nematode GABA receptor complex. GABA receptor subunits, expressed in the ventral nerve cord in nematodes, are known to be important for somatic muscles function. It is thought that the action of macrocyclic lactones with GABA receptor leads to hyperpolarization of the GABA-gated channels and somatic muscle paralysis. Based on studies by Li *et al* (2014)[6] and Moreno *et al* (2010)[7] summarized above (see section 3.1.1), Wolstenholme *et al* (2016)[8] proposed that the lack of expression of GluCl genes in the motor nervous system of *B. malayi* suggests that worm paralysis in the presence of macrocyclic lactones may be due to other targets, such as neuromuscular GABA-gated channels.

3.1.3. ABC-transporters

Ivermectin is known to interact with efflux ABC transporters, such as P-glycoprotein (PgP) in vertebrate hosts as well as nematode and arthropod parasites leading to increased drug efflux. *In vitro* studies show that moxidectin has a lower affinity for mammalian PgP compared to ivermectin (Lespine *et al*., 2007[9]). Also, moxidectin, unlike ivermectin, is a poor substrate for PgP (Study Report no. RPT-71499; for details see Clinical Pharmacology review). However, moxidectin is a substrate for another transporter protein, breast cancer resistance protein (BCRP) (Perez *et al*., 2009[10]).

[7] Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, and Geary TG. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of Brugia malayi. *Proc Natl Acad Sci USA* (2010) 107: 20120–20125.

[8] Wolstenholme AJ, Maclean MJ, Coates R, and McCoy CJ, and Reaves BJ. How do the macrocyclic lactones kill filarial nematode larvae? *Invert Neurosci* (2016) 16 (3): 7.

[9] Lespine A, Martin S, Dupuy J, Roulet A, Pineau T, Orlowski S, and Alvinerie M. Interaction of macrocyclic lactones with P-glycoprotein: structure affinity relationship. *Eur J Pharm Sci* (2007) 30: 84–94.

[10] Perez M, Blazquez AG, Real R, Mendoza G, Prieto JG, Merino G, Alvarez AI. In vitro and in vivo interaction of moxidectin with BCRP/ABCG2. *Chem Biol Interact* (2009**,**)*180* (1): 106-112.

3.1.4. Immunomodulatory effects

Nematodes, including the filaria parasites, are known to secrete immunomodulatory proteins, metabolites and microRNAs which may alter the immune system and help the parasites to evade immune response. Treatment with ivermectin has been shown to reduce secretory proteins (Moreno *et al*., 2010[7]). Studies show increased binding of granulocytes and mononuclear cells and antibody-dependent adherence, in the presence of ivermectin, to the microfilariae of *Acanthocheilonema viteae* (previously known as *Dipetalonema viteae*), *Litomosoides sigmodontis* (previously known as *L. carinii*) and *Dirofilaria immitis* (Rao *et al*. 1987[11]; Zahner *et al*. 1997[12]; Vatta *et al*. 2014[13]). Njoo *et al* (1993[14]; 1994[15]) reported that ivermectin treatment caused neutrophil activation and increased C-reactive protein as well as IL-6 levels in onchocerciasis patients. The immunomodulatory properties could also occur due to the presence of *Wolbachia* associated with the filaria parasites (Gillette-Ferguson *et al*., 2004[16]). Overall, the studies suggest the immunomodulatory properties of ivermectin could contribute to the mode of action as well as adverse effects of the treatment. The immunomodulatory activity of moxidectin has not been tested.

Comments
The mechanism by which moxidectin exhibits its effect against O. volvulus has not been studied. However, studies with other nematodes including B. malayi, a filaria parasite, suggest that moxidectin, like ivermectin, may exhibit it effect by binding to GluCl, GABA receptors and/or ABC transporters leading to an increase in permeability, hyperpolarization, influx of chloride ions, muscle paralysis, reduction in motility and excretion of immunomodulatory proteins as well as the fertility of both male and female adult worms (Figure 2). The role of immunomodulatory effects of moxidectin either directly or indirectly by inhibiting secretion of proteins or enhancing antibody- or cell- mediating adherence, in conferring protection cannot be ruled out.

[11] Rao UR, Chandrashekar R, and Subrahmanyam D. Effect of ivermectin on serum dependent cellular interactions to *Dipetalonema viteae* microfilariae. *Trop Med Parasitol* (1987) 38: 123–127.

[12] Zahner H, Schmidtchen D, and Mutasa JA. Ivermectin-induced killing of microfilariae in vitro by neutrophils mediated by NO. *Exp Parasitol* (1997) 86: 110–117.

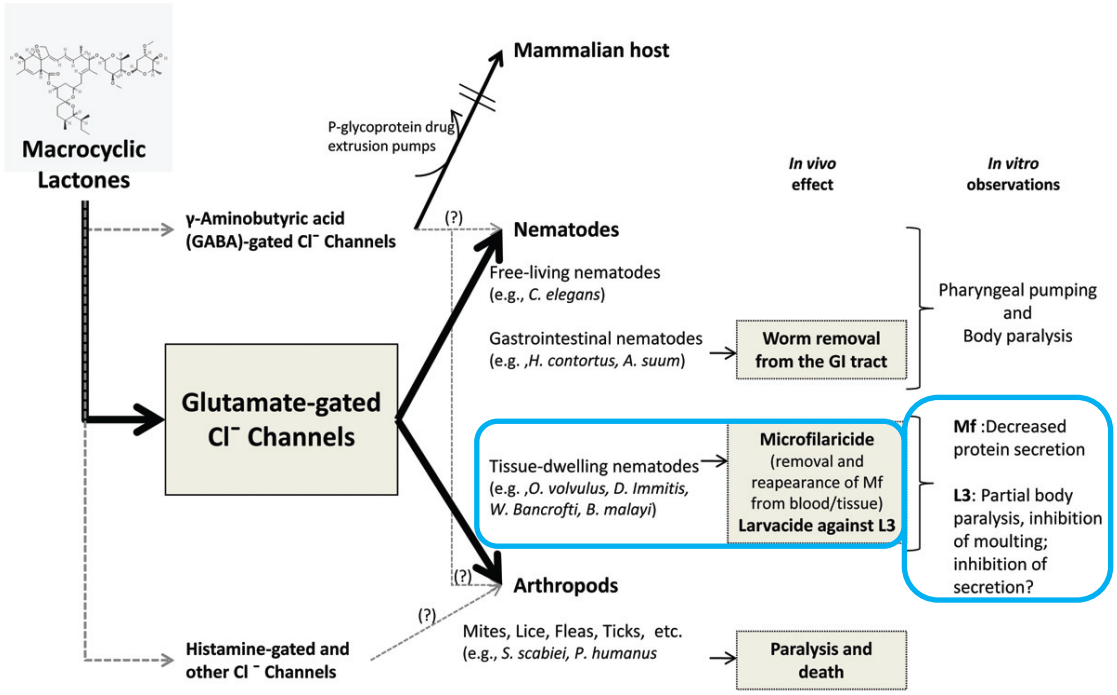
[13] Vatta AF, Dzimianski M, Storey BE, Camus MS, Moorhead AR, Kaplan RM, and Wolstenholme AJ. Ivermectin-dependent attachment of neutrophils and peripheral blood mononuclear cells to *Dirofilaria immitis* microfilariae in vitro. *Vet Parasitol* (2014) 206: 38–42.

[14] Njoo FL, Hack CE, Oosting J, Stilma JS, and Kijlstra A. Neutrophil activation in ivermectin-treated onchocerciasis patients. *Clin Exp Immunol* (1993) 94 (2): 330-333.

[15] Njoo FL, Hack CE, Oosting J, Stilma JS, and Kijlstra A. C-reactive protein and interleukin-6 are elevated in onchocerciasis patients after ivermectin treatment. *J Inf Dis* (1994) 170 (3): 663-668.

[16] Gillette-Ferguson I, Hise AG, McGarry HF, Turner J, Esposito A, Sun Y, Diaconu E, Taylor MJ, and Pearlman E. *Wolbachia*-induced neutrophil activation in a mouse model of ocular onchocerciasis (River Blindness). *Inf Immun* (2004) 72 (10): 5687-5692.

Figure 2: Summary of the state-of-knowledge about macrocyclic lactone pharmacology in target organisms and hosts.



Source: Geary TG and Moreno Y (2012)[17]

3.2. Activity *in vitro*

The activity of moxidectin was reported against the microfilariae of two of the *Onchocerca* species as well as the microfilariae and/or adult worms of another filarial species, *B. malayi*.

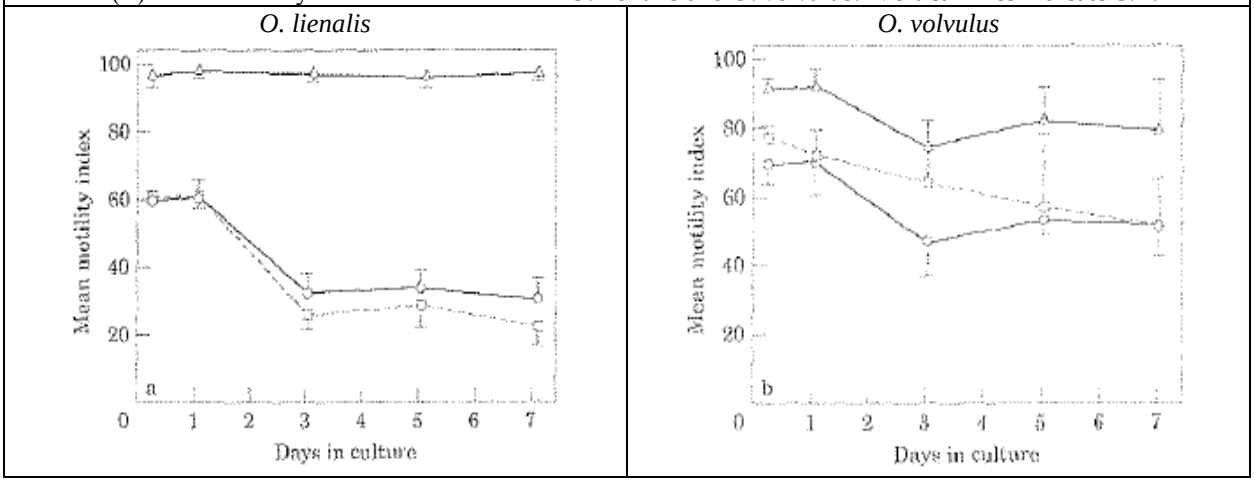
3.2.1. Onchocerca species – microfilariae

The *in vitro* activity of moxidectin was reported against the microfilariae of *O. volvulus* and *O. lienalis* (Tagboto and Towson, 1996[18]). Briefly, the parasites were cultured in medium containing LLCMK2 monkey kidney cells (feeder layer), with and without drug, and the motility of the microfilariae followed for 7 days; medium was changed every 2 to 3 days. Over 96% of the *O. lienalis* and 79% of *O. volvulus* microfilariae were viable in drug free medium over the 7-day period of observation. On Day 7, at a concentration of 0.1 µM, both moxidectin (0.06 µg/mL) and ivermectin were effective in decreasing the motility index of *O. lienalis* and *O. volvulus* microfilariae to 22% and 50%, respectively (Figure 3). *O. lienalis* appears to be more sensitive to both moxidectin and ivermectin than *O. volvulus.*

[17] Geary TG and Moreno Y Macrocyclic lactone anthelmintics: Spectrum of activity and mechanism of action (2012) *Curr Pharma Biotech* (2012) 13: 866-872.

[18] Tagboto SK and Townson S. *Onchocerca volvulus* and *O. lienalis*: the microfilaricidal activity of moxidectin compared with that of ivermectin *in vitro* and *in vivo*. *Ann Trop Med Parasitol* (1996) 90 (5): 497-505.

Figure 3: Effect of incubation in medium with 10^{-7} M moxidectin (□) or ivermection (○) or in drug free medium (Δ) on the motility of skin microfilariae of *O. lienalis* and *O. volvulus*. Vertical lines indicate S.E.



3.2.2. *Brugia malayi* - microfilariae and adult worms

The activity of moxidectin against the microfilariae and/or adult worms of *B. malayi* was reported in three studies (Stitt *et al*., 2011[19]; Tomkins *et al*., 2010[20]; Verma *et al*., 2014[21]). The experimental design and the results varied among the studies (for details see Appendix-1). Overall, the studies suggest that moxidectin is effective in decreasing the motility of microfilariae as well as male and female adult worms; also, release of microfilariae from the adult worms, was reduced *in vitro*. In one study (Stitt *et al*., 2011[19]), male and female adult worms were paralyzed in the presence of moxidectin by Days 10 and 8, respectively; however, moxidectin was not effective in paralyzing the microfilariae although the motility was reduced.

The moxidectin 50% inhibitory concentrations (IC_{50}) were reported in one study (Verma *et al*., 2014[21]). The moxidectin IC_{50} against male and female worms (121.6 µg/mL and 153.6 µg/mL, respectively) were lower than for microfilariae (518.3 µg/mL) suggesting lower sensitivity of microfilariae to moxidectin compared to adult worms under the experimental conditions tested.

3.2.3. *Brugia malayi - Wolbachia*

Tomkins *et al*., 2010[20] reported the effect of moxidectin on the expression of *Wolbachia* (*wsp*) gene in adult worms and microfilaria of *B. malayi* that survived the motility experiment. The results show that moxidectin decreased the *wsp* expression in all stages of the parasite compared to the parasites in drug free medium. It is unclear if this is due to direct activity of the drug against bacteria or separation of the bacteria from the dead worms.

Comments

The methods to measure in vitro sensitivity of microfilaria and different stages of the Onchocerca and other filaria species varied. In each of the study, testing was based on motility

[19] Stitt LE, Tompkins JB, Dooley LA, and Ardelli BF. ABC transporters influence sensitivity of *Brugia malayi* to moxidectin and have potential roles in drug resistance. *Exptal Parasitol* (2011) 129: 137-144.

[20] Tomkins JB, Stitt LE, and Ardelli BF. *Brugia malayi*: *In vitro* effects of ivermectin and moxidectin on adults and microfilariae. *Exptal Parasitol* (2010) 124: 394-402.

[21] Verma M, Pathak M, Shahab M, Singh K, Mitra K, and Misra-Bhattacharya S. Moxidectin causes adult worm mortality of human lymphatic filarial parasite *Brugia malayi* in rodent models. *Folia Parasitolgica* (2014) 61 (6) 561-570.

of a single strain/isolate of the parasite. Microfilariae of O. lienalis appear to be more sensitive to both moxidectin and ivermectin than O. volvulus under the experimental conditions tested.

Studies suggest that moxidectin is effective in reducing the motility of the adult worms and the microfilariae of B. malayi. One study (Stitt et al., 2011[19]) reported that the motility of B. malayi microfilariae in the presence of moxidectin was significantly lower than that of the non-treated controls. None of the drug concentrations tested paralyzed or killed microfilariae; however, male and female adult worms were paralyzed under the experimental conditions tested. Adult worms appear to be more sensitive to moxidectin than microfilariae.

The mammalian cells such as the Vero monkey kidney cells are 28- to 94-fold less sensitive to moxidectin compared to microfilariae and adult worms of B. malayi.

3.3. Activity *in vivo (animal models)*

The *in vivo* activity of moxidectin was reported against *Onchocerca* species as well as other filarial species that include *B. malayi*, *Brugia pahangi*, *L. sigmodontis*, *Monanema martini*, *D. immitis*, and *Dirofilaria repens*.

