

## Review

## Ivermectin: An Anthelmintic, an Insecticide, and Much More

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Here we tell the story of ivermectin, describing its anthelmintic and insecticidal actions and recent studies that have sought to reposition ivermectin for the treatment of other diseases that are not caused by helminth and insect parasites. The standard theory of its anthelmintic and insecticidal mode of action is that it is a selective positive allosteric modulator of glutamate-gated chloride channels found in nematodes and insects. At higher concentrations, ivermectin also acts as an allosteric modulator of ion channels found in host central nervous systems. In addition, in tissue culture, at concentrations higher than anthelmintic concentrations, ivermectin shows antiviral, antimalarial, antimetabolic, and anticancer effects. Caution is required before extrapolating from these preliminary repositioning experiments to clinical use, particularly for Covid-19 treatment, because of the high concentrations of ivermectin used in tissue-culture experiments.

### Ivermectin, the Anthelmintic and Insecticide

Ivermectin is a mixture of more than 80% 22,23-dihydroavermectin B1a and B1b (Figure 1). It is a remarkably potent anthelmintic and insecticide when given orally at therapeutic doses of 150 or 200 µg/kg to ruminants, pigs, horses, or humans where it yields  $C_{max}$  plasma concentrations of 11–54 ng/ml or 13–63 nM [1,2]. It is generally safe, with acute  $LD_{50}$  (see Glossary) toxicities seen at 24 000 µg/kg in monkeys [3] and 80 000 µg/kg in beagles [4]; it has a wide spectrum of action against gastrointestinal (GI) parasitic nematodes, lungworms, lice, and mange, but it is not effective against cestodes or trematodes. It is, however, very effective at low plasma concentrations against microfilaria, and it impairs adult filaria fertility for long periods without killing most adult filaria. Here we review ivermectin's mechanism of action as an anthelmintic, as an insecticide, and recent studies that have sought to reposition ivermectin as an antiviral, antimalarial, and anti-diabetic drug (Table 1).

### Standard Theory of the Ivermectin Mode of Action

#### Ivermectin and Analogs Are Allosteric Modulators of GluCl Ion Channels

Ivermectin and its analogs are **positive allosteric modulators (PAMs)** that selectively open inhibitory glutamate-gated chloride ion channels in the membranes of pharyngeal muscles, motor nerves, female reproductive tracts, and the **excretory/secretory (ES)** pores of nematodes and of muscle and nerves of insects and crustaceans [5,6]. The effect is: (i) inhibition of pharyngeal pumping (Figure 2) when the pharyngeal muscle is the target; (ii) inhibition of motility when motor nerves are the main target; (iii) inhibition of egg or microfilaria release when the female reproductive tract is the target site; and (iv) loss of host immunosuppression when the ES pore cannot open to release host immunosuppressants.

Figure 2A–E shows a two-micropipette recording of the effects of glutamate on membrane potential and the conductance of pharyngeal muscle in *Ascaris suum*. When glutamate is added to the preparation, **glutamate-gated chloride (GluCl) ion channels** open, the resistance of the pharyngeal muscle decreases, and the membrane potential hyperpolarizes as

### Highlights

Ivermectin and analogs are remarkable broad-spectrum anthelmintics and insecticides, but resistance is now a real concern. Resistance mechanisms have been proposed but they do not appear to account for the resistance seen in parasitic nematodes.

Ivermectin is very effective for controlling microfilaria at low doses but it has limited effects on adult filaria for unclear reasons.

Ivermectin is a positive allosteric modulator of glutamate-gated chloride channels found in both nematodes and insects, and it binds to the channels in their lipid phase.