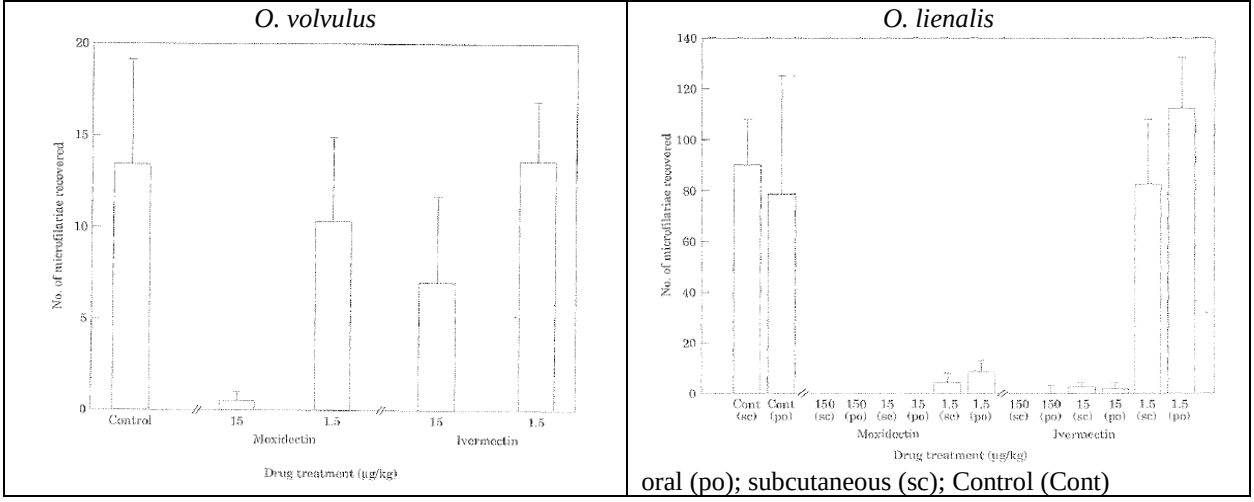
3.3.1. *Onchocerca* species

The activity of moxidectin was measured against the *Onchocerca* species in experimentally infected rodents (mice and jirds) and naturally infected cattle and horses.

3.3.1.1. Experimentally infected rodents

Tagboto and Towson, 1996[18] reported the activity of moxidectin in CBA/Ca mice infected subcutaneously (S/C) with 5000 **microfilariae** of *O. volvulus* or *O. lienalis.* Treatment with different doses of moxidectin or ivermectin was initiated on Day 3 post-infection, by either oral or S/C route. Drugs were administered either as a single dose on Day 3 or 5 daily doses. On Day 18 post-infection, mice were necropsied and ears processed for measuring microfilariae density. The results show that a single dose of moxidectin and ivermectin, administered orally or S/C, was effective in reducing *O. volvulus* or *O. lienalis* microfilariae; such an effect was dose-dependent (Figure 4).

Figure 4: Mean recoveries of skin microfilariae from mice treated with moxidectin, ivermectin, or drug-free diluent (Control) as a single dose on Day 3 post-infection. Vertical lines indicate SD.



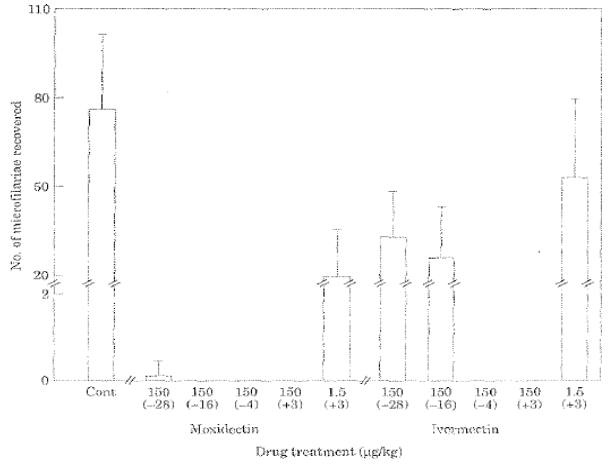
oral (po); subcutaneous (sc); Control (Cont)

At a low dose of 1.5 µg/kg, moxidectin was more effective than ivermectin; no microfilariae were observed at doses of ≥15 µg/kg of either of the drugs in *O. lienalis* infected mice. In *O. volvulus* infected mice, few residual microfilariae (~2-3 in moxidectin and ~15 in ivermectin groups) were reported at a dose of 15 µg/kg; higher doses were not tested. Microfilariae of *O. lienalis* appear to be more sensitive to both drugs than *O. volvulus.*

In mice treated for 5 days, moxidectin was more effective than ivermectin, between the doses of 0.2 and 3.2 µg/kg, in reducing *O. lienalis* or *O. volvulus* microfilariae density.

In another experiment, treatment was administered on either Day -28, -16, -4, or 3 post-infection in *O. lienalis* infected mice. The results show that moxidectin at a dose of 150 µg/kg, administered on Day 28 or 16 prior to infection, was more effective than ivermectin in clearing microfilariae. However, the activity of both moxidectin and ivermectin was similar when administered on Day -4 or +3 (Figure 5). Similar experiment was not done in *O. volvulus* infected mice.

Figure 5: Mean recoveries of *O. linealis* microfilariae from mice treated with moxidectin or ivermectin.



Cont: Drug-free diluent

Moxidectin, ivermectin or diluent administered as a single subcutaneous dose on 28, 16, or 4 days before (-28, -16 and -4, respectively) or 3 days after (+3) infection.

Vertical lines indicate SD

3.3.1.2. Naturally infected calves and horses

In calves naturally infected with *O. ochengi*, moxidectin administered either monthly or every 3 months for 22 months was effective in clearing microfilariae and suppressing the development of nodules in cattle; 24 months after treatment discontinuation, the prevalence of nodules and microfilariae were lower in moxidectin treated cattle compared to those treated with ivermectin (Njongmeta *et al*., 2004[22]).

In another study (Langworthy *et al*., 2000[23]), moxidectin administered as a single dose or once a month for 7 months, was effective in eliminating microfilariae from the skin and reducing embryogenesis; however, there was no effect on nodule diameter as well as the adult worm

[22] Njongmeta LM, Nfon CK, Gilbert J, Makepeace BL, Tanya VN, and Trees AJ. Cattle protected from onchocerciasis by ivermectin are highly susceptible to infection after drug withdrawal. *Int J Parasitol* (2004) 34: 1069-1074.

[23] Langworthy NG, Renz A, Mackenstedt U, Henkle-Duhrsen K, de C Bronsvoort MB, Tanya VN, Donnelly MJ, and Trees AJ. Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. *Proc Roy Soc London B* (2000) 267: 1063-1069.

number, motility or viability up to 12 months post-treatment (for details see Table 2 and Appendix-2).

In horses naturally infected with *O. cervicalis*, no microfilariae were detected in skin snips on Day 14 after treatment with a single dose of moxidectin (Monahan *et al*., 1995[24]). Similar observations were reported in majority of the horses in another study (Mancebo *et al*., 1997[25]); however, the duration of treatment or follow-up was not specified.

3.3.2. Filaria species other than *Onchocerca species*

3.3.2.1. Experimentally infected rodents and dogs

Several studies reported the activity of moxidectin in rodents and dogs infected with different filaria species that include *B. malayi* (Verma *et al.*, 2014[21]), *B. pahangi* (McCall, 1999[26]; McCall, 2001[27]), *L. sigmodontis* (Breton *et al* 1997[28]), *M. martini* (Breton *et al* 1997[28]), and *D. repens* (Genchi *et al*., 2010[29]). Animals were infected with either adult worms or the infective larvae (L_3) and treatment with moxidectin was initiated when the animals became microfilariae positive (for details see Table 2 and Appendix-2). Most of the studies show that moxidectin was effective in inhibiting or reducing microfilariae as well as the number, motility, and viability of adult worms; embryogenesis was reduced. Male worms appear to be less sensitive than female worms to moxidectin treatment.

3.3.2.2. Naturally infected dogs

In an epidemiological study, moxidectin was reported to prevent heart worm infection in dogs naturally infected with *D. immitis* (Glickman *et al*., 2006[30]).

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[24] Monahan CM, Chapman MR, French DD, and Klei TR. Efficacy of moxidectin oral gel against *Onchocerca cervicalis* microfilariae. *J Parasitol* (1995) 8: 117-118.

[25] Mancebo OA, Verdi JH, and Bulman GM. Comparative efficacy of moxidectin 2% equine oral gel and ivermectin 2% equine oral paste against *Onchocerca cervicalis* (Railliet and Henry, 1910) microfilariae in horses with naturally acquired infections in Formosa (Argentina). *Vet Parasitol* (1997) 73: 243-248.

[26] Study Report TDR-980389. Professor John W McCall, Georgia, USA. Experimental chemotherapy of filariasis and screening of filaricides. December 2, 1999.

[27] Study Report TDR-980386. Professor John W McCall, Georgia, USA. Comparison of the antifilarial activity of moxidectin with ivermectin in dogs with induced lymphatic infections of *Brugia pahangi*. October 5, 2001.

[28] Breton B, Diagne M, Wanji S, Bougnoux ME, Chandre F, Marechal P, Petit G, Vuong PN, and Bain O. Ivermectin and moxidectin in two filarial systems: resistance of *Monanema martini;* inhibition of *Litomosoides sigmodontis* insemination. *Parasitologia* (1997) 39:19-28.

[29] Genchi M, Pengo G, and Genchi C. Efficacy of moxidectin microsphere sustained release formulation for the prevention of subcutaneous filarial (*Dirofilaria repens*) infection in dogs. *Vet Parasitol* (2010) 170: 167-169.

[30] Glickman LT, Glickman NW, Moore GE, Lok JB, McKall JW, and Lewis HB. Comparative effectiveness of sustained-release moxidectin (ProHeart 6) and ivermectin (Heartgard Plus) for the prevention of heartworm infection in dogs in the United States. *Int J Appl Res Vet Med* (2006) 4 (4): 339-354.

Table 2: Summary of studies supporting activity of moxidectin against different stages of filarial parasites *in vivo*

Filaria species (Reference)	**Parasite stage for initiation of infection (Host)**	**Comments**
Experimentally infected rodents		
O. volvulus and *O. lienalis* (Tagboto and Towson, 1996[18])	**Mf** (CBA/Ca mice); treatment initiated on Day 3	Mf in ear reduced; Mf of *O. lienalis* appear to be more sensitive to both drugs than *O. volvulus.*
B. malayi (Verma *et al.*, 2014[21])	**AW** (Jirds); treatment initiated when animals were mf^{+ve}	AW: reduction in the number, motility and viability. Degenerative changes in about 60-70% female AW that include deformed and degenerated eggs and embryos in the uteri. Mf in peritoneal cavity: reduction in density and motility.
B. malayi *(Verma et al., 2014[21])*	**L_3** (*Mastomys* - mice); treatment initiated when animals were mf^{+ve}	AW: reduction in number and embryogenesis. Mf in blood: reduced.
B. pahangi (McCall, 1999[26])	**L_3** (Jirds); treatment initiated when animals were mf^{+ve}	AW and mf in blood reduced after treatment every 4 weeks for up to 12 weeks.
L. sigmodontis (Breton *et al* 1997[28])	**L_3** (Jirds); treatment initiated when animals were mf^{+ve}	AW: Reduction in number and abnormal embryogenesis; no effect on male AW. Mf in blood: all animals became mf^{-ve} on Day 2 post-treatment that was followed by an increase; however, mf density was lower than untreated controls.
M. martini (Breton *et al* 1997[28])	**L_3** (Mice); treatment initiated when animals were mf^{+ve}	AW: reduction in number, abnormal embryogenesis. Male AW normal. Mf in blood: no effect.
Experimentally infected dogs		
B. pahangi (McCall, 2001[27])	**L_3** (Beagle dogs); treatment initiated when animals were mf^{+ve}	AW: reduction in live worms. Mf in blood: reduction in density within 24 h of treatment and continued to decrease.
D. repens (Genchi *et al*., 2010[29])	**L_3** (Dogs): animals infected 180 days after treatment with a single dose (Prophylaxis study)	AW: no worms in tissues. It appears that the presence of mf was not examined.
Naturally infected animals		
D. immitis (Glickman *et al*., 2006[30])	Dogs	Post-marketing epidemiology study based on million electronic medical records for dogs visiting >500 Banfield veterinary hospitals. Prevention of heart worm infection
O. ochengi (Njongmeta *et al*., 2004[22])	Cattle	Mf in skin: No mf detected. Nodules: suppressed.
O. ochengi (Langworthy *et al*., 2000[23])	Cattle	Mf in skin: No mf detected. Nodule: No effect on nodule diameter, worm number, worm motility, and viability. Reduction in embryogenesis.
O. cervicalis (Monahan *et al*., 1995[24])	Horses	Mf in skin: all horses were mf^{-ve}.
O. cervicalis (Mancebo *et al*., 1997[25])	Horses	Mf in skin: 90% of the ponies were mf^{-ve}; mf density reduced in the reaming 10%.
MF=microfilariae; AW=adult worms; L_3= infective larvae		

Comments

Overall, the studies in experimentally infected rodents suggest that moxidectin treatment is active against the microfilariae of Onchocerca species; moxidectin was more effective than ivermectin. In one study, O. lienalis microfilariae were reported to be more sensitive to both

drugs than O. volvulus thereby suggesting some difference in sensitivity of microfilariae to drugs among the Onchocerca species. Similar observations were made in vitro.

No studies were available supporting the activity of moxidectin against the adult worms or the L_3 of the Onchocerca species in experimentally infected rodents. However, studies in naturally infected calves and horses suggest that moxidectin is active against microfilariae and inhibits/reduces embryogenesis. Similar observations were reported against other filarial species in rodents and dogs infected with the L_3 or adult worms of different filaria species.

3.4. Drug Resistance

None of the studies to evaluate a potential for development of resistance or the mechanism of resistance were performed using *Onchocerca* species. Most of the studies, were performed using nematode parasites other than the filaria parasites; few studies were performed with the filarial parasites, *B. malayi* and *D. immitis*.

3.4.1. Potential for development of drug resistance

3.4.1.1. *In vitro*

A potential for develop of resistance to moxidectin *in vitro* has not been measured.

3.4.1.2. *In vivo*

A potential for development of resistance in animals infected with *Onchocerca* species has not been measured. However, studies with other nematodes such as a gastro-intestinal nematode, *Haemonchus contortus*, show a potential for development of resistance. For example, the 95% effective doses (ED_{95}) of moxidectin and ivermectin were shown to be increased by 5.3 and 9.7-fold, respectively, in lambs infected with the infective larvae of the strain selected after serial passages, compared to the parental strain (Wang *et al*., 1995[31]). Similar observations were made in sheep infected with *H. contortus* strain selected after 22 passages (Ranjan *et al*., 2002[32]) and in naturally infected lambs (Lloberas *et al*., 2013[33]).

No data for prevalence of resistance to moxidectin in subjects with onchocerciasis is available. The methods to assess resistance in subjects with filarial infections are not available. Based on modeling, Churcher *et al* (2009)[34] proposed that resistance be based on variability in the rate at which *O. volvulus* microfilariae re-populate host's skin following ivermectin treatment. The

[31] Wang GT, Berger H, Simkins K, and Rock D. The rate of resistance development by *H. contortus* to endectocides. *Proceedings of the XV International Conference of the World Association for the Advancement or Veterinary Parasitology. Yokohama. Japan*. 1995: 104. The publication not available. Summarized by Xu *et al*., 1998 (*Mol Biochem Parasitol* 91: 327-335[40]).

[32] Ranjan S, Wang GT, Hirschlein C, and Simkins KL. Selection for resistance to macrocyclic lactones by *Haemonchus contortus* in sheep. *Vet Parasitol* (2002) 103: 109-117.

[33] Lloberas M, Alvarez L, Entrocasso C, Virkel G, Ballent M, Mate L, Lanusse C, and Lifschitz A. Comparative tissue pharmacokinetics and efficacy of moxidectin, abamectin and ivermectin in lambs infected with resistant nematodes: Impact of drug treatments on parasite P-glycoprotein expression. *Int J Parasitol: Drugs and Drug Resistance* (2013) 3: 20-27.

[34] Churcher TS, Pion SDS, Osei-Atweneboana MY, Prichard RK, Awadzi K, Boussinesq M, Collins RC, Whitworth JA, and Basanez M-G. Identifying sub-optimal responses to ivermectin in the treatment of River Blindness. *Proc Nat Acad Sci* (2009) 106: 16716-16721.

model estimates a single skin rebound/re-population rate for every host sampled, allowing reports of sub-optimal responses to be statistically compared with responses from populations with no prior exposure to ivermectin. Statistically faster rates of skin rebound were observed in 3 Ghanaian villages (treated 12–17 times), despite the wide variability in rebound rates observed in ivermectin-naïve populations. Another village previously thought to have high rates of skin rebound was shown to be indistinguishable from the normal treatment response. The model was used to generate testable hypotheses to identify whether atypical rates of skin rebound by microfilariae could result from low treatment coverage alone or provide evidence of decreased ivermectin efficacy. Limitations of the skin-snipping method for estimating parasite load indicates that changes in the distribution of microfilarial rebound rates, rather than their absolute values, may be a more sensitive indicator of emerging ivermectin resistance.