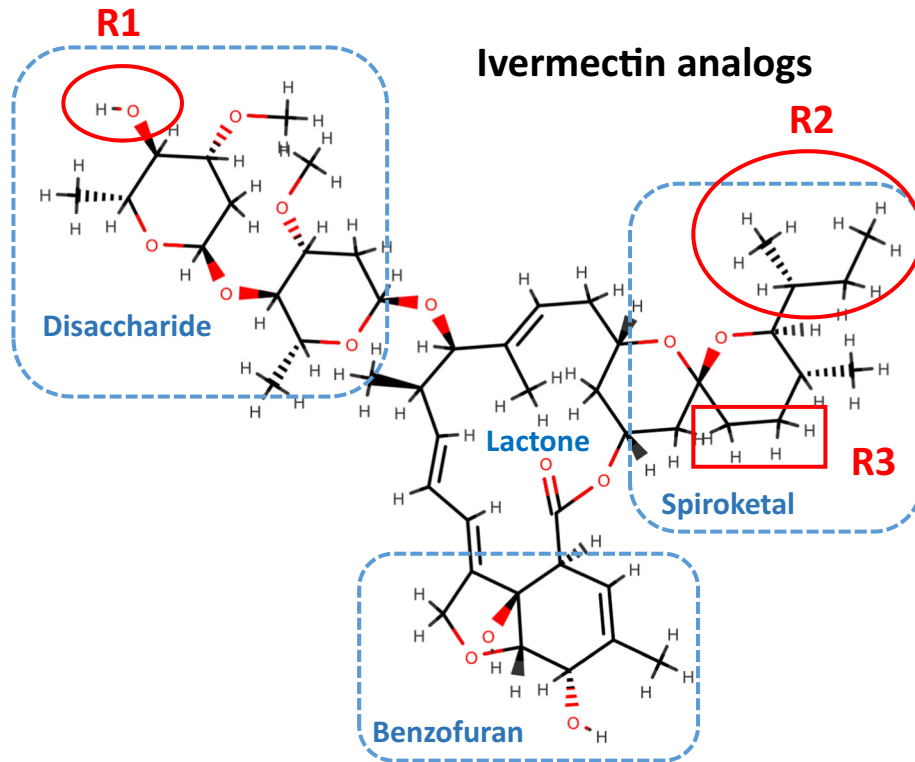
Ivermectin and analogs also modulate other ion channels and have effects on the mammalian host brain when the blood–brain barrier is impaired.

Preliminary repositioning studies of ivermectin show antiviral, antimalarial, antimetabolic, and anticancer effects at concentrations higher than anthelmintic concentrations in tissue culture.

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Drug	R1	R2	R3
Ivermectin	-OH	-CH(CH <sub>3</sub> ) <sub>2</sub> and -CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -
Abamectin	-OH	-CH(CH <sub>3</sub> ) <sub>2</sub> and -CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	-CH=CH-
Doramectin	-OH	Cyclohexyl	-CH=CH-
Eprinomectin	-CH <sub>3</sub> CONH	-CH(CH <sub>3</sub> ) <sub>2</sub> and -CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	-CH=CH-
Emamectin	-CH <sub>3</sub> NH	-CH(CH <sub>3</sub> ) <sub>2</sub> and -CH(CH <sub>3</sub> )C <sub>2</sub> H <sub>5</sub>	-CH <sub>2</sub> =CH-
Moxidectin	Aglycone	-CH=CHCH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub>	-C=C(NOCH <sub>3</sub> )-
Milbemycin D	Aglycone	-CH(CH <sub>3</sub> ) <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -

#### Trends in Parasitology

**Figure 1. Structure of Ivermectin and Analogs.** The molecule is divided into four regions: the disaccharide, the lactone, the spiroketal, and the benzofuran. Different modifications to this structure at the positions R1, R2, and R3 give rise to ivermectin, abamectin, doramectin, eprinomectin, emamectin, moxidectin, and milbemycin.

Cl<sup>-</sup> enters the pharynx (Figure 2D,E). This is reversible on washing. Ivermectin behaves similarly, but is slower in onset, and because it is lipophilic (sticky) it does not wash off from the preparation after application (Figure 2F). When ivermectin is applied to active pumping pharyngeal muscle cells of *Trichostrongylus colubriformis*, the electropharyngeogram shows the reduction in electrical activity (Figure 2G).

#### Glossary

**Allosteric site:** a binding site on the GluCl receptor channel that is not the same site on the receptor as the glutamate agonist. Ivermectin binds to the allosteric site to increase the opening of the channel.

**EC<sub>50</sub>:** the concentration of a drug that produces 50% of the maximum response.

**Excretory/secretory (ES):** nematodes have an esophageal gland near the head region that releases ES substances; they include a range of active substances that, in animal parasitic nematodes, have immune-suppressant actions, anticoagulant effects, protease inhibitory effects, and microvesicles containing RNA.

**γ-aminobutyric acid (GABA):** an inhibitory transmitter that binds to pentameric transmembrane ion channels to open them; the channels then conduct Cl<sup>-</sup> ions to hyperpolarize the cell. These ion channels are referred to as GABA receptors; they are present on mammalian nerve cells and on nematode muscle and nerve cells.

**G-protein-coupled inwardly rectifying potassium (GIRK) channels:** when potassium ion channels open they hyperpolarize the cell as K<sup>+</sup> leaves the cell, producing inhibition (relaxation of muscle cells and reduced electrical activity of nerve cells). There are a number of different types of potassium ion channel. The GIRK channel is coupled to a G-protein receptor. Its current voltage relationship shows inward rectification, allowing potassium to move into the cell more easily than out of the cell.

**Glutamate-gated chloride (GluCl) ion channels:** recognized target sites of ivermectin. They are pentameric Cl<sup>-</sup> ion channels that are opened by the transmitter, glutamate, and by an allosteric effect of ivermectin. They are present only in invertebrates (insects, crabs, and nematodes) – not in flukes or tapeworms or mammalian hosts.

Opening of the GluCl channels by ivermectin produces inhibition of muscle, nerve, and other tissues. Eight genes, identified in nematodes, encode different subunits of the pentameric GluCl ion channels. These genes are referred to as: *avr-14*, *avr-15*, *glc-1*, *glc-2*, *glc-3*, *glc-4*, *glc-5*, and *glc-6*.

**Glycine receptors (GlyRs):** receptors present on host nerve cells in the brain and nerve ganglia, but not in the nematode parasite. They are Cl<sup>-</sup> ion-

Table 1. Experimental Settings for Studying the Effects of Ivermectin on Ion Channels, FXR, NHR-8, Viruses, Malaria, Cancer, and Asthma

Target	Experimental preparation	Concentration: ng/ml (nM)	Refs and comment
AVR-15b <i>Haemonchus contortus</i> channel opening	HEK293 expressed	EC <sub>50</sub> 19 (22 nM)	[28]
Abamectin on GluCl <sub>s</sub> of <i>Ascaris suum</i> pharynx inhibition	Dissected whole worm pharynx	EC <sub>50</sub> 350 (400 nM)	[37]
<i>Brugia malayi</i> mf protein release, ES inhibition by Bma-AVR-14A GluCl	Whole <i>Brugia</i> and microfilaria	<88 (<100 nM)	[31]
Downregulation of <i>B. malayi</i> meiosis genes	Whole <i>Brugia</i>	<88 (<100 nM)	[8]
<i>A. suum</i> GABA ion-channel inhibition	Dissected preparation	<175 (<200 nM)	[27]
Histamine-gated Cl channel activation	Expressed channel from <i>Drosophila melanogaster</i>	875 (1000 nM) used	[33]
pH-gated Cl channel activation	<i>Sarcoptes scabiei</i> oocyte expressed receptor	Single conc 10 000 (11 429 nM)	[34]
Nematode nAChRs	Expressed channels and <i>A. suum</i> preparation	EC <sub>50</sub> 368 (420 nM)	[35,37]
FXR activation and transcription	FXR and ivermectin Crystal structure and mice, serum glucose and cholesterol decrease	1300 µg/kg per day for 14 days	[69–72] Repurpose or find more potent analog
NHR-8 transcription factor	NHR-8 of <i>Caenorhabditis elegans</i> and <i>H. contortus</i>	Knockout in <i>C. elegans</i> reduced ~2 the EC <sub>50</sub> for the larval development assay (from 1.63 to 0.96 nM)	[104] Low level of resistance
Dengue; West Nile virus; Venezuelan equine encephalitis virus inhibition	Experiments used tissue culture and showed virus inhibition was associated with the inhibition of transport by nuclear transporter IMPα/β1	IC <sub>50</sub> s 875–3500 (1000–4000 nM)	[55–61] More potent analog required to be effective
SARS-Covid-2 inhibition	Tissue culture	4375 (5000 nM), ~ 100x	[107]
<i>Plasmodium</i> inhibition	Culture: inhibition of <i>Plasmodium</i> in liver inhibits nuclear import of signal recognition particles of <i>P. falciparum</i>	IC <sub>50</sub> ~438 (~500 nM)	[65–67] More potent analog required to be effective. Ivermectin has effects on mosquitoes
Cancer	Tissue culture and mice experiments by different mechanisms	<8000 nM	[76,77,79–83,105] High concentrations required for effects
Asthma	Mice: reduction of cellular and humoral responses to antigen in asthma model	2000 µg/kg	[89] High µg/kg dose

Although equivalent recordings from the nerves, reproductive tract, and the ES pore of parasitic nematodes have not been made it is expected that similar observations would be made from all their tissues that have GluCl channels. The different tissues in the different parasitic nematodes have molecularly diverse GluCl channels and therefore different sensitivities to ivermectin. The most ivermectin-sensitive tissues, and the phenotypic effects of ivermectin, vary with the parasite species.

channel receptors that are opened by the transmitter glycine. Glycine, as an inhibitory transmitter, binds to the pentameric glycine transmembrane ion channels to open them; the channels then conduct Cl<sup>-</sup> ions to hyperpolarize the cells.

**hERG:** the alpha subunit of the ether-a-go-go potassium ion channel. This is another type of potassium channel that repolarizes the cardiac action potential. It is sensitive to some xenobiotic drugs that produce cardiac arrhythmias.

**IC<sub>50</sub>:** the concentration of a drug that inhibits 50% of the maximum response.

**LD<sub>50</sub>:** the dose, usually µg/kg or mg/kg, that has been measured to kill half the population of the test animals. LD<sub>50</sub> usually refers to a single oral dose but the conditions (acute, chronic, animal, and mode of administration) will affect the LD<sub>50</sub> value.