A study by Pion *et al* (2013[35]) in ivermectin-naïve and a frequently treated population from Cameroon reported that the rebound rate post-treatment was higher, between 15 and 80 days, in the frequently treated group than the ivermectin-naïve group; however, the rate was the same at Day 180. Also, the number of degenerating microfilariae were lower in the frequently treated group than the ivermectin naive group. Oocyte production and the number of intermediate embryos (morulae and coiled microfilariae) was similar in the two groups (Nana-Djeunga *et al*., 2014[36]). Overall, the studies suggest a potential for development of resistance to ivermectin. Similar studies with moxidectin have not been conducted.

Comments
Studies in animals infected with H. contortus suggest a potential for development of resistant to moxidectin. No data for prevalence of resistance to moxidectin in subjects with onchocerciasis is available.

The methods to assess resistance in subjects with filarial infections are not available. Efforts have been made to assess resistance by measuring microfilarial density, the rate of decline in microfilarial density as well as the rate at which relapse i.e., rebound of microfilariae, occurs. For example, a study conducted in Cameroon, suggests a possibility of development of resistance to ivermectin; the rebound rate post-treatment was higher early on (2 to 8 weeks) in frequently treated population compared to the ivermectin-naïve group; however, the rate was the same at Month 6. Also, the number of degenerating microfilariae were lower in the frequently treated group than the ivermectin naive group. Molecular testing to identify the genotype and correlating the findings with poor responses to treatment should be useful in evaluating resistance.

Moxidectin has a long half-life compared to ivermectin; therefore, it is likely that fewer treatment selection pressure will be applied and this may lead to a lower potential for development of resistance.

[35] Pion SDS, Nana-Djeunga HC, Kamgno J, Tendongfor N, Wanji S, Njiokou F, Prichard RK, and Boussinesq M. Dynamics of *Onchocerca volvulus* microfilarial densities after ivermectin treatment in an ivermectin-naïve and a multiply treated population from Cameroon. *PLoS Neglected Tropical Diseases* (2013) 7 (2): e2084.

[36]Nana-Djeunga HC, Bourguinat C, Pion SD, Bopda J, Kengne-Ouafo JA, Njiokou F, Prichard RK, Wanji S, Kamgno J, and Boussinesq MB. Reproductive status of Onchocerca volvulus after ivermectin treatment in an ivermectin-naïve and a frequently treated population from Cameroon. *PLoS Neglected Tropical Diseases* (2014) 8 (4): e2824.

3.4.2. Mechanism of drug resistance

3.4.2.1. Role of glutamate-gated ion channels and GABA receptors

Mutations in the GluClα3 subunit of GluCl channels of *C. oncophora* (a gastro-intestinal nematode) expressed in *Xenopus laevis* oocytes, were associated with 3-fold decrease in sensitivity to ivermectin and moxidectin (Njue *et al*., 2004[3]). In *C. elegans*, mutations in multiple GluCl units were reported to be associated with ivermectin resistance (McCavera *et al*., 2007[37]). Blackhall *et al* (2003)[38] reported genetic variations, measured by allele frequencies, of the GABA receptor in the *H. contortus* strains obtained after 17 passages in sheep that were treated with moxidectin or ivermectin compared to the parenteral strain passaged in sheep but not treated with any drug.

3.4.2.2. Role of ABC transporters (efflux pumps)

In *O. volvulus*, several PgP and half-transporters are reported (reviewed by Lespine *et al*., 2012[39]). However, the mechanism of resistance to any of the macrocyclic lactones has not been evaluated in *O. volvulus*. Studies with other parasites such as *B. malayi* suggest a role of PgP in conferring resistance. Stitt *et al* (2011)[19] reported the effect of moxidectin on *B. malayi* adult worms and microfilariae in the presence and absence of inhibitors of drug efflux. Briefly, the gene expression profiles of ABC systems following treatment was performed by real-time reverse transcriptase (RT)-PCR. On Days 6 and 12 of culture of adult worms (n = 5/culture, per time point), the anterior and posterior ends of the worms were removed (to ensure no contamination by microfilariae or sperm) and used for RNA extraction and transcriptional analysis. For microfilariae, the transcriptional profiles of ABC systems were assessed on Days 15 and 30 using 10 pooled samples of 1000 microfilaria. For each ABC system, the real time RT-PCR experiment was performed in triplicate for each of the male and female adult worms and microfilariae using the same amount of cDNA from each life cycle stage. The results show an increased expression of ABC transporters in subfamilies A, B, C, and G following incubation of adult worms and microfilariae with moxidectin. Incubation of moxidectin with inhibitors of ABC transporter function did not enhance sensitivity to moxidectin in male worms; however, sensitivity was significantly enhanced in female worms and microfilariae.

A bioassay was performed to determine effects of inhibitors of drug efflux and drug resistance on *B. malayi*; motility was used as the indicator of susceptibility and was assessed over 12 days for adults and 7 days for microfilariae. The ability of multi-drug resistance (MDR) transporters to confer resistance can be reduced or reversed by substrates that are competitive inhibitors (e.g., verapamil) or that block their function directly; treatment of MDR or sensitive organisms with inhibitors restores or enhances drug sensitivity. *B. malayi* adult worms and microfilariae were exposed to inhibitors that are known to interfere with transporter function and the capacity of these inhibitors to potentiate the effects of moxidectin was measured. Adult worms were incubated with verapamil, cyclosporine A, vinblastine, and daunorubicin while microfilariae

[37] McCavera S, Walsh TK, and Wolstenholme AJ. Nematode ligand-gated chloride channels: an appraisal of their involvement in macrocyclic lactone resistance and prospects for developing molecular markers. *Parasitol* (2007) 134: 1111-1121.

[38] Blackhall WJ, Prichard RK, and Beech RN. Selection at a gammaaminobutyric acid receptor gene in *Haemonchus contortus* resistant to avermectins/milbemycins. *Mol Biochem Parasitol* (2003) 131:137–145.

[39] Lespine A, Menez C, Bourguinat C, and Prichard RK. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: Prospects for reversing transport-dependent anthelmintic resistance. *Int J Parasitol: Drugs and Drug Resistance* (2012) 2: 58-75.

were incubated with verapamil, quinidine, quinine, vincristine, vinblastine, colchicine, actinomycin D, daunorubicin, doxorubicin, etoposide, rhodamine, and forskolin. For each experiment with adult worms and microfilariae, samples were divided into four groups which consisted of a drug-free control (with no drugs but DMSO), a moxidectin control (at 5 µg/mL), a group with the inhibitor (at 5 µg/mL) and a group with moxidectin and the inhibitor (both at 5 µg/mL). The results show that the motility of male worms was similar when incubated with either moxidectin alone or in combination with verapamil or daunorubicin (Figures 6A1 and 6B1). Incubation of females worms with verapamil or daunorubicin in combination with moxidectin decreased the motility compared to moxidectin alone (Figure 6A2 and 6B2). For both males and females, motility of worms incubated with vinblastine + moxidectin was not significantly different from that of worms incubated with moxidectin alone.

The motility of microfilariae incubated with moxidectin in combination with verapamil, vinblastine, colchicine, etoposide and forskolin was significantly lower than that of microfilariae incubated with moxidectin or inhibitor alone (Figure 6B). Quinidine, quinine, vincristine, actinomycin D, daunorubicin, doxorubicin, and rhodamine had greater effects on motility in comparison to moxidectin alone or in combination with the inhibitor.

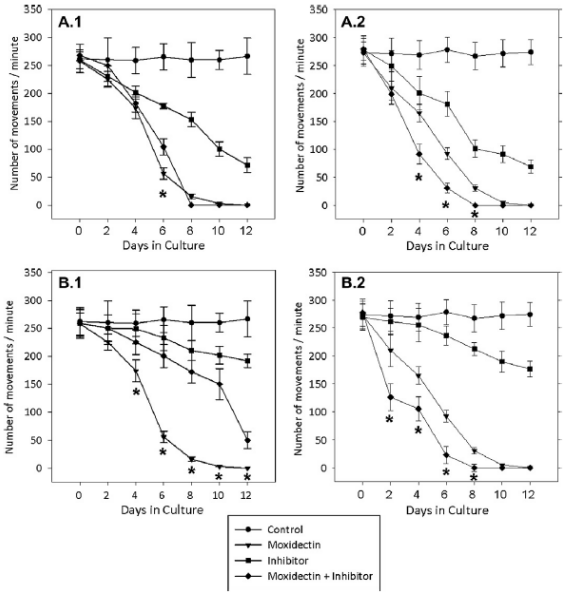
The study suggests that ABC transporters influence sensitivity to moxidectin and have a potential role in drug resistance.

Similar observations were reported by Xu *et al*., 1998[40] in immunocompromised jirds infected with L_3 of *H. contortus*; the strain selected for the study was obtained under moxidectin drug pressure after 14 passages. Treatment with moxidectin or ivermectin, with or without verapamil (MDR reversing agent), was initiated 10 days post-infection. The animals were necropsied on Day 13 to determine worm count. The results show an improvement in activity when moxidectin or ivermectin were administered in combination with verapamil (Table 3). The results also suggest a possibility of cross-resistance between moxidectin and ivermectin.

[40] Xu M, Molento M, Blackhall W, Ribeiro P, Beech R, and Prichard R. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Mol Biochem Parasitol* (1998) 91: 327-325.

Figure 6: Motility in *B. malayi* adult worms and microfilariae following co-administration of moxidectin with inhibitors of drug efflux.

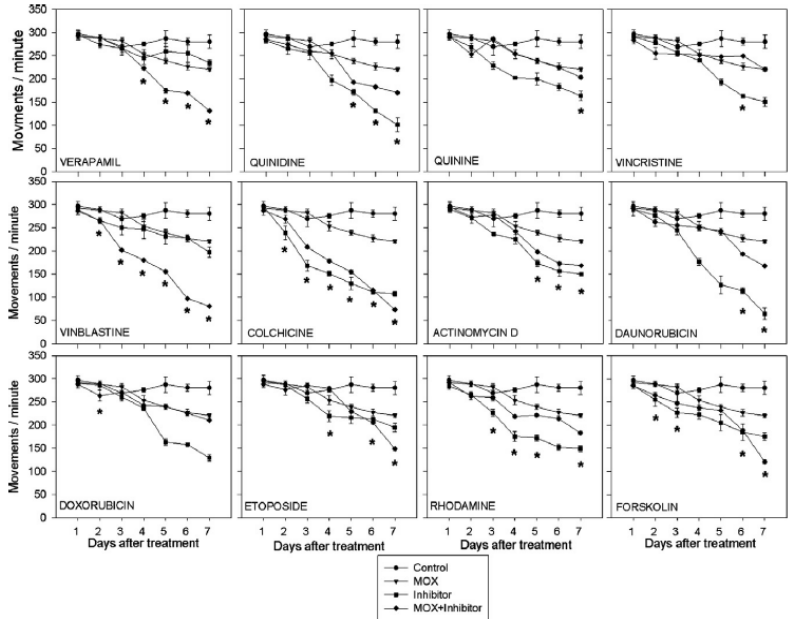
A: Adult worms



Males (A1 and B1) and females (A2 and B2) following co-administration of moxidectin with verapamil (A) or daunorubicin (B).

The y-axis indicates the number of movements/ minute and the x-axis indicates the days after treatment. An asterisk (*) indicates a significant difference between adults administered moxidectin alone in comparison to those co-administered moxidectin with an inhibitor.

B: Microfilariae



The y-axis indicates the number of movements/ minute and the x-axis indicates the days after treatment. An asterisk (*) indicates a significant difference between microfilariae administered moxidectin alone in comparison to those co-administered moxidectin with an inhibitor

Table 3: Effect of moxidectin and ivermectin treatment in the presence and in the absence of verapamil against the moxidectin-selected (MOF14) strain of *H. contortus* in the jirds

Treatment	Worm count	±S.E.	Efficacy (%)
Control	46	7	0
VRP	80	9	0
MOX	14	3	70
MOX + VRP	2	1	96
IVM	9	1	80
IVM + VRP	3	1	93

The combination of VRP with MOX was significantly different from the MOX-treated group ($P < 0.012$), and the IVM plus VRP-treatment group was significantly different from IVM alone ($P < 0.02$).

Studies in naturally infected lambs suggest that resistance to ivermectin and not moxidectin in adult worms of *H. contortus* was associated with multidrug ABC transporters, especially PgP efflux pumps (Lloberas *et al*., 2013[33]). Similar observations were reported *in vitro* studies (Godoy *et al*., 2016[41]).

Molento and Prichard (2001)[42] reported, using an *in vitro* larval migration assay, that the activity of moxidectin as well as ivermectin was reversed by verapamil against moxidectin and ivermectin resistant strains of *H. contortus* selected after several passages in sheep.

Ardelli and Prichard (2007)[43] reported a possibility of selection of ivermectin resistance, based on reduction in polymorphism, in subjects with *O. volvulus* in West Africa where prevalence and intensity of infections were markedly reduced after years of vector control and ivermectin distribution; moxidectin was not tested.

Bourguinat *et al* (2008)[44] reported that repeated ivermectin treatment of subjects with *O. volvulus* in Cameroon, selected for specific alleles of PgP like protein, known as the half-sized ABC transporter, in the adult worms. PgP was also implicated in ivermectin resistance in *D. immitis* (Bourguinat *et al*., 2011[45]).

Bygarski *et al* (2014)[46] reported PgP protein is an important resistance mechanism in *C. elegans* against both ivermectin and moxidectin although there were some similarities and differences in the resistance mechanisms between the two drugs. Mani *et al* (2016)[47] reported some similarities and differences in the binding sites for ivermectin and moxidectin of *D. immitis* PgP-11; the avermectins (e.g., ivermectin) bind the 'R' binding site unlike the milbemycins (e.g., moxidectin), whereas both sub-classes of macrocyclic lactones might interact with the 'H' site of *D. immitis* PgP-11.

Comments
The mechanism of resistance to moxidectin may be multifactorial that include changes in the target GluCl, GABA receptor, and/or ABC transporter.

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[41] Godoy P, Che H, Beech RN, and Prichard RK. Characterisation of P-glycoprotein-9.1 in *Haemonchus contortus*. *Parasites and Vectors* (2016) 9: 52.

[42] Molento MB and Prichard RK. Effect of multidrug resistance modulators on the activity of ivermectin and moxidectin against selected strains of *Haemonchus contortus* infective larvae. *Pesq Vet Bras* (2001) 21 (3): 117-121.

[43] Ardelli BF and Prichard RK. Reduced genetic variation of an *Onchocerca volvulus* ABC transporter gene following treatment with ivermectin. *Trans Roy Soc Trop Med Hyg* (2007) 101 (12): 1223-1232.

[44] Bourguinat C, Ardelli BF, Pion SDS, Kamgno J, Gardon J, Duke BOL, Boussinesq M, and Prichard RK. P-glycoprotein-like protein, a possible genetic marker for ivermectin resistance selection in *Onchocerca volvulus*. *Mol and Biochem Parasitol* (2008) 158: 101-111.

[45] Bourguinat C, Keller K, Blagburn B, Schenker R, Geary TG, and Prichard RK. Correlation between loss of efficacy of macrocyclic lactone heartworm anthelmintics and P-glycoprotein genotype. *Vet Parasitol* (2011) 176 (4): 374-381.

[46] Bygarski EE, Prichard RK and Ardelli BF. Resistance to the macrocyclic lactone moxidectin is mediated in part by membrane transporter P-glycoproteins: Implications for control of drug resistant parasitic nematodes. *Int J Parasitol: Drugs and Drug Resistance* (2014) 4: 143-151.

[47] Mani T, Bourguinat C, Keller K, Ashraf S, and Blagburn B. Interaction of macrocyclic lactones with a *Dirofilaria immitis* P-glycoprotein. *Int J Parasitol* (2016) 46 (10) 631-640.