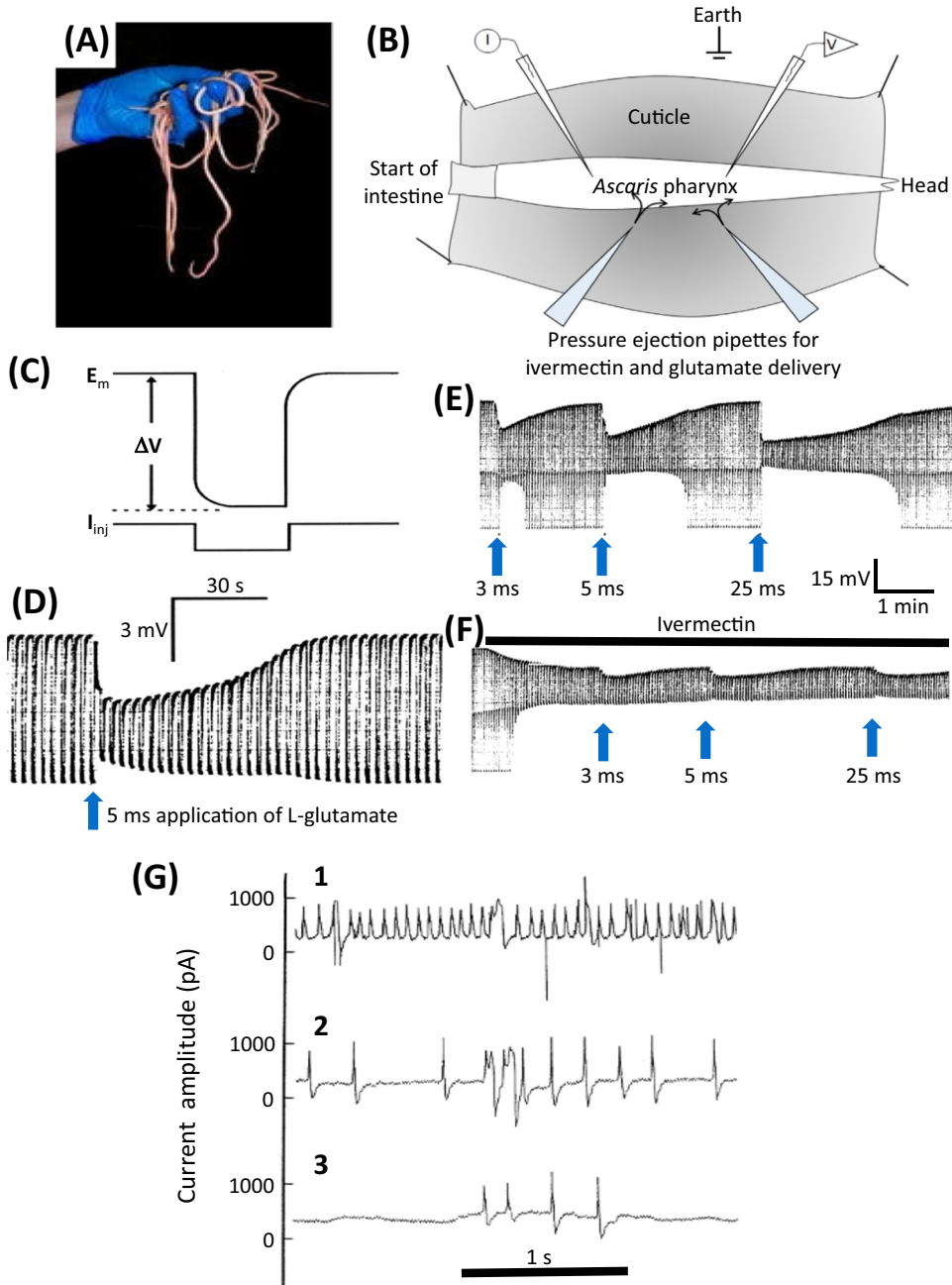
**Mass drug administration (MDA):** a strategy used to control neglected tropical diseases by treating entire at-risk populations with a chemotherapeutic agent, like the use of ivermectin to control onchocerciasis. Usually the administration is made once or twice a year. It can result in a maintained reduction of the diseases.

**P-glycoprotein (Pgp):** a plasma membrane transporter that acts on xenobiotic drugs to export them out of the cells. There are many Pgps, each having a selective preference for the xenobiotic that they export. They are energy-dependent and require ATP.

**Positive allosteric modulators (PAMs):** pharmacological receptors can be activated by agonists binding to the normal physiological orthosteric site or they may be activated by ligands binding to another, usually nonphysiologic site known as an allosteric site. A PAM is such a ligand; it binds to the allosteric site to activate the receptor.

**Quantitative trait loci (QTL):** a section of DNA, on a chromosome, that correlates with a quantitative phenotypic trait (motility, drug-resistance, size, etc.) of a population of nematode parasites (or other organisms).

**Ryanodine receptor (RyR):** an intracellular calcium-permeable channel found in the membrane of the endoplasmic reticulum or sarcoplasmic reticulum of cells. It is a major cellular mediator of calcium-induced calcium release in animal cells and is activated pharmacologically by ryanodine.



**Signal recognition particles (SRPs):** conserved ribonuclear proteins that recognize and target translating proteins to the protein-conducting channel (translocon) in the endoplasmic reticulum in eukaryotes during protein synthesis.

**Figure 2. Electrophysiologic Effects of Ivermectin in Parasitic Nematodes.** (A) *Ascaris suum* is a large nematode that is tractable for electrophysiologic recordings. (B) A diagram of the dissected *A. suum* pharynx. Two glass micropipettes are placed in the pharynx, one for injecting rectangular current pulses (I) and one for recording the membrane potential (V). Two ‘puffing’ microelectrodes are used to apply, separately, glutamate or ivermectin at different time intervals. (C) Higher time resolution recording showing the injection of a hyperpolarizing rectangular current pulse ( $I_{inj}$ ) and the membrane potential ( $E_m$ ) and response to the injected current ( $\Delta V$ ) allowing the input conductance ( $= 1/resistance$ ) of the pharynx to be measured. (D) A 5 ms puff of 0.5 M glutamate produces a hyperpolarization due to the entry of  $Cl^-$  (upper envelope of the trace) and a reduction in the width of the trace as the size of the membrane potential change ( $\Delta V$ ) is reduced as the glutamate-gated chloride (GluCl) channels, once activated, open and increase the conductance of the pharyngeal muscle membrane. (E) Effects of glutamate. The trace shows

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Trends in Parasitology

Li *et al.* [7] used *in situ* hybridization to localize tissue expression of the *Brugia malayi* GluCl channel gene *avr-14* (*Bma-AVR-14*) in adult filarial worms. Splice variant subunits of *Bma-AVR-14* were expressed in female worms in the ovary, in developing embryos, in the lateral hypodermal chords, and in the uterus wall adjacent to stretched microfilariae. The genes were also expressed in adult male worms in the spermatogonia, in the vas deferens, and in the somatic muscles adjacent to the vas deferens. The tissues of the ovary and other reproductive cells are nonexcitable, so the GluCl channels may serve a paracrine function, inhibiting the growth and development of these tissues like other ligand-gated ion channels in the nonexcitable tissues of vertebrates. The activation of GluCl channels in the reproductive tracts of nematodes, by a paracrine function, could change gene expression producing the longer-term inhibitory effects of ivermectin on the developing embryos and reproductive tissues of filaria and suppression of microfilaria production by female worms. The significance of the effect of ivermectin on the reproductive tract was also shown by RNAseq analysis that revealed downregulation of meiosis genes in female *B. malayi* by low (100 nM–1  $\mu$ M) concentrations of ivermectin [8]. The evidence of GluCl channel expression in the nematode reproductive tract of filaria may explain the effects of ivermectin reducing their fertility but not why this effect is so slow in onset. It is pointed out that this effect is prolonged and persists long after the ivermectin has been cleared from the host and is no longer detectable.

#### Composition of the GluCl Ion Channels

GluCl ion channels consist of a ring of five protein subunits that may arrange in homogeneous or heterogeneous combinations (Figure 3C). In the free-living nematode *Caenorhabditis elegans*, there are six GluCl genes encoding channel subunits – *avr-14*, *avr-15*, *glc-1*, *glc-2*, *glc-3*, and *glc-4* – that contribute to the ivermectin sensitivity in *C. elegans* [9–14]. The GluCl channel subunit gene family is divergent in parasitic nematodes and varies between the species. In the intestinal parasite of sheep, *Haemonchus contortus*, and in the human hookworms *Ancylostoma ceylanicum* and *Necator americanus*, there are no *glc-1* nor *avr-15* orthologs<sup>1</sup>. *glc-1* is not present and is also missing in the filarial parasites *B. malayi* and *Onchocerca volvulus*. In *H. contortus* the subunit genes known to be present are: *avr-14*, *glc-2*, *glc-3*, *glc-4*, *glc-5*, and *glc-6* [15]. Thus, different nematode species have different GluCl channel gene homologs.

The sensitivity to ivermectin varies between the different homomeric and heteromeric GluCl channels. A few amino acid changes in the sequence of any of the subunits may alter the ivermectin sensitivity of the channel significantly. It is not surprising that there are striking differences, even between closely related species: *A. ceylanicum* is much more sensitive to ivermectin than the new world hookworm, *N. americanus*, both *in vitro* and *in vivo* [16,17]. Other explanations for the difference in sensitivity are also possible.