3.4.3. Cross-resistance between moxidectin and ivermectin

Some of the studies (Craig *et al*., 1992[48]; Tyrell *et al*., 2002[49]; Lloberas *et al*., 2013[33]) reported that moxidectin was more effective than ivermectin in sheep or lambs infected with an ivermectin resistant strain of *H. contortus* suggesting lack of cross-resistance. However, Ranjan *et al* (2002[32]) reported decreased sensitivity to ivermectin or moxidectin resulted in resistance to moxidectin suggesting a possibility of cross-resistance between the two drugs. The rates of resistance development differed between the two drugs; resistance occurred more slowly with moxidectin than with ivermectin. Similarly, Shoop *et al* (1993[50]) reported cross-resistance between ivermectin and moxidectin in sheep infected with ivermectin-resistant isolates of the nematodes, *Ostertagia circumcincta* and *Trichostrongylus colubriformis.* Menez *et al* (2016)[51] reported cross-resistance between moxidectin and ivermectin using *C. elegans* and *H. contortus* in a larval development assay.

Njue *et al*., 2004[3] reported that a mutation in the GluClα3 subunit of GluCl channels of *C. oncophora* was associated with 3-fold decrease in sensitivity to both ivermectin and moxidectin, suggesting a possibility of cross-resistance.

Bygarski *et al*., 2014[46] reported PgP protein is important resistance mechanism in *C. elegans* against both ivermectin and moxidectin and there were some similarities in resistance mechanisms; the similarities in resistance mechanism may lead to some degree of cross-resistance to moxidectin.

Comments
Overall, the studies suggest a possibility of development of cross-resistance between ivermectin and moxidectin.

4. CLINICAL MICROBIOLOGY

The Applicant submitted two studies supporting the efficacy of moxidectin conducted in sub-Saharan Africa. Over 99% of patients with onchocerciasis are found in this region. The communities in the sites selected, for the two studies, had not previously participated in ivermectin mass drug administration programs.

4.1. Study 1 (Phase II)

This was a randomized, single-ascending-dose, ivermectin-controlled, double blind trial to evaluate the safety, tolerability, pharmacokinetics, and efficacy of moxidectin in subjects with *O. volvulus* infection [Protocol 3110A1-200-GH (Wyeth, a Pfizer company), B1751004 (Pfizer), OCRC 33 (OCRC, TDR/WHO)]. The study was conducted in inpatients/outpatients between

[48] Craig TM, Hatfield TA, Pankavich JA, and Wang GT. Efficacy of moxidectin against an ivermectin-resistant strain of *Haemonchus contortus* in sheep. *Vet. Parasitol* (1992) 41: 329-333.

[49] Tyrrell KL, Dobson RJ, Stein PA, and Walkden-Brown SW. The effects of ivermectin and moxidectin on egg viability and larval development of ivermectin-resistant *Haemonchus contortus*. *Vet Parasitol* (2002) 107: 85–93.

[50] Shoop WL, Haines HW, Michael BF, and Eary CH. Natural resistance to avermectins and milbemycins: oral activity of ivermectin and moxidectin against ivermectin-resistant and -susceptible nematodes. *Vet Res* (1993) 133: 445–447.

[51] Menez C, Alberich M, Kansoh D, Blanchard A, and Lespine A. Acquired tolerance to ivermectin and moxidectin after drug selection pressure in the nematode 2 *Caenorhabditis elegans. AAC* (2016) 60 (8): 4801-4819.

September 6, 2006 and November 29, 2009 at a single site in Ghana. No vector control measures were implemented in the region as it was a forested area at the time.

Primary objective
To determine the safety and tolerability of orally administered moxidectin in subjects with *O. volvulus* infection, as measured by the incidence of clinical adverse events and clinically significant laboratory test results.

Primary endpoints
1. The reduction from baseline (mean change) in skin microfilarial density (mf/mg skin) at 18 months after the administration of test article.
2. Adverse events, vital signs, laboratory parameters and ECGs.

Secondary objectives
1. To determine doses that effectively eliminate microfilariae and prevent their re-accumulation in the skin as measured by the skin microfilarial loads at Day 8, Months 1, 2, 3, 6, 12, and 18. after treatment.
2. To determine the viability and fertility of adult worms at Month 18.
3. To assess the pharmacokinetics of moxidectin in male and female adult subjects.

Secondary endpoints
1. Reduction from baseline in skin microfilarial density at Day 8 and Months 1, 2, 3, 6 and 12.
2. Proportion of subjects with undetectable skin microfilariae at Months 1, 6, 12 and 18.
3. Percent change from baseline in the mean skin microfilarial density at Months 1, 6, 12, and 18.
   - Area under the curve (AUC) of the percent reduction in skin microfilarial density from baseline to 12 months post treatment.
4. Ocular microfilariae at Days 3, 7 and 14, Months 1, 2, 3, 6, 12, and 18.
5. Viability and fertility of macrofilariae at Month 18.

Study design

Inclusion criteria
Men and women in good general health, with *O. volvulus* infection and the following:
1. Written, signed (or thumb-printed) and dated informed consent.
2. Age between 18 to 60 years.
3. Body weight ≥ 40 kg for women and ≥ 45 kg for men.
4. Non-pregnant, non-breastfeeding women. Women of child-bearing potential must agree to use birth control during the first 150 days after treatment.
5. Healthy, as determined by a physician on the basis of a physical examination, ECG, and a thorough review of the medical history and clinical laboratory results.
6. Skin microfilarial density within the required range for subjects with mild, moderate and severe infection:
   - *Mild*: 0 and <10 microfilariae/mg of skin.
   - *Moderate*: 10 to 20 microfilariae/mg of skin and the sum of microfilariae in the two eyes ≤10.
   - *Severe* (>20 microfilariae/mg of skin) infection.

   Each range of infection was for the 3 dose groups – thus there were 9 cohorts.
7. Adequate hematologic, renal, and hepatic function, defined as:
   - WBC count ≥ 2,800 and ≤ 11,300 cells/mL.

- Hemoglobin: ≥ 11.0 g/dL for men and ≥ 10.0 g/dL for women.
- Platelet count: ≥ 110,000 mm^3.
- Serum creatinine: ≤ 1.25 x upper limit of normal (ULN).
- Total bilirubin: ≤ 1.25 x ULN.
- Aspartate aminotransferase/serum glutamic oxaloacetic transaminase (AST/SGOT) ≤1.25 x ULN.
- Alkaline phosphatase (AP): ≤ 1.25 x ULN.
- Prothrombin time within normal limits (WNL).
- Urinalysis WNL.

Exclusion criteria

1. Participation in any studies other than purely observational ones, within 4 weeks before test article administration.
2. Any vaccination within 4 weeks before test article administration.
3. Acute infection requiring therapy within the last 10 days before test article administration.
4. Administration of any medication with the exception of medication required to treat any reactions during the screening fluorescein angiography (chlorpheniramine or paracetamol) or herbal preparation within 10 days prior to test article administration or any condition currently requiring regular medication.
5. Clinically significant ECG abnormalities or history of cardiac abnormality.
6. Past or current history of neurological or neuropsychiatric disease or epilepsy.
7. Subjects with orthostatic hypotension at the screening evaluation.
8. History of drug or alcohol abuse or regular use of ≥3 cigarettes per day.
9. Use of alcohol or other drugs of abuse within 72 hours before test article administration.
10. Any condition, in the investigator's opinion, that places the subject at undue risk.
11. Subjects who have donated blood within 8 weeks before study entry.
12. Subjects with ocular onchocerciasis in cohorts intended to enroll subjects with mild infection (0 and <10 microfilariae/mg of skin). Ocular onchocerciasis was defined by presence of live or dead microfilariae, onchocercal punctate opacities, onchocercal lesions of the posterior segment or lesions that mimic those seen in onchocerciasis.
13. Subjects with hyper-reactive onchodermatitis.
14. Antifilarial therapy within the previous 5 years.
15. Coincidental infection with *Loa loa*.
16. Female subjects of childbearing potential with a contraindication to depo-medroxy-progesterone acetate (DMPA) if not on Norplant®.
17. Any other condition which the investigator feels would exclude the subject from the study.

Subjects were administered a single oral dose of 2 mg, 4 mg or 8 mg or moxidectin (n=127) or ivermectin 150 µg/kg (n=45) by severity of infection and followed as in-patients for the first 18 days and as out-patients for the remainder of the 18-month study for clinical and laboratory parameters that included parasitological measurements at different time points (Table 4).

Table 4: Phase II study - Schedule of events

	Visit Number																								
Clinical Planned Events	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	Study Day																									
Study Procedures	-4 to -2[j]	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1 mo ±1 wk	2 mo ±1 wk	3 mo ±1 wk	6 mo ± 1 mo	12 mo ±1 mo	18 mo ±1 mo
Informed consent[a]																										
Demographics	X																									
Admission	X																									
Medical history	X																									
Medication history	X																									
Physical examination	X																				X	X	X	X	X	X
Height	X																									
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X		X[b]	X	X					X																
Ocular examination	X				X				X							X					X	X	X[c]	X	X	X
Retinal Colour Photographs and Fluorescein angiogram[c]	X				X				X							X					X	X	X	X	X	X
Interim physical examination			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
Hematology[d]	X		X	X		X				X					X					X	X	X	X	X	X	X
Serum chemistry	X		X	X		X				X					X					X	X	X	X	X	X	X
Pregnancy test		X																								
Urinalysis[d]	X		X	X		X				X					X					X	X	X	X	X	X	X
Skin snips	X									X											X	X	X	X	X	X
Randomization		X																								
Drug packaging and dispensing		X																								
Double-blind drug administration			X[e]																							
Moxidectin Plasma Samples		X[f]	X[g]	X		X				X					X					X	X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DMPA[h]													X									X				
Discharge																				X						
Nodulectomy																										X[i]
Clinical Planned Events	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

a. Informed consent was obtained in the village prior to transport of potential study participants to the study center and before any study-related procedures performed. Informed consent was to be obtained prior to day-4.
b. ECG were done approximately 4 hours after study drug administration and may have been repeated on days 4 through 7 if clinically indicated.
c. For all subjects until month 3, then only in subjects with lesions or visual defects.
d. All blood and urine samples were filtered and stained for microfilariae.
e. Was given after an overnight fast and 2 hours before breakfast. The administration of the test article(s) may have been delayed for up to 2 days to permit all predose examinations to be performed on subjects.
f. Day -1 sample was taken approximately 2 hours before drug administration.
g. Day 1 samples was taken at 1, 2, 4, and 8 hours after drug administration.
h. Depo-medroxyprogesterone acetate (DMPA) on women of childbearing potential not already receiving a parenterally administered contraceptive.
i. All located nodules were processed for histopathology and slides read by 1 or more blinded observers.
j. Day -4 to -2 procedures may have been performed as late as Day -1, but were performed prior to double-blind drug administration.

Parasitological assessments:

Parasitological assessments included measurement of *O. volvulus* microfilaria density in skin snips and the eye (ocular examination) at different time points. Also, the adult worms in the palpable nodules were examined at Month 18.

The parasitological assessments were performed on-site and there was no shipping of samples for testing at a central laboratory. The study was conducted under WHO Operational Guidelines

for Good Clinical Laboratory Practice (2007). Formal training and monitored compliance was performed. (b) (4)

Evaluation of microfilariae in the skin snips
A total of 4 skin snips were taken at each time point (one from each iliac crest and calves) using a corneoscleral punch (Walser or Holth-type). Each snip was weighed, incubated overnight in isotonic saline and the microfilariae that emerged were counted using an inverted microscope at a magnification of 60X, and if needed at 100X. The results were expressed as microfilariae/mg of skin. If the microfilariae density was high (>150/well) or the technician felt that the counting was not reliable, the skin snips were moved to another well and diluted or an aliquot from the well was further diluted. The Applicant states that microfilarial counts can sometimes be checked by double counting either by the same or another individual. Whenever a second reader was used for microfilarial counts, both observers had to agree on the findings by discussion, else a third observer was required.

In the Standard Operating Procedure (SOP), it is stated that the microfilariae can be preserved in formalin and the plate submitted for recounting some days after the original counting was performed. However, it appears that the microfilaria counting after fixation was not performed.

Any *Mansonella streptocerca* microfilariae detected were counted separately.

Evaluation of microfilariae in the ocular region
Counting of the number of live microfilariae in the anterior chamber (MFAC) and the live and dead microfilariae in the cornea were performed by the ophthalmologist, after head-down positioning for 5 minutes, using a slit lamp microscope, provided by the Applicant, using 16 to 25 x magnification. If there were > 50 microfilaria then it was recorded as 50.

Evaluation of adult worms in the nodules
Fresh impression smears of the nodules were examined visually for the presence of live worms and microfilariae by a single observer (b) (4) according to standard operating procedures (SOPs) and standardized criteria. In wet mount, the viability of the developmental stages was assessed by examining their morphology and motility. The developmental stages of female adult worms and spermatozoa from male adult worms stick on to the slide. The different developmental stages (normal and abnormal oocytes and embryonic stages i.e., early and late morula, embryos and stretched microfilariae) of the parasites were counted and sex of the adult worms determined. The impression smears were also stained with Giemsa for detailed assessment.

Isolation of female adult worms, due to their large size, was performed by collagenase digestion and not by direct dissection of nodules; however, male worms were isolated by direct dissection of nodules. The uterine contents of female worms were released into an isotonic solution, to quantify the number of ova and embryonic stages with the aid of a light microscope and a counting chamber.

Histopathological evaluation for adult worms in the nodules was performed by the method of Buttner *et al.*, 1988[52]. Briefly, 4 mm sections were obtained, in triplicate, along the longest axis

[52] Buttner DW, Albiez EJ, von Essen J, and Erichsen J. Histological examination of adult *Onchocerca volvulus* and comparison with the collagenase technique. *Trop Med Parasitol* (1988) 39 (Suppl 4): 390-417.

of each nodule in a way that each third of a nodule was sampled for histological assessments that included the reproductive status of female and male adult worms and extent of embryogenesis and sperm production.

Nodules were processed on site, the slides read before being sent off for further reading and comparison of results, if required. Whenever a second reader was used for microfilarial counts or the interpretation of histology slides, both observers had to agree on the findings by discussion else a third observer was required.

Other parasitological assessments:
Subjects were screened for other parasitic co-infections in blood, urine, and other specimens in accordance with standardized SOPs.

Comments
For microfilariae density in the skin, the microfilariae were counted directly in the wells of the microtiter plates containing skin snips after overnight incubation in an isotonic solution. The entire well was examined using an inverted microscope. Appropriate training and quality control measures were in place. It appears that the microfilaria counting, after fixation, was not performed. It is unclear how active and motile the microfilariae are after overnight incubation and whether parasites will float, from one field to another, when the entire well of the microtiter plate was examined. It will be useful to check the reliability of the microfilariae count during inspection; this was communicated to Drs John Lee and Janice Pohlman, Office of Scientific Investigations.

Results
Of the 172 subjects enrolled, 166 completed the study and were included in the evaluable modified intent-to treat (e-MITT) population. The number of subjects with mild, moderate and severe baseline infection intensity was similar in the ivermectin and two of the moxidectin (2 mg and 4 mg) groups; there were fewer subjects with severe baseline infection intensity in the 8 mg moxidectin group.

Effect of treatment on microfilariae in the skin
The results show a decrease in microfilaria density within 8 days of treatment with moxidectin or ivermectin; decrease in microfilariae density was highest in subjects treated with 8 mg moxidectin and there was a dose-dependent effect (Table 5 and Figure 7). The decrease in microfilariae density persisted until Month 6 in all treatment groups.

At Months 12 and 18 post-treatment, there was a trend towards an increase in microfilariae density as well as a decrease in the number of subjects that remained microfilariae negative. The rebound of microfilariae was slower in moxidectin treated subjects compared to those treated with ivermectin (Table 5 and Figure 7). A higher proportion of subjects were microfilariae negative in the moxidectin treated groups than the ivermectin treated group at all the time points post-treatment (Table 5).