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the membrane potential and input conductance responses to 3 ms, 5 ms, and 25 ms 'puffs' of 0.5 M glutamate from a micropipette, which produces maximal changes in input conductance of 16  $\mu$ S, 33  $\mu$ S, and 60  $\mu$ S, respectively. Longer applications of glutamate did not produce an increase in response in this experiment, showing that the 25 ms puff led to a maximum response. F) The effect of a continuous 'puff' of 100  $\mu$ M ivermectin applied also by pressure ejection from a micropipette shows that ivermectin mimics the effect of glutamate but has a slower onset. Once the conductance change produced by the ivermectin reached a stable position of 32.7  $\mu$ S, glutamate was again applied in the same controlled way using the same application times; this produced a maximum conductance change of 28  $\mu$ S with a 25 ms puff application. The maximum conductance change obtained with the coapplication of glutamate and ivermectin was 60  $\mu$ S, virtually the same conductance (cf. 60  $\mu$ S) change produced after a high-dose application of glutamate. Co-application of glutamate and ivermectin did not produce an effect greater than the high dose of glutamate alone. This was interpreted as indicating that these two substances activate the same ion channel. If there were two separate ion channels, one activated by glutamate and another activated by ivermectin, then these high concentrations would have produced an additive response. (G) Electropharyngeograms from *Trichostrongylus colubriformis* before, 5 min after, and 15 min after adding 100 nM ivermectin to the bath (from [106]). A patch-pipette was used to record the currents elicited by the pumping pharynx by sucking the head of the worm into the patch-pipette. (1) Control recording before application of 100 nM ivermectin; (2) 5 min after application of 100 nM ivermectin; (3) 15 min after application of 100 nM ivermectin.



In many insects, a single GluCl channel gene, *GluCl $\alpha$* , is present but its transcripts are modified by mRNA splicing and editing [18–20]. The presence of gene splicing adds an additional layer of diversity to the GluCl channel and its sensitivity to ivermectin. An example of the effects of ivermectin in insects is seen with *Drosophila melanogaster*, where GluCl $\alpha$  mediates sensitivity to ivermectin. The presence of GluCl channel targets in insects and arthropod parasites allows ivermectin to be used for the treatment of scabies and lice in humans [21]. Interestingly, ivermectin also has inhibitory effects on mosquitoes, and its use has been suggested to reduce the mosquito vectors in malaria [22]. In humans, it has been shown to be effective for treatment of head lice, (*Pediculus humanus capitis*), scabies (*Sarcoptes scabiei*) prevention of myiasis, and biting by mosquitoes [23]. In animals, a range of preparations of ivermectin are used and are effective against lice, mites, screw worms, bot flies, and warbles.

#### GluCl Channels Have Separate Orthosteric (Glutamate) and Allosteric (Ivermectin) Binding Sites

Each subunit of the pentameric GluCl ion channel is about 500 amino acids long with an N terminal and C terminal on the extracellular surface (Figure 3A). Each subunit has four transmembrane  $\alpha$ -helices: M1, M2, M3, and M4. The five subunits of the GluCl channel may be identical (homogeneous) or nonidentical (heterogeneous). Glutamate binds to the orthosteric agonist sites (Figure 4C) which are located at the interface between two subunits [24]. There are two to five orthosteric agonist sites on each channel, depending on the subunit composition of the GluCl channel. Figures 3C and 4C show the orthosteric binding sites where glutamate binds to the channel. When glutamate binds, the channel opens, and each channel opening can be seen under single-channel patch-clamp recording of the *A. suum* pharynx (Figure 3D, top trace) with current pulses of about 2 pA ( $2 \times 10^{-12}$  A) as the channel changes from the closed state to the open state.

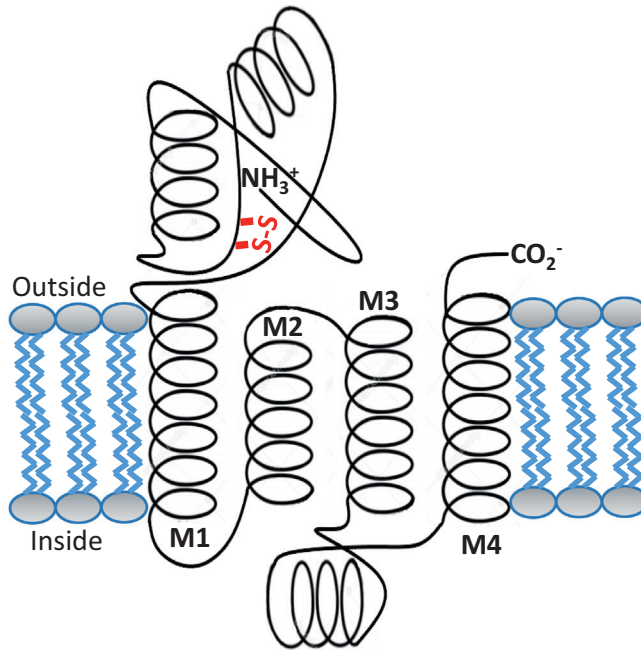
The site of action of ivermectin is an **allosteric site** (Figures 3B,C and 4C), a location that is different to the physiologic glutamate ligand-binding site. Ivermectin binds to the GluCl channel within the transmembrane region of the ion channel (Figure 3B). FRAP (fluorescence recovery after photobleaching) experiments – using fluorescent bodipy (boron-dipyrromethene) ivermectin [25] quenched with trypan blue – show that ivermectin locates in the outer membrane of the phospholipid bilayer to exert its effects on the chloride channels (Figure 3B). The activation of the GluCl channels is progressive and slow, increasing over minutes following application of ivermectin to the preparation (Figure 2F), indicating diffusion into the lipid membrane and diffusion to the ion channel binding site.

Crystal structures of homopentameric GluCl channels [26] provide further details of the molecular binding sites of glutamate and ivermectin to the receptor within the outer phospholipid layer of the membrane. The pentameric GluCl channels form barrel-like staves around their central anion-selective pore. Recall that each of the pentameric subunits has four  $\alpha$ -helix transmembrane regions, M1, M2, M3, and M4 (Figure 3A). Ivermectin can insert deeply into the subunits to make contact with the M2 pore-lining  $\alpha$ -helix and the M2 and M3 loops because it is lipophilic (Figure 3B,C). This stabilizes the open-pore conformation of the ion channel, thereby increasing the time the ion channel stays open (Figure 3B,D). Single-channel experiments in *A. suum* [27] show that low concentrations of dihydroavermectin produce progressive opening of channels, giving rise to a ‘staircase’ effect. The conductance of these channels was 9–15 pS. Similar conductances were observed with expressed GluCl channels from *H. contortus* [28].

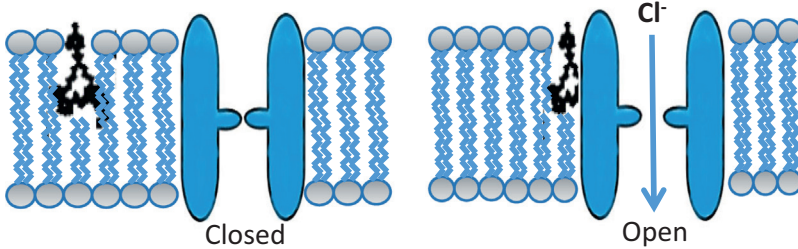
#### Ivermectin Inhibits the Nematode Parasites’ Ability to Suppress the Host Immune Response: Immune Modulation Theory

Nematode parasites can modulate the immune response of their host by releasing a complex mixture of immune modulatory compounds [29,30] and thereby survive within the host. Loss of the ability to release the immune modulators would contribute to the elimination of the parasite.

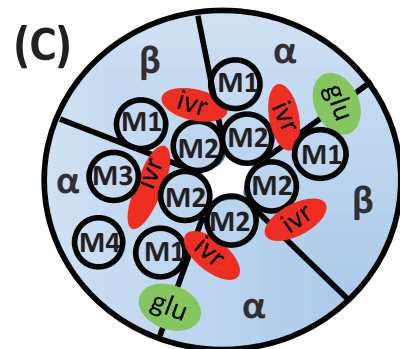
(A)



(B)

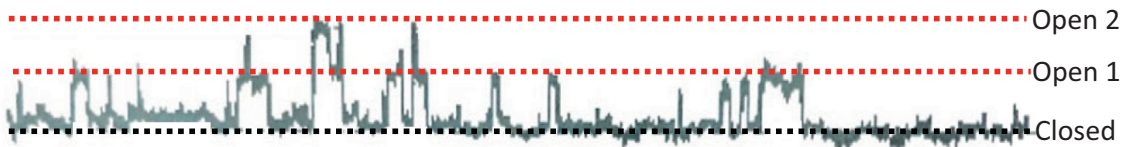


(C)

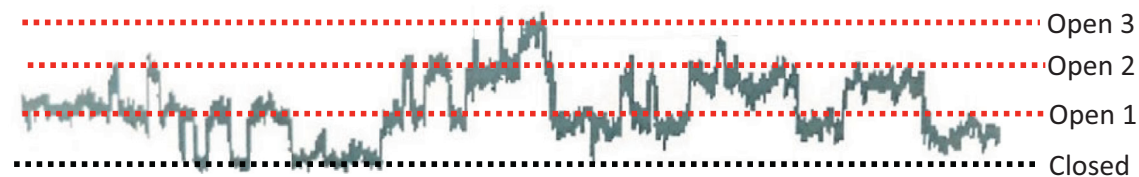


(D)