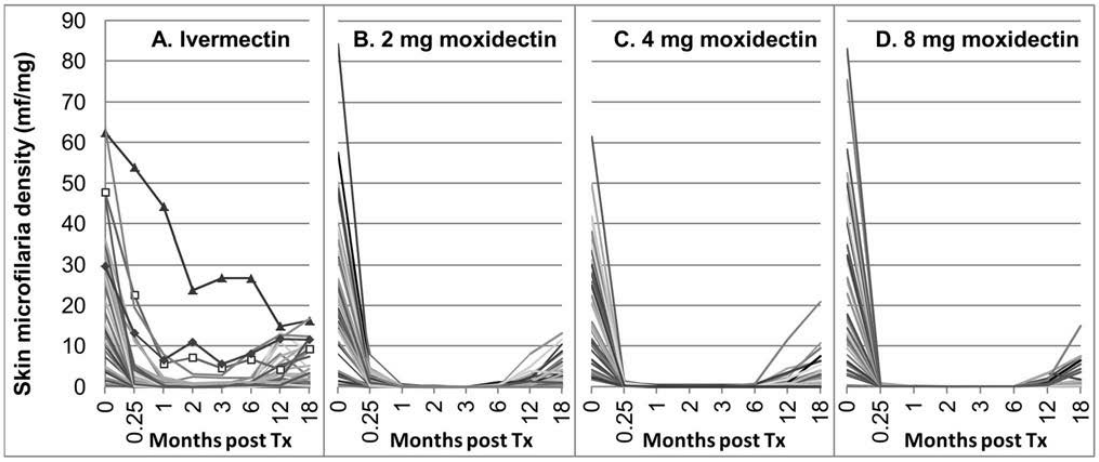
Table 5: Phase II study – summary of parasitological findings in skin snips in the e-MITT population

Treatment group (N)	Microfilariae/mg skin			Mf $^{-ve}$ subjects n (%)
	Mean	Median	Mean change from baseline*	
Baseline				
Moxidectin 2 mg (42)	24.0	22.0	Not applicable	
Moxidectin 4 mg (45)	20.6	20.5		
Moxidectin 8 mg (37)	22.9	14.5		
Ivermectin 150 µg/kg (42)	21.2	16. 9		
Day 8				
Moxidectin 2 mg (42)	1.4	0.8	-22.6*	6 (14.3)
Moxidectin 4 mg (45)	0.4	0.3	-20.2*	13 (28.9)
Moxidectin 8 mg (37)	0.2	0.1	-22.7*	18 (48.6)
Ivermectin 150 µg/kg (42)	4.4	1.1	-16.8	4 (9.5)
Month 1				
Moxidectin 2 mg (42)	0.1	0.0	-23.9*	33 (78.6)
Moxidectin 4 mg (45)	0.0	0.0	-20.6*	44 (97.8)
Moxidectin 8 mg (37)	0.0	0.0	-22.9*	36 (97.3)
Ivermectin 150 µg/kg (42)	1.8	0.0	-19.5	23 (54.8)
Month 2				
Moxidectin 2 mg (41)	0.0	0.0	-22.5*	39 (95.1)
Moxidectin 4 mg (45)	0.0	0.0	-20.6*	43 (95.6)
Moxidectin 8 mg (37)	0.0	0.0	-22.9*	36 (97.3)
Ivermectin 150 µg/kg (42)	1.2	0.0	-20.0	28 (66.7)
Month 3				
Moxidectin 2 mg (42)	0.0	0.0	-23.9*	40 (95.2)
Moxidectin 4 mg (45)	0.0	0.0	-20.6*	45 (100)
Moxidectin 8 mg (37)	0.0	0.0	-22.9*	37 (100)
Ivermectin 150 µg/kg (42)	1.1	0	-20.1	23 (54.8)
Month 6				
Moxidectin 2 mg (42)	0.1	0.0	-23.9*	34 (81.0)
Moxidectin 4 mg (45)	0.0	0.0	-20.6*	41 (91.1)
Moxidectin 8 mg (37)	0.0	0.0	-22.9*	37 (100)
Ivermectin 150 mg/kg (42)	1.6	0.4	-19.6	13 (31.0)
Month 12				
Moxidectin 2 mg (42)	0.9	0.2	-23.0*	15 (35.7)
Moxidectin 4 mg (45)	0.8	0.2	-19.8*	18 (40.0)
Moxidectin 8 mg (37)	0.4	0.0	-22.4*	22 (59.5)
Ivermectin 150 µg/kg (42)	3.4	1.3	-17.8	8 (19.0)
Month 18				
Moxidectin 2 mg (42)	2.8	1.3	-21.2	7 (16.7)
Moxidectin 4 mg (45)	2.2	0.3	-18.5*	12 (26.7)
Moxidectin 8 mg (37)	1.8	0.3	-21.0*	13 (35.1)
Ivermectin 150 µg/kg (42)	4.0	1.8	-17.2	6 (14.3)

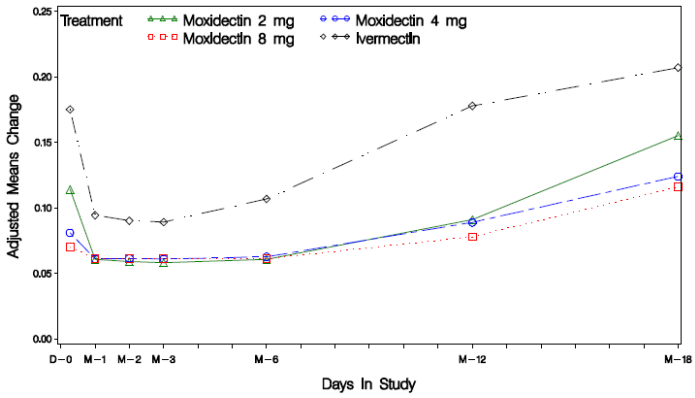
Mf=microfilariae; N= number of subjects; n=number of mf^{-ve} subjects in the treatment group.
*Statistically different from ivermectin;

Figure 7: Phase II study – (A) skin microfilariae density in individual patients and (B) mean change from baseline at the different times pre- and post-treatment (e-mITT population) in the different treatment groups.

A: Microfilariae density (mf/mg skin) in individual participants at the different times pre- and post-treatment



B: Adjusted mean change from baseline in microfilarial densities (mf/mg skin)



Source: NDA

Effect of treatment on ocular microfilariae

Ocular involvement was low among the subjects enrolled (22%); the number of microfilariae in the anterior chambers across both eyes varied between 1 and 39. The presence of corneal microfilariae [alive (1 to 5 microfilariae in 2 subjects) or dead (1 to 3 microfilariae in 3 subjects)] and punctate opacities was rare. Time to clearance of microfilariae in the anterior chamber of eye in subjects treated with 8 mg moxidectin was lower (4 days) compared to other treatment groups (Table 6). No live or dead microfilaria were detected in the cornea at Day 15 after treatment in any of the treatment groups.

Table 6: Phase II study - Time (in days) from baseline to maximum persistent reduction of microfilariae in the anterior chamber by dose group (e-MITT population).

Therapy Group	Number of Patients	Median (days)	95% CI	P-Value[a]
Moxidectin 2 mg	9	31.00	14.0, 35.0	
Moxidectin 4 mg	10	30.50	15.0, 58.0	
Moxidectin 8 mg	10	4.00	3.0, 4.0	
Ivermectin	8	33.50	14.0, 18.0	<0.0001

a. p-value is calculated using the log-rank test.
Abbreviations: CI: confidence interval; e-mITT: evaluable modified intent to treat
Source: 3110-200 EFF-TIMETO-EMITT - 03DEC10 13:47

Effect of treatment on viability and fertility of adult worms in the nodules at Month 18

Not all palpated and excised nodules, collected at Month 18, were onchocercal; other nodules included lipomas, lymph nodes, and granulomas around foreign bodies. Based on the worms excised from the onchocercal nodules, the results show a trend towards a decrease in the number

of live microfilariae and adult worms in the moxidectin (8 mg) or ivermectin treated subjects however, there were no differences in the viability or reproductive capacity of adult male or female worms (Table 7; Figure 8). Also, there was no correlation between the number of nodules or live male or female adult worms excised and microfilariae density at Month 18 (Figure 8).

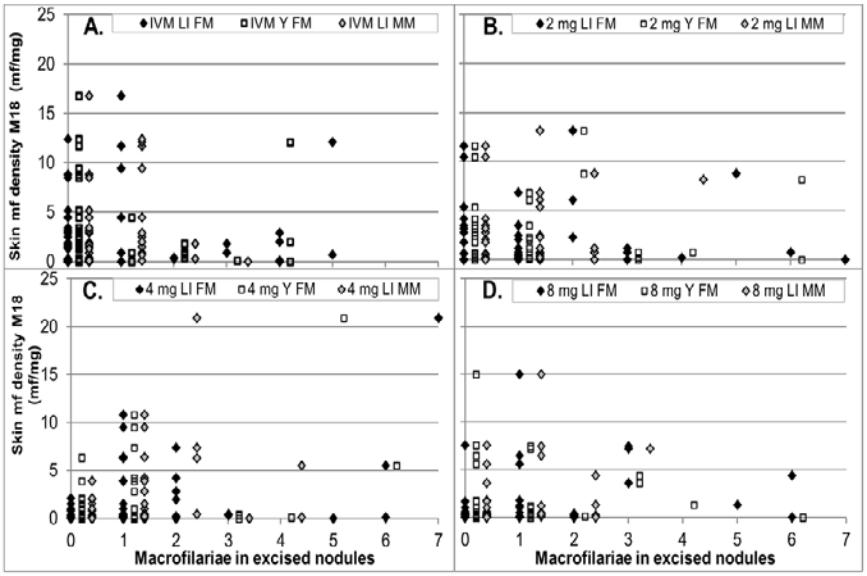
The Applicant states that the number of palpable nodules examined at Month 18 was higher than at the pretreatment visit. This could be due to increased awareness of the subjects enrolled to the nodule sites after pre-treatment examination, formation of new nodules due to the presence of worms at early developmental phase (due to long incubation period), presence of dead or calcified worms in the nodules prior to treatment, palpable onchocercal nodules not representative of all adult worm burden in the body, or re-infections due to high endemicity.

Table 7: Phase II study - Viability and reproductivity of the adult worms at Month 18

	Moxidectin 2 mg	Moxidectin 4 mg	Moxidectin 8 mg	Ivermectin	P-Value
Number of nodules excised	66	57	49	48	
- Number of nodules with microfilariae in capsule (% of nodules excised)	10 (15.2%)	19 (33.3%)	6 (12.2%)	11 (22.9%)	0.114
- Number of nodules without male worms (% of nodules excised)	33 (50.0%)	21 (36.8%)	22 (44.9%)	24 (50.0%)	0.656
Female worms					
Total Number of female worms(alive, dead or moribund)	100	79	60	74	
- Number of live worms (% of total number of female worms)	73 (73.0%)	61 (77.2%)	47 (78.3%)	46 (62.2%)	0.435
- Number of moribund or dead worms (% of total number of female worms)	27 (27.0%)	18 (22.8%)	13 (21.7%)	28 (37.8%)	0.435
- Number of dead and calcified worms (% of total number of female worms)	15 (15.0%)	11 (13.9%)	5 (8.3%)	18 (24.3%)	0.726
Live female worms					
Total number of live female worms	73	61	47	46	
- Number producing embryos (% of live female worms)	18 (24.7%)	32 (52.5%)	18 (38.3%)	19 (41.3%)	0.197
- Number with degenerate embryos (% of number producing embryos)	1 (5.6%)	12 (37.5%)	9 (50.0%)	7 (36.8%)	0.019
- Number not producing embryos (% of live female worms)	55 (75.3%)	29 (47.5%)	29 (61.7%)	27 (58.7%)	0.197
- Number with relict/degenerate embryos (% not producing embryos)	0 (0.0%)	1 (3.4%)	1 (3.4%)	0 (0.0%)	0.431
- Number with sperm in uteri (% of live female worms)	22 (30.1%)	19 (31.1%)	6 (12.8%)	12 (26.1%)	0.128
Male worms					
Total number of male worms	37	39	23	23	
- Number of live (% of total number of male worms)	35 (94.6%)	39 (100%)	19 (82.6%)	23 (100%)	0.302
- Number of moribund or dead worms (% of total number of male worms)	2 (5.4%)	0 (0.0%)	4 (17.4%)	0 (0.0%)	0.302
- Number of dead and calcified worms (% of total number of male worms)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Live male worms					
Total number of live male worms	35	39	19	23	
- Number with normal spermatogenesis (% of total number of live male worms)	35 (100%)	38 (97.4%)	19 (100%)	23 (100%)	0.548

NOTE: P-values are based on an ANCOVA analysis with proportions at the subject-level (rather than group-level presented
in the table) as the response variable, with Baseline infection intensity and gender included as covariates.
Source: 3110-200 WORM5-VIAB - 30NOV10 15:09

Figure 8: Phase II study - Number of live female macrofilaria, live young female macrofilaria and live male macrofilaria in excised nodules vs skin mf density at Month 18 in different treatment groups.
A. Ivermectin, B. 2 mg moxidectin, C. 4 mg moxidectin, D. 8 mg moxidectin.



For display reasons, the x axis maximum was set to 7.

The data for one participant treated with 2 mg moxidectin who had 13 live female AW and a skin mf density of 8.2 mf/mg are thus not displayed.

Participants with 0 AW in excised nodules either had no palpable nodules or all excised nodules were non-onchocercal based on histological evaluation.

LI FM: live female macrofilariae.
Y FM: live young female macrofilariae.
LI MW: live male macrofilariae.

Source: NDA

Comments

Overall, the study suggests that moxidectin at any of the doses (2, 4 and 8 mg) tested was more effective than ivermectin in reducing skin microfilariae density; decrease in microfilaria density in the moxidectin treated groups was dose-dependent. The skin microfilariae density was decreased in higher proportion of moxidectin treated subject and to a greater degree for a longer duration than the ivermectin treatment group. The proportion of microfilaria negative subjects was higher in the moxidectin treated subjects compared to those treated with ivermectin at all the time points. A decrease in skin microfilariae density within 8 days of treatment may reflect both the microfilaricidal and embryostatic effects of the drug. A continued decrease in microfilariae up to Month 6 may reflect the effect of treatment on adult worms especially fertility and embryogenesis.

Ocular involvement was low (22%) in the subjects enrolled. Both moxidectin and ivermectin reduced microfilariae in the anterior chamber of the eye. The presence of corneal microfilariae (alive or dead) and punctate opacities was rare. The median time to reduction of ocular microfilaria was lowest in subjects treated with 8 mg moxidectin compared to other treatment groups.

Based on excised adult worms and histological findings of the onchocercal nodules, the results suggest a trend towards a decrease in the number of live microfilariae and adult worms as well as an increase in degenerative embryos at Month 18 in the moxidectin (8 mg) or ivermectin treated subjects compared to low dose moxidectin treated subjects; however, there were no differences in the reproductive capacity of adult male or female worms in the onchocercal nodules collected from subjects in the different treatment groups. These results should be interpreted with caution as the number of worms in the excised nodules may not reflect worm burden in the host. Also, the presence of extra-nodular adult worms cannot be ruled out. The pretreatment nodules were not processed for histological evaluation. The formation of new nodules due to the presence of worms at early developmental phase (due to long incubation period) post-treatment, presence of dead or calcified worms in the nodules prior to treatment,

palpable onchocercal nodules not representative of all adult worms in the body, or re-infections due to high endemicity cannot be ruled out.

4.2. Study 2 (Phase III)

This was a phase III multicenter, randomized, double-blind study comparing the efficacy, safety, and tolerability of a single dose of orally administered moxidectin versus ivermectin in subjects with *O. volvulus* infection [Protocol Number: 3110A1-3000-AF (Wyeth), B1751006 (Pfizer) ONCBL60801 (OCRC, WHO)]. The study was conducted at four sites in Africa: two sites (Butembo, Rethy) in Democratic Republic of Congo, one site (Bolahun) in Liberia and one site (Hohoe) in Ghana; the Ghana site was also the location of the Phase II study. It is unclear if the vector control measures were implemented in these regions.

Primary objectives:

To compare the safety, tolerability, and parasitological efficacy, as measured by skin microfilariae density at Month 12.

Primary endpoints:

Efficacy: The skin microfilariae density (mf/mg) at Month 12.
Safety: Adverse events, vital signs, laboratory parameters and ECGs.

Secondary objectives:

To compare additional measures of parasitological efficacy of a single oral dose of moxidectin versus ivermectin in subjects infected with *O. volvulus* as measured by skin microfilariae levels at additional time points and microfilariae levels in the eye.

Secondary endpoints:

- Skin microfilariae density at Months 1, 6, and 18.
- Percent reduction from baseline in the mean skin microfilariae density at Months 1, 6, 12, and 18.
- Area under the curve (AUC) of the percent reduction in skin microfilariae density from baseline to 12 months post treatment.
- Proportion of subjects with undetectable levels of skin microfilariae at Months 1, 6, 12 and 18.
- Percentage (%) reduction from baseline in microfilariae levels in the anterior chamber of the eyes at 12 months after study drug administration in subjects with the sum of microfilariae in the anterior chamber of both eyes >10 at baseline.

Other efficacy endpoints:

- Proportion of subjects achieving a nadir with respect to skin microfilariae density at Months 1, 6, 12, and 18.
- Proportion of subjects with an increase in skin microfilariae density from an achieved nadir.
- Proportion of subjects achieving and sustaining < 1 mf/mg.
- Number of palpable nodules at baseline and at Months 12 and 18.

Exploratory efficacy endpoints:

Efficacy against other coincidental helminths (e.g., intestinal helminths, *Wuchereria bancrofti*).

Post-hoc efficacy endpoints:

- Sustained responders with respect to undetectable levels of skin microfilariae.
- Ocular non-responders.

Study design

Inclusion criteria

1. Male or female subjects ≥12 years of age and weighing ≥ 30 kg.
2. Subjects with *O. volvulus* infection, ≥10 microfilariae/mg by skin snip.
3. All female subjects not surgically sterile or post-menopausal must have agreed and committed to use a reliable method of birth control for 6 months after investigational medicinal product (IMP) administration. A woman of childbearing potential was one who was biologically capable of becoming pregnant. This included women who were using contraceptives or whose sexual partners were sterile or used contraceptives.

Exclusion criteria

1. Prior treatment with anthelmintics (e.g., diethylcarbamazine [DEC], suramin, ivermectin or albendazole) within 6 months before planned IMP administration.
2. Pregnant or breastfeeding women.
3. Low probability of residency in the area (based on subject's assessment) over the next 20 months.
4. Subjects with loiasis.
5. Subjects with lymphatic filariasis with an intensity of infection >100 microfilariae/mL.
6. Acute or uncontrolled disease process (e.g., acute pneumonia that required therapy or end-stage acquired immune deficiency syndrome (AIDS) within 7 days before IMP administration. Patients with stable chronic diseases (e.g., no change in medication for past month) were permitted.
7. Received any investigational drugs or investigational devices within 4 weeks before administration of IMP that may have confounded safety and/or efficacy assessments.
8. Known or suspected allergy to moxidectin or ivermectin or other compounds related to these classes of medication.
9. Any concomitant condition that, in the opinion of the Investigator, would preclude an evaluation of a response or would place subject's health at undue risk.

Subjects were administered a single oral dose of either moxidectin (8 mg) or ivermectin (approximately 150 μg/kg) and followed at different time intervals for up to 12 months for clinical response and laboratory parameters that include parasitological assessments (Table 8). The 18-month assessment only occurred for those subjects whose visit was completed prior to the approval of Amendment 3 of the protocol or took place prior to 31 Dec 2011, whichever was the later date (i.e., Amendment 3 removed the 18-Month assessment visit).

Table 8: Phase III study - Schedule of events

Study Procedures	Days -30 to -1	Day 1	Day 2	Day 3	Day 4	Day 6 ± 1D	Day 14 ± 2D	Month 1 ± 5D	Month 3 ± 2W	Month 6 ± 1M	Month 12 ± 1M	Month 18 ± 2M
Demographics	X											
Medical history	X											
Medication history	X											
Assessment of *Loa loa* infection	X											
Pregnancy test[a]	X[j]							X[k]	X[k]	X[k]		
Skin snips[l]	X							X		X	X	X
Nodule palpation	X										X	X
Height	X											
Weight	X							X	X	X		
Complete physical examination	X[j]											
Interim physical examination		X	X	X	X	X	X	X	X	X	X	X
Vital signs[b]	X[j]	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG	X[j]		X[c]									
Ocular examination	X			X[c]				X		X	X	X
Hematology[d]	X[j]					X	X	X	X	X		
Serum chemistry[e]	X[j]					X	X	X	X	X		
Urinalysis[f]	X[j]					X	X	X	X	X		
Lymphatic filariasis evaluation[g]	X							X		X	X	X
Assessment of intestinal helminths infection[h]	X							X				
Test article administration[i]		X										
Adverse events collection	----------X----------											

ECG=electrocardiogram.

a. For women of childbearing potential, a negative urine pregnancy test result is required before randomization.
b. Vital signs on day 1 performed before and after test article administration.
c. 12-Lead ECG on day 2 or day 3; ocular exam on day 3 or day 4.
d. Hematology includes complete blood count (CBC) (consisting of total white blood cell (WBC) count with differential, platelet count, hemoglobin, and hematocrit).
e. Serum chemistry includes sodium, potassium, chloride, glucose, blood urea nitrogen (BUN) or urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase (AP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), aspartate aminotransferase (SGPT) (AST), and alanine aminotransferase (SGPT) (ALT).
f. Urinalysis included specific gravity, pH, albumin (protein), glucose, ketones, hemoglobin, bilirubin, urobilinogen, nitrite and leukocyte esterase. Microscopic evaluation of red blood cells (RBC), WBC, epithelial cells, bacteria, casts, and crystals performed at baseline and thereafter only at investigator's discretion as medically indicated.
g. Lymphatic filariasis (LF) evaluation in areas co-endemic for LF or where endemicity is unknown. Pretreatment LF evaluation: immunochromatographic card test for *W. bancrofti* (ICT) in subjects without clinical signs and symptoms. If positive, these subjects (and all subjects with signs and symptoms of LF) will have night blood evaluation for diagnosis and quantification of LF infection. Post-treatment LF evaluation (night blood evaluation) only in subjects who are positive at baseline.
h. Collection of feces and evaluation for intestinal helminths was optional.
i. Drug administration after an overnight fast.
j. Test results obtained within 1 week before test article administration.
k. Pregnancy testing offered to women of reproductive potential.
l. Skin snips could be obtained with the 30-day Screening period or up to 2 months prior to IMP administration.

When a subject discontinued from the study or was withdrawn, the investigator notified the sponsors and, when possible, the following procedures performed: vital signs, including supine and standing blood pressure and pulse rate; interim physical examination; collection of adverse events; laboratory evaluation, including hematology, serum chemistry and urinalysis (if discontinuation or withdrawal occurs before the month 6 visit); quantification of skin microfilarial levels by skin snip.

*The 18 Month assessment only occurred for those subjects whose visit was completed prior to the approval of Amendment 3 or took place prior to 31 December 2011, whichever was the later date (i.e., Amendment 3 removed the 18 Month assessment visit).

Parasitological assessments

Parasitological assessments included quantification of skin microfilarial density in skin snips and ocular examination. The parasitological methods used were the same as for the Phase II study

except that nodulectomy was not performed. Testing was conducted at each of the 4 sites. The SOPs used at each site were identical.

Similar to the Phase II study, (b) (4) Both the parasitologist and laboratory head from the other 3 sites (one in Liberian, and the two in Democratic Republic of Congo) underwent training by (b) (4) in Ghana during the conduct of the Phase II study. This included recounting of microfilariae under direction for some Phase II samples to confirm understanding and performance. The Ghana laboratory was not accredited, but had equipment calibrated annually by the Ghana Standard Board, all staff were fully trained and the laboratory participated in the External Quality Control program organized by WHO for clinical chemistry.

The Applicant provided identical equipment to each of the clinical sites, and there were regular quality checks by the study monitors. Each instrument used in testing for the study operated as expected and the site and equipment was in conformance with local regulatory and Good Clinical Laboratory Practice (GCLP) guidelines. The laboratory quality control records were maintained on site.

Assessment of intestinal helminthic infections was optional.

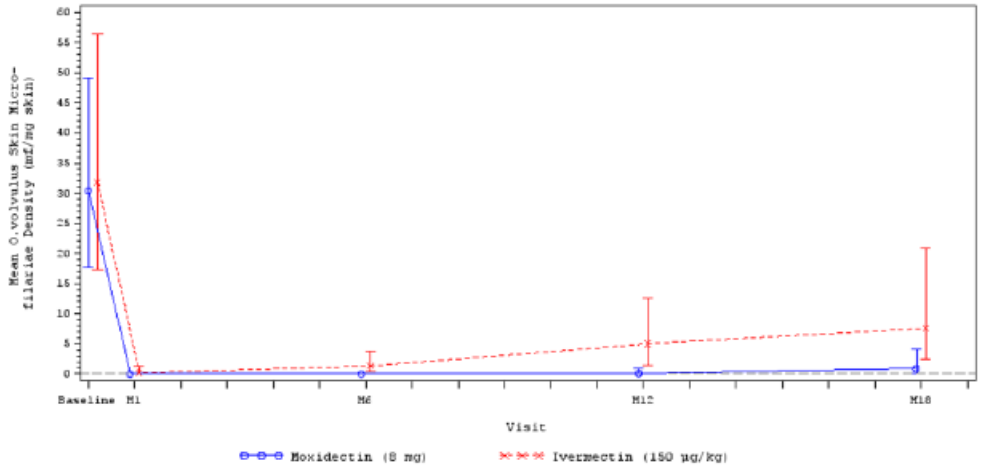
Results
Of the 1499 randomized subjects, 943 subjects in the moxidectin arm and 478 in the ivermectin arm completed the 12-month follow-up for the microfilariae in the skin snips and were included in the e-MITT population. The number of palpable nodules were similar between the two treatment groups.

Effect on microfilariae in the skin
The mean baseline microfilariae density was similar between the two treatment groups. Approximately, 70% of the subjects had at least 20 microfilariae/mg skin at baseline and there was no difference between the two treatment groups. There was a slightly higher proportion of subjects with baseline microfilariae density ≥ 50 microfilariae/mg skin in the ivermectin group compared to the moxidectin group (32% vs 24%). Subjects enrolled at Site 2 (one of the site in Democratic Republic of Congo - Rethy, Ituri Nord) had a higher mean microfilariae density of 49.60 microfilariae/mg skin (SD ±38.96) at baseline compared to the other 3 sites.

The results show a decrease in microfilaria density within Month 1 of treatment with moxidectin or ivermectin; decrease in microfilariae density was highest in subjects treated with moxidectin compared to ivermectin (Table 9 and Figure 9). The decrease in microfilaria density persisted until Month 6 in both groups. At Months 12 and 18 post-treatment, there was a trend towards an increase in microfilariae density as well as a decrease in the number of subjects that remained microfilariae negative. A higher proportion of subjects were microfilariae negative in the moxidectin treated group than the ivermectin treated group at all the time points post-treatment (Table 9).

Table 9: Phase III study – summary of parasitological findings in skin snips in the e-MITT population				
Treatment group (N)	**Microfilariae/mg skin**			**Mf $^{-ve}$ subjects n (%)**
	Mean	**Median**	**Mean change from baseline**	
Baseline				
Moxidectin 8 mg (943)	39.1	30.8	Not applicable	
Ivermectin 150 µg/kg (478)	41.1	31.8		
Month 1				
Moxidectin 8 mg (939)	0.1	0.0	-38.9	782 (83.3)
Ivermectin 150 µg/kg (476)	2.3	0.2	-38.7	203 (42.6)
Month 6				
Moxidectin 8 mg (938)	0.0	0.0	-39.0	860 (91.7)
Ivermectin 150 mg/kg (478)	3.6	1.3	-37.5	53 (11.1)
Month 12				
Moxidectin 8 mg (943)	1.3	0.1	-37.8	440 (46.7)
Ivermectin 150 µg/kg (478)	10.0	5.1	-31.2	24 (5.0)
Month 18				
Moxidectin 8 mg (758)	4.3	0.9	-35.3	213 (28.1)
Ivermectin 150 µg/kg (381)	15.3	7.6	-26.2	15 (3.9)
Mf=microfilariae; N= number of subjects; and n=number of mf^{-ve} subjects in the treatment group.				

Figure 9: Phase III study - Unadjusted mean *O. volvulus* skin microfilariae density (mf/mg skin) by visit (AR-mITT)



Source: Figure 14.2.1.4
Median values across all subjects of the mean *O. volvulus* skin microfilariae densities are plotted. Vertical bars represent the upper and lower quartiles of that visit.

There appears to be a trend towards decreased efficacy in subjects with higher microfilariae density at baseline in both moxidectin and ivermectin treatment groups (Table 10).

Table 10: Phase III study - Analysis of mean *O. volvulus* skin microfilariae density (mf/mg skin) at Month 12 by baseline intensity of infection (e-MITT)

Group	Baseline intensity of infection mf/mg skin	N	Mean[a] (±SD)	LS geometric mean[b] (95% CI)	Ratio of LS geometric[c] (95% CI)	P value
Moxidectin	< 20	283	0.37 (1.001)	0.253 (-0.002, 0.574)	0.416 (0.361, 0.480)	< 0.0001
Ivermectin		149	3.40 (5.963)	2.010 (1.365, 2.830)		
Moxidectin	≥ 20 to < 50	427	1.22 (2.808)	0.544 (0.235, 0.929)	0.307 (0.271, 0.348)	< 0.0001
Ivermectin		178	7.11 (8.485)	4.030 (2.791, 5.372)		
Moxidectin	≥ 50 to < 80	154	2.30 (4.204)	1.025 (0.592, 1.575)	0.181 (0.151, 0.216)	< 0.0001
Ivermectin		101	15.16 (12.895)	10.196 (7.684, 13.435)		
Moxidectin	≥ 80	79	2.54 (4.556)	1.036 (0.561, 1.654)	0.096 (0.075, 0.124)	< 0.0001
Ivermectin		51	28.84 (18.192)	20.189 (14.843, 27.339)		

Source: Tables 14.2.1.5.1 and 14.2.1.5.3

[a] Raw mean value

[b] LS Geometric Means, CIs, and p-values are obtained from a mixed-effects model on mf/mg skin at Month 12 with baseline mf/mg skin, sex, treatment group and treatment-by-baseline mf/mg skin as fixed effects and site as a random effect.

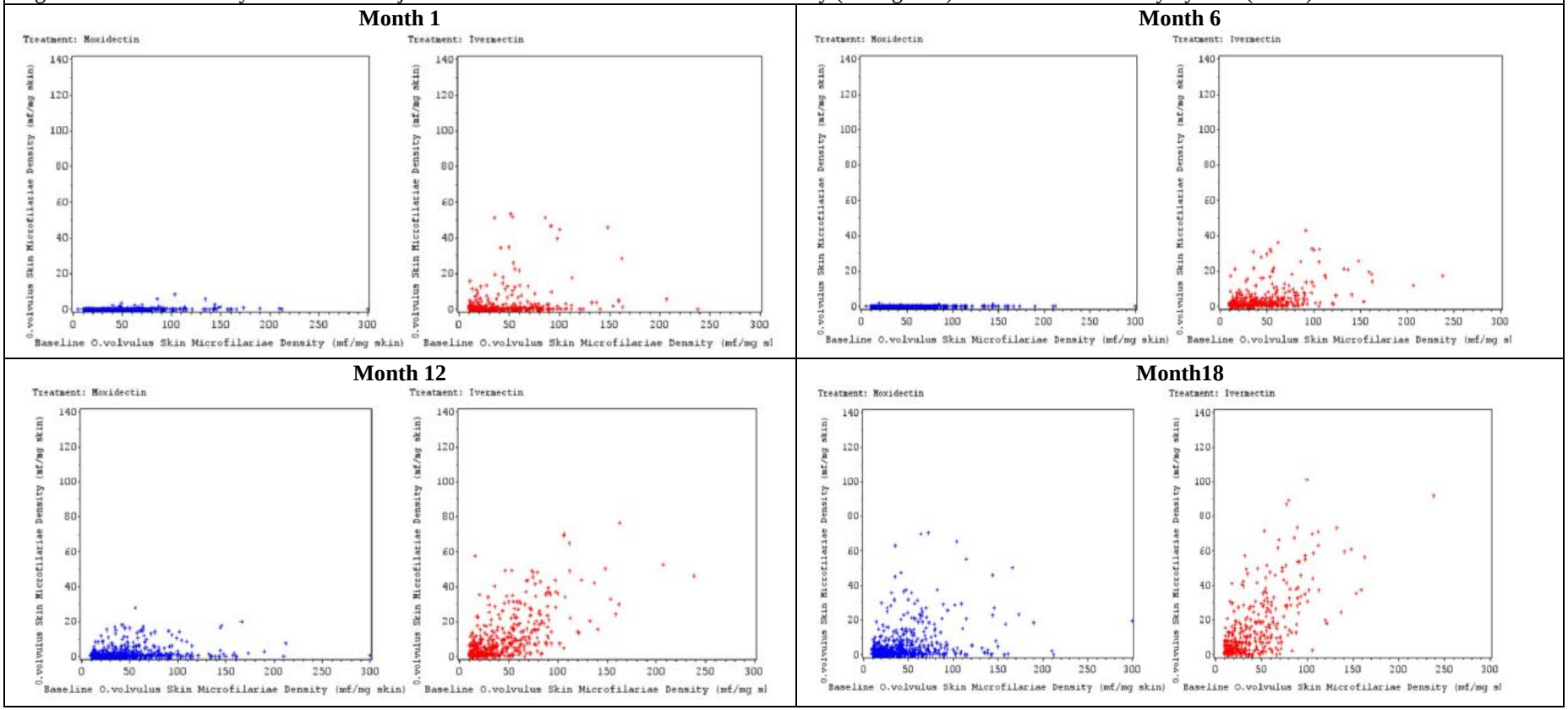
[c] Ratio is derived by back-transforming the difference in LS means on the log transformed data to give a ratio of (X+1)/ (Y+1). Therefore, this will not be identical to the ratio of the LS Geometric Means.

Baseline is defined as the last assessment reported prior to IMP administration on Day 1.

Data were transformed using ln (y+1) before analysis; LS Geometric Means and CIs have been back-transformed to the original units. Note the 1 has been subtracted.

The inter-subject variability appears to be larger in subjects treated with ivermectin compared to moxidectin (Figure 10). Rebound of skin microfilariae occurred in both treatment groups; however, the reappearance microfilaria was slower in subjects treated with moxidectin compared to ivermectin (Table 9 and Figure 10).

Figure 10: Phase III study - Individual subject mean *O. volvulus* skin microfilariae density (mf/mg skin) versus baseline density by visit (mITT)



Effect on ocular microfilariae

Ocular involvement was examined in majority of the subjects. Approximately 40% of the subjects enrolled were microfilariae positive (count > 0) in the anterior chamber of both eyes. Microfilariae count of >10 was reported in 135 (13.8%) subjects in the moxidectin group and 78 (15.7%) subjects in the ivermectin group. There was no change in the proportion of microfilariae positive subjects, in the anterior chamber or cornea, up to Month 1 after treatment with moxidectin or ivermectin; at Month 6 onwards, there appears to be a trend towards an increase in the proportion of microfilariae negative subjects in both moxidectin and ivermectin treated groups (Table 11).

Table 11: Phase III study - Summary of ocular findings in the anterior chamber and cornea of subjects in the e-MITT population

Treatment group	Ocular microfilariae					
	Anterior chamber		Cornea			
	Range	n/N (% negative)	Live	n/N (% negative)	Dead	n/N (% negative)
Baseline/Screening						
Moxidectin	0-102	572/939 (60.9)	0-42	764/939 (81.4)	0-22	876/939 (93.3)
Ivermectin	0-92	281/475 (59.2)	0-29	385/475 (81.1)	0-25	453/475 (95.4)
Day 3						
Moxidectin	0-100	452/813 (55.6)	0-21	701/813 (86.2)	0-38	674/813 (82.9)
Ivermectin	0-120	235/420 (56.0)	0-35	358/420 (85.2)	0-30	346/419 (82.6)
Month 1						
Moxidectin	0-75	660/933 (70.7)	0-17	890/934 (95.3)	0-32	815/934 (87.3)
Ivermectin	0-100	336/472 (71.2)	0-28	450/472 (95.3)	0-28	412/472 (87.3)
Month 6						
Moxidectin	0-20	882/932 (94.6)	0-1	931/932 (99.9)	0-13	921/932 (98.8)
Ivermectin	0-49	452/474 (95.4)	0-9	470/474 (99.2)	0-29	467/474 (98.5)
Month 12						
Moxidectin	0-22	919/937 (98.1)	0	936/936 (100)	0-1	933/936 (99.7)
Ivermectin	0-54	453/474 (95.6)	0-1	473/474 (99.8)	0	474/474 (100)
Month 18						
Moxidectin	0-6	741/751 (98.7)	0-2	750/751 (99.9)	0-2	750/751 (99.9)
Ivermectin	0-77	362/378 (95.8)	0	378/378 (100)	0-1	377/378 (99.7)

N=number of subjects per treatment group; n=number of microfilariae negative subjects

Efficacy against other coincidental helminths and treatment with other anthelminthic drugs

Efficacy against other coincidental helminths was evaluated by species at Month 1 post-treatment. Overall, the cure rates at Month 1 were similar in the two treatment groups (Table 12). However, these results should be interpreted with caution as these subjects were administered other anthelminthic drugs e.g., praziquantel and mebendazole.

Some of the nonclinical and clinical studies (Denham *et al*., 1978[53]; Rivas-Alcala *et al*., 1981[54]; 1984[55]) suggest moxidectin to be effective in reducing microfilaria density. The decrease in

[53] Denham DA, Suswillo RR, and Rogers R. Studies with *Brugia pahangi* 19. Anthelmintic effects of mebendazole. *Trans Roy Soc Trop Med Hyg* (1978) 72 (5) 546-547.

[54] Rivas-Alcala R, Greene BM, Taylor HR, Domiguez-Vasquez A, Ruvalba-Macias AM, Lugo-Pfeiffer C, Mackenzie CD, and Beltran FH. Chemotherapy of onchocerciasis: A controlled comparison of mebendazole, levamisole, and diethylcarbamazine. *The Lancet* (1981) 8245: 485-490.

[55] Rivas-Alcala R, Mackenzie CD, Gomez-Rojo E, Greene BM, and Taylor HR. The effects of diethylcarbamazine, mebendazole and levamisole on Onchocerca volvulus in vivo and in vitro. *Tropenmedizin und Parasitologie* (1984) 35 (2): 71-77.

microfilariae density in the moxidectin and ivermectin treated subjects treated with mebendazole compared to those not treated with mebendazole was similar (for details see clinical and statistics review).

Table 12: Phase III study - Summary of efficacy against other coincidental helminths at Month 1

Species	**Comments**
Mansonella streptocerca	4 subjects (one in the moxidectin and three in the ivermectin group) with microfilariae present at baseline; all 4 subjects became microfilaria negative in the skin.
Strongyloides stercoralis	4 subjects (all in the moxidectin group) and all considered cured.
Schistosoma mansoni[a]	• 146 subjects in the moxidectin arm: 92 (63%) cured. • 68 subjects in the ivermectin arm: 36 (53%) cured.
Soil transmitted helminths	
Whipworm [b]	• 13 subjects in the moxidectin group,10 (77%) were considered cured. • 6 subjects in the ivermectin group, 5 (83%) were considered cured.
Round worm[c]	• 34 subjects in the moxidectin arm, 33 (97%) cured. • 10 subjects in the ivermectin arm: all cured.
Hookworm	• 498 subjects in the moxidectin arm: 234 (47%) cured. • 264 subjects in the ivermectin arm: 76 (29%) cured.

[a]Praziquantel was given to treat *Schistosoma mansoni* in 11 subjects in the moxidectin group and 6 subjects in the ivermectin group in the first month post IMP administration.
[b]Mebendazole was given to treat Hookworm in 47 subjects in the moxidectin group and 39 subjects in the ivermectin group in the first month post IMP administration. Apart from 3 ivermectin subjects and 2 moxidectin subjects this was confirmed in all cases to have been given after the Kato-Katz test had been performed at Month 1.
[c] Mebendazole was given to treat round worm in 1 subject in the moxidectin group and 4 subjects in the ivermectin group in the first month post IMP administration. This was confirmed in all cases to have been given after the Kato-Katz test had been performed at Month 1.

Comments
The methods used for parasitological observations were same as for the Phase II study.

The results of the phase III study suggest that moxidectin is more effective than ivermectin in reducing the skin microfilariae density. A decrease in microfilaria density occurred within Month 1 of treatment and persisted until Month 6 in both groups. At Months 12 and 18 post-treatment, there was a trend towards an increase in microfilariae density as well as a decrease in the number of subjects that remained microfilariae negative. The rebound of microfilariae was slower in moxidectin treated subjects compared to ivermectin treated subjects. A higher proportion of subjects were microfilariae negative in the moxidectin treated group than the ivermectin treated group at all the time points post-treatment. Reduction in microfilariae in the anterior chamber and cornea of the eye was observed at Month 6; also, the proportion of microfilariae negative subjects increased. The efficacy of moxidectin and ivermectin in the ocular region appears to be similar. The reasons for slower and decreased effectiveness of treatment in the eye compared to skin are not known.

5. INTERPRETIVE CRITERIA/BREAKPOINTS

The Applicant has not requested any interpretive criteria in the labeling. This is appropriate as the tests to measure *in vitro* sensitivity of *O. volvulus* parasites are not standardized and their use is limited to research laboratories.

6. THE LABELING

6.1. Applicant's version of the microbiology section of the labeling

12.1 Mechanism of action

(b) (4) (See Microbiology (12.4)).

12.4 Microbiology

Mechanism of Action

(b) (4) excretion of immunomodulatory proteins and (b) (4) the fertility of both male and female adult worms. (b) (4)

(b) (4)

Resistance (b) (4)

(b) (4)

6.2. Comments

Changes are recommended in Sections 12.1 and 12.4 based on the current Division practice, clarity as well as accuracy of the information reviewed.

6.3. FDA's version of the labeling

(Additions marked as double-underlined and deletions as striked out)

12.1 Mechanism of action

Moxidectin, a macrocyclic lactone, is an anthelminthic drug (b) (4) *ee Microbiology (12.4)*~~)~~].

12.4 Microbiology

Mechanism of Action

The mechanism by which moxidectin exhibits its effect against *O. volvulus* is not known. (b) (4) S~~s~~tudies with other nematodes suggest (b) (4) that moxidectin binds to glutamate-gated chloride channels (GluCl), gamma-aminobutyric acid (GABA) receptors and/or ATP-binding cassette (ABC) transporters. This leads to increased permeability, influx of chloride ions, hyperpolarization and muscle paralysis. Additionally, there is a reduction in motility of all stages of the parasite, (b) (4) excretion of immunomodulatory proteins and (b) (4)

the fertility of both male and female adult worms (b) (4)

(b) (4)

Antimicrobial activity

Moxidectin is active against the microfilariae of *O. volvulus [see Clinical Studies (14)]*. Studies suggest that moxidectin is not active against the adult worms, however, it is effective in inhibiting intra-uterine embryogenesis and release of microfilariae from the adult worms.

Resistance (b) (4)

Studies in vitro and infected animals suggest a potential for development of resistance to moxidectin and cross-resistance with other macrocyclic lactones, such as ivermectin. However, the clinical relevance of these findings is not known.

The mechanism of resistance may be multifactorial that include alteration in the target GluCl, GABA receptors and/or ABC transporters.

(b) (4)

[See appended electronic signature page]
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Appendix-1

Summary of studies supporting the *in vitro* activity of moxidectin against filarial species and *Wolbachia*

Parasite stage - Species (Reference) and experimental design	Species	IC_{50}
Microfilariae – Onchocerca species (Tagboto and Towson, 1996[18]) Freshly isolated mf *O. lienalis* (from naturally infected cattle) and cryopreserved *O. volvulus* skin mf (from human volunteers) were added to cultures (50 mf/culture) of LLCMK2 monkey kidney cells (feeder layer) and incubated with 0.1 µM of moxidectin or ivermectin. Culture medium was renewed every 2 to 3 days. The motility of mf was followed for 7 days, using an inverted microscope. The motility of the mf was scored at a scale of 0 to 3: normal (scored as 3), marginally impaired (scored as 2), severely impaired (scored as 1) and immotile (scored as 0). The results were expressed as motility index (MI). The results show that both moxidectin and ivermectin were effective in decreasing the motility of *O. lienalis* and *O. volvulus* mf. Over 96% of the *O. lienalis* and 79% of *O. volvulus* mf were viable in drug free medium over the 7-day period.	*O. volvulus*	IC_{50} not determined. Decrease in motility at moxidectin concentration of 0.06 µg/mL MI: 80% at 4h; 50% between Days 3 and 7.
	O. lienalis	IC_{50} not determined. Decrease in motility at moxidectin concentration of 0.06 µg/mL MI: 60% at 4 h; 22% between Days 3 and 7.
Microfilariae and adult worms – Brugia malayi (Stitt *et al*., 2011[19]) *B. malayi* AW (n=10/culture) were cultured for 14 days in the presence and absence of moxidectin (5-20 µg/mL) and medium, with and without drug, was changed every 2 days. Mf (~50000/culture; isolated from the peritoneal cavity of jirds) were cultured for 30 days. All cultures performed in triplicate. The results show that the motility of AW was significantly reduced ($p < 0.001$) at all concentrations of moxidectin tested in comparison to controls. While motility of non-treated controls (both male and female) continued to decline throughout the course of the experiment, worms were still motile 12 days post-culture. All AW were paralyzed by 8 (females) and 10 (males) days post moxidectin exposure. The motility of mf exposed to 15 and 20 µg/mL moxidectin was significantly lower than that of the non-treated controls, none of the concentrations tested paralyzed or killed mf. All four drug concentrations inhibited release of mf and by Day 8 their release had ceased in all female worms.	*B. malayi*	IC_{50} not determined. Female worms paralyzed by Day 8. Male AW paralyzed by Day 10. Mf motility reduced but not paralyzed
Microfilariae and adult worms - Brugia malayi and Wolbachia (Tomkins *et al*., 2010[20]) *B. malayi* AW (n=3/culture) were cultured in the presence and absence of moxidectin (0.15-5000 µg/mL); medium, with and without drug, was changed every 2 days. All cultures performed in triplicate. The motility of AW was assessed by counting the number of posterior end movements of the AW that occurred in one minute using a microscope that was interfaced with a computer. To begin the measurements, the plate containing the worms was moved to the stage of the microscope. The microscope was focused on one worm, an automatic timer was set for one minute and then the number of movements was recorded using a digital counter. The experiment was terminated when motility had ceased for all experimental groups or if movement of controls differed significantly from that recorded for the previous sampling period. Each experiment was performed in triplicate. The effect of drug on motility of mf released into the medium of AW cultures was measured. Approximately 50,000 mf (isolated from the peritoneal cavity of jirds) were cultured in flasks and 5 mf in 50 µL medium were examined for motility as for the AW. The results show both moxidectin and ivermectin decreased the motility of male and female AW as well as mf (both in culture as well as those released into the culture of female AW until Day 3). There was a decrease in fertility of treated female AW as no mf were produced after Day 3 in culture; however, the uterus was not examined. Compared to female AW, male worms were more sensitive to the drug their motility declined faster, in comparison to females, and males were non-motile by day six. Neither males	*B. malayi*	IC_{50} not determined. On Day 7: All female and male AW immotile at ≥310 µg/mL Mf nonmotile at ≥1250 µg/mL

Parasite stage - Species (Reference) and experimental design	Species	IC_{50}
nor females recovered from paralysis (i.e., after being removed to drug-free medium) and were considered dead. After completion of the motility experiment, AW and mf were frozen and then processed for extraction of total RNA to examine the *Wolbachia* (*wsp*) gene expression by real time RT-PCR. As the density of *Wolbachia* RNA is low in comparison to *Brugia* RNA, 3 adult males and females worms and 10,000 mf were pooled prior to RNA extraction. In cultures not exposed to the drug, the expression of *wsp* gene was higher in female AW compared to male AW and mf. Exposure to the drug decreased the *wsp* expression in all stages of the parasite; such an effect was concentration dependent.		
Microfilariae and adult worms – Brugia malayi (Verma *et al*., 2014[21]) AW and mf of *B. malayi*, isolated from the peritoneal cavity of infected jirds were washed; mf suspension was filtered to remove host cells using 5 µM filter. Viable AW were cultured in 24-well plates (1 male or female worm/well) in the presence or absence of drug for 10 days and medium (with and without drug) replaced every 48 hours. Viable mf (100/well) were cultured in 48-well plates. Duplicate cultures were set up at each concentration (0.15-5.0 µM i.e., 95.97-3.20 µg/mL) and each experiment was repeated three-times. Motility of both the stages of the parasite was observed at every 24 h under an inverted microscope and scored as very high (4+, 0% inhibition in worm motility), moderate (3+, 1–49% inhibition), slow (2+, 50–74% inhibition), paralyzed (1+, 75–99% inhibition) and completely immotile (D, 100% inhibition). IC_{50}, CC_{50} (effect on Vero monkey kidney cell line) and selectivity index (SI) a ratio of CC_{50}/IC_{50}, was calculated. The drugs showing SI ≥ 10 were considered safe for *in vivo* follow up. The adult worms were also processed for viability assay using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye. On Day 10, the AW that became completely immotile were transferred to the fresh drug-free medium (37°C for 2 hours) to confirm the non-reversal of the motility. Parasites in control cultures were motile until Day 10. However, within 2 and 5 days of culture with 5 µM (3.20 µg/mL) moxidectin inhibited the adult female and male worm motility; mf motility was inhibited by Day 7. Lower concentrations were also effective in inhibiting motility between Days 3-10. The CC_{50} against Vero cells was 22.6 µM (14.46 µg/mL). The SI at the IC_{50}s was higher against the AW (93.5 against the female worms 121.7 against the male worms) compared to that against mf (27.8). The moxidectin and ivermectin IC_{50}s were comparable against the AW and mf. The moxidectin SIs were lower than the ivermectin SIs; this may be due to higher ivermectin CC_{50}s against the Vero cells.	*B. malayi*	Based on motility: Female AW: 0.24 µM (153.56µg/mL) Male AW: 0.19 µM (121.57 µg/mL) Mf: 0.81µM (518.25 µg/mL)
mf=microfilariae; AW=adult worm; h=hour; IC_{50}=50% inhibitory concentration; CC_{50}=50% cytotoxicity)		

Appendix-2

Summary of nonclinical studies supporting activity of moxidectin in animals infected with filarial species

Parasite species (Reference)	**Study summary**
	Rodents
Onchocerca volvulus *Onchocerca lienalis* (Tagboto and Towson, 1996[18])	CBA/Ca mice were infected S/C with 5000 **mf** of *O. volvulus* or *O. lienalis.* Treatment with different doses of moxidectin or ivermectin was initiated on Day 3 PI, by either oral or S/C route. Drugs were administered either as a single dose on Day 3 or 5 daily doses. On Day 18 PI, mice were necropsied and ears processed for measuring intensity of mf. The results show that a single dose of moxidectin and ivermectin, administered orally or S/C, was effective in reducing *O. volvulus* or *O. lienalis* mf; such an effect was dose-dependent. At a low dose of 1.5 µg/kg, moxidectin was more effective than ivermectin; no mf was observed at doses of ≥15 µg/kg of either of the drugs in *O. lienalis* infected mice. In mice infected with *O. volvulus* mf, few residual mf (~2-3 in moxidectin and ~15 in ivermectin groups) were reported at a dose of 15 µg/kg; higher doses were not tested. Mf of *O. lienalis* appear to be more sensitive to both drugs than *O. volvulus.* In mice treated for 5 days, moxidectin was more effective than ivermectin, between the doses of 0.2 and 3.2 µg/kg, in reducing *O. lienalis* or *O. volvulus* mf density. In another experiment, treatment was administered on either Day -28, -16, -4, or 3 PI in mice infected with *O. lienalis*. The results show that moxidectin at a dose of 150 µg/kg administered on Day 28 or 16 prior to infection was more effective than ivermectin in clearing mf. However, the activity of both moxidectin and ivermectin was similar when administered on Day -4 or 3 PI. Similar experiment was not done in *O. volvulus* infected mice.
Brugia malayi (Verma *et al.*, 2014[21])	Jirds (*Meriones unguiculatus* Milne-Edwards): **AW** (10 female and 5 male) were transplanted into the peritoneal cavity. On Day 7, a drop of peritoneal fluid was aspirated to check for the presence of mf. Mf^{+ve} jirds were treated S/C or orally with a single dose of moxidectin (10-40 mg/kg). Jirds were necropsied on Day 50 and the peritoneal cavity examined for the presence of residual parasites (mf and AW) and their motility. Mastomys (*Mastomys coucha* Smith): Approximately 100 **infective larvae (L_3)** were inoculated S/C. Mf^{+ve} animals were treated, S/C, 5 to 8 months later with a single dose (20 mg/kg) of moxidectin. The mf in blood were measured prior to treatment and on post-treatment Days 8 and 15 and every other week until Day 90. Animals were necropsied on Day 90 and different tissues (lungs, heart, testes, lymph nodes) examined for the presence of AW. The recovered females were teased individually in a drop of PBS to observe embryostatic effect, if any. Experiments were performed in triplicate and each group consisted of 5-6 animals. The results show that moxidectin was effective in reducing the number of residual AW, their motility and viability, as well as the mf density by 40%-60% in jirds; motility of mf in the peritoneal cavity was reduced and about 15% of the mf were dead. Degenerative changes were observed in about 60-70% female AW from treated animals, that include deformed and degenerated eggs and embryos in the uteri. In Mastomys, moxidectin was effective in reducing the AW by 50%. Embryogenesis of female worms decreased by 54%. The decrease in mf density in blood gradually increased from Day 7 (18%) to Day 90 (71%). In animals treated with diethylcarbamazine, the mf density decreased between Days 7 and 45; this was followed by an increase.
Brugia pahangi (McCall, 1999[26])	Jirds were infected with 100 **L_3** by S/C route and oral treatment with different doses (125, 500, or 2000 µg/kg) of moxidectin initiated 10 weeks PI. Jirds were treated every 4 weeks for 1, 3, 6, or 12 treatments. Blood was collected for measuring mf density at different time intervals. Animals from each dose group as well as control group were necropsied at weeks 8, 16, 24, and 348 after initiation of treatment. The results show a reduction in mf and AW count that was dose-dependent.
Litomosoides sigmodontis (Breton *et al* 1997[28])	*L. sigmodontis*, previously known as *L. carinii*, is maintained in the experimental host, the jird *Meriones unguiculatus.* The experimental vector is the mite *Ornithonyssus bacoti.* Rodents are inoculated with 25 to 200 **L_3**. AW live in the pleural cavity, and less commonly in the peritoneal cavity. The prepatent phase lasts over six months, and in blood, mf densities are high from the second month PI. Four months PI, when the mf density was increasing or high and stable, S/C treatment with moxidectin (0.2 mg/kg) was initiated, as a single dose or for 5 days. Jirds were followed for mf in blood and necropsied on Days 42-90 after treatment; the coelomic cavities examined for AW. Tissues were processed for histopathological examination.

Parasite species (Reference)	Study summary
	The results show that treatment with moxidectin or ivermectin decreased mf; no mf was detected on Day 2 post-treatment; however, the animals became mf^{+ve} at Day 14 (remained at about 25% of its initial value until Day 70, then decreased to 6% at Day 90). There was a reduction in the number of AW recovered compared to the control group; male worms were normal suggesting no effect on male AW. However, abnormal embryogenesis was reported in 80% of the female worms. Ivermectin appears to be less effective in inducing worm damage although there was a reduction in the number of worms recovered. All treated and control rodents had similar lesions and microfilarial localizations (mainly intra-lymphatic) and densities. Most of the lesions belonged to the inflammatory process.
Monanema martini (Breton *et al* 1997[28])	*M. martini* is maintained in its natural host, the African murid rodent *Lemniscomys striatus.* It has dermal microfilariae and induces *Onchocerca*-like lesions in its host. The experimental vector is the hard tick *Hyalomma truncatum.* Animals were inoculated with 80 **L_3**, exceptionally with 15, 30 or 60 L_3. The filarial worms live in the lymphatic vessels of the large intestine and caecum, however, a small proportion of about 4% can be recovered in the right heart and pulmonary arteries. Mf are concentrated in the ear pinna. The prepatent phase lasts two months, and the patent phase at least one year. Microfilariae densities are maximal between the sixth and ninth month post-inoculation. Animals were selected for experimentation when the mf density was increasing or high and stable, i.e., 5 ± 1 months PI. Treatment with moxidectin (5 mg/kg) and ivermectin was as summarized above for *L. sigmodontis* infected jirds and mf density was measured in the skin (ear pinna). Necropsies were performed on Days 42-90 after treatment and different tissues examined for worms and histopathological evaluations. The results show no effect on mf during the 42-day period post-treatment with a single dose moxidectin (single dose) or ivermectin (single dose or 5-day treatment). There was a reduction in the number of AW recovered compared to the control group; male worms were normal whereas abnormal embryogenesis was reported in 80% of the female worms. Ivermectin appears to be less effective in inducing worm damage although there was a reduction in the number of worms recovered. All treated and control rodents had similar lesions and microfilarial localizations (mainly intra-lymphatic) and densities. Most of the lesions belonged to the inflammatory process.
	Dogs
Brugia pahangi (McCall, 2001[27])	Beagle dogs were infected S/C with 100 **L_3** of *B. pahangi* and blood was collected at frequent interval for the presence of mf. Treatment with a single dose of moxidectin (250 µg/kg) or ivermectin (250 µg/kg) orally was initiated in mf^{+ve} dogs (~3.5 months PI); dogs with evidence of lymphedema were excluded. Blood was collected for determining mf density at regular intervals until Day 333 after initiation of treatment. Dogs were necropsied on Day 334 after treatment and any live or dead worms from tissues were counted. The results show that moxidectin was more effective than ivermectin in reducing mf density in the blood; decrease in mf was observed within 24 h of treatment and continued to decrease in treated dogs. Six of the 8 dogs treated with moxidectin became mf^{-ve}; none of the ivermectin treated dogs became mf^{-ve} whereas one control group dog became mf^{-ve}. The number of dogs with live worms at Day 334 post-treatment was reduced in the moxidectin treated group (1/8) compared to ivermectin (4/8) and control (5/8) groups. Most of the dogs harboring live worms at necropsy had a low intensity of infection (<10 worms).
Dirofilaria immitis -Heart worm (Glickman *et al*., 2006[30])	Moxidectin and ivermectin were reported to be effective in the **prevention of heartworm infection**. This is based on the nationwide, post-marketing, epidemiological study using >11 million electronic medical records for dogs visiting >500 Banfield veterinary hospitals. The authors state that the effectiveness lasts longer in dogs after discontinuation of moxidectin compared to ivermectin.
Dirofilaria repens -Heart worm (Genchi *et al*., 2010[29])	Beagle dogs were tested for circulating *D. repens* and *D. immitis* microfilariae by a concentration test (Knott's test) and by a commercial ELISA kit Canine Heartworm Antigen Test Kit (IDEXX PerChekTM HTWM PF, IDEXX, USA) to exclude natural filarial infections. Mf and antigen negative dogs were administered microsphere sustained release formulation of moxidectin (0.17 mg/kg) or saline. After 180 days of drug administration, dogs were inoculated with 50±5 **L_3**. Animals were euthanized on Day 380 (7 months PI) and tissues examined for the presence of pre-adult and AW. Skin was immersed in warm water and both tissues and water examined for the presence of parasites. The results show that no parasites were detected in treated dogs whereas AW were found in all the dogs in the control group suggesting prophylactic effect of moxidectin. It appears that the presence of mf was not examined.

Parasite species (Reference)	Study summary
Calves/cattle	
Onchocerca ochengi (Njongmeta *et al*., 2004[22])	The effect of moxidectin as a prophylactic agent was measured **in naturally infected calves** in Cameroon. This model has similarities to *O. volvulus* as the AWs worms inhabit intradermal nodules and multiple infections occur; sequential nodulectomies can be done to reveal the kinetics of the response of the AW to drugs. In phase 1 of the study, moxidectin (200 µg; n=10 per group) or ivermectin (150 µg; n=10 per group) were administered either monthly or every 3 months for 22 months after exposure (Phase 1). In the second phase of the study, the non-treated control group (n=14), was replaced by a new group of naive (non-exposed) calves control-2 (n=8 per group), which had been reared in fly-proof accommodation from birth and treated S/C with a single dose of 150 µg/kg ivermectin at two months of age. Exposure of the naïve controls started when they were aged 5 months, and 3 months after the last drug treatment in the previously exposed cattle; this point was designated as time zero. *O. ochengi* nodule and mf load was determined by implanting a transponder S/C adjacent to a tattooed nodule if palpated on a third consecutive examination. Every month for the first 12 months, and thereafter at 3-monthly intervals, skin mf prevalence and density were determined. At the end of the experiment, 10% of total nodules on each animal (but not more than 10 nodules per animal) were stab-incised, their contents squeezed into 4% formal saline, and the identity of the female worms confirmed by microscopic examination. The results show that moxidectin administered either monthly or every 3 months was effective in suppressing the development of nodules; no mf were detected. Control group of animals developed nodules and were mf^{+ve} in phase 1 of the study; the minimum times to first detection of nodules and mf were 10 and 12 months post-exposure (m.p.e.), respectively. After discontinuation of treatment (Phase 2 study), the prevalence of nodules and mf density at 24 m.p.e. was less in moxidectin (88-90%) treated calves compared to control (100%) or ivermectin (100%) treated calves.
Onchocerca ochengi (Langworthy *et al*., 2000[23])	**Naturally infected cattle**, with over 20 *O. ochengi* nodules, were treated with either moxidectin (200 µg/kg: single dose or once a month for 7 months) or oxytetracycline (10 mg/kg, twice weekly for 3 weeks; after a 6 week pause 20 mg/kg was administered twice weekly for 2 weeks and after a 4 week pause once a month for 4 months). Four nodules from each animal and the AWs examined for motility by the MTT assay, embryogenesis and presence of micro-organisms by electron microscopy or by *Wolbachia* gene expression and histological evaluation. Skin were processed for determining mf density. Moxidectin was effective in eliminating mf from the skin and embryogenesis; the authors state that there was no effect on nodule diameter, worm number, worm motility or MTT reduction up to 12 months post-treatment (data not shown). Oxytetracycline reduced the motility of AW and this was related to reduction in *Wolbachia.*
Horses	
Onchocerca cervicalis (Monahan *et al*., 1995[24])	**Naturally infected,** mf^{+ve} (in skin snips) ponies, in southern Louisiana or Mississippi, were treated with a single oral (as a gel, behind the tongue) dose [300 (n=4), 400 (n=10), 500 µg/kg (n=4)]; all ponies were mf^{-ve} on Day 14. The 5 untreated ponies remained mf^{+ve}.
Onchocerca cervicalis (Mancebo *et al*., 1997[25])	**Naturally infected** mf^{+ve} (skin snips) horses in the northeast Province of Formosa (Argentina), were treated with either 2% oral gel moxidectin (0.4 mg/kg; n=20), ivermectin (0.2 mg/kg; n=20); 5 horses were untreated and served as control. In the moxidectin treated group, 18 of the 20 horses were mf^{-ve} and mf were reduced to 1-2 in 2 horses by Day 14; the outer structure of mf in 2 horses was altered that include a rough surface and disintegration - these horses were mf^{-ve} on Day 21. All ivermectin treated horses were mf^{-ve} on Day 14. All the control group horses remained mf^{+ve} although there was a slight reduction in mf count: mean 430 (Day 0) to 330 mf/g (Day 14).
Mf=microfilariae; AW=adult worms; S/C=subcutaneous; PI=post-infection	

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/s/

SHUKAL BALA
03/14/2018

AVERY C GOODWIN
03/14/2018