800  $\mu$ M glutamate;  $p_{Open} = 0.08$



800  $\mu$ M glutamate + 1  $\mu$ M ivermectin;  $p_{Open} = 0.14$



1 pA  
25 ms

Trends in Parasitology

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Moreno *et al.* [31] have pointed out that ivermectin, at submicromolar concentrations, produces a rapid loss of microfilaria in host blood but fails to affect their motility in culture. An explanation for this contradictory observation is that the ivermectin-sensitive GluCl channels of microfilaria are localized on a muscle that surrounds the microfilarial ES vesicle; release of immune-modulator proteins from the ES vesicle is therefore under the control of GluCl channels. Moreno *et al.* [31] showed that ivermectin reduced protein release from the ES apparatus and that the loss of the ES substances could reduce the ability of the parasite to secrete proteins that allow evasion of the host immune system. Observations [32] with *Dirofilaria immitis* microfilariae support this loss of immune modulation by the parasite and show that ivermectin produces attachment of mononuclear cells and neutrophils. These observations imply a direct effect, that is, that ivermectin prevents the parasites' ability to release substances that inhibit the host's immune response. The effect of ivermectin on ES substances in nonfilarial nematodes remains to be explored.

### Effects of Ivermectin on Channels Other Than GluCl Channels in Nematode Parasites and Insects: Additional Channel Theory

Although it is often assumed that ivermectin only acts therapeutically as a PAM on different GluCl channels of nematodes, insects, and arthropods, it also has PAM and inhibitory negative allosteric (NAM) effects on other ion channels. These actions may support the actions of ivermectin on GluCl channels. It has inhibitory effects on  **$\gamma$ -aminobutyric acid (GABA)** channel conductances at low concentrations of  $<0.2 \mu\text{M}$  in *A. suum* [27], but at higher ( $10 \mu\text{M}$ ) concentrations it has potentiating effects. Ivermectin also acts as a PAM on the histamine-gated  $\text{Cl}^-$  channels in *D. melanogaster* [33], the pH-gated  $\text{Cl}^-$  channel in *Sarcoptes scabiei* [34], and the nematode pyrantel and levamisole nicotinic acetylcholine receptors (nAChRs) [35,36].

### Derquantel and Abamectin Interact and Have Synergistic Effects on Nematode nAChRs

The regular use of anthelmintics and ivermectin has led to concerns about the development of drug resistance. Drug combinations may be more effective than single drugs and may delay the onset of resistance. A combination of the nicotinic antagonist, derquantel, and the ivermectin analog, abamectin, has been found to have synergistic anthelmintic effects against GI nematode parasites. In *Ascaris* muscle-strip contraction and electrophysiologic experiments [35,37], derquantel and abamectin have potent and synergistic antagonistic effects on muscle nAChRs (Figure 4A,B). The effects of abamectin on expressed nAChRs are complex [36]. At low concentrations there is an inhibition of the nicotinic responses, but this is overcome at high concentrations. The biphasic effects of abamectin suggest that it acts at two allosteric sites (Figure 4C): one, a high-affinity NAM site decreasing the agonist response, and another, lower-affinity, PAM site [35]. The observations illustrate the complex effects of macrocyclic lactones on heteromeric ion channels and that careful adjustment of concentration ratios is necessary when using combinations with other anthelmintics.

**Figure 3. Glutamate-Gated Chloride (GluCl) Channel Structure, Ivermectin and Glutamate Binding Sites, and Channel Currents.** (A) Diagram of a GluCl channel subunit. It is about 500 amino acids long and begins at the N terminal on the extracellular face of the membrane. The subunits that contain vicinal cysteines are recognized as  $\alpha$ -subunits. Each subunit has four  $\alpha$ -helical transmembrane regions: M1, M2, M3, and M4. The M2 region lines the pore of the transmembrane ion channel. Between the M3 and M4 regions there is an extended cytoplasmic loop. The C terminal is also found at the extracellular surface of the membrane. (B) Five subunits are arranged like the staves of a barrel around the central pore of the ion channel to form a pentamer. Ivermectin binds to the channel in the transmembrane region of the ion channel in the outer bilayer of the lipid bilayer membrane and can open the channel. (C) The five subunits of the GluCl channel viewed from above. If the five subunits of the ion channel are all the same, then the ion channel receptor is referred to as homomeric. If it is made of nonidentical subunits it is referred to as heteromeric. The orthosteric ligand-binding site, where glutamate binds, is located between two adjacent subunits (green) and in the extracellular region of the ion channel. The ivermectin binding site is deep in the ion channel which involves contact with the M2 region near the pore, and contacts the M3 region as well. (D) The upper trace shows a cell-attached patch recording at  $-50 \text{ mV}$  with  $800 \mu\text{M}$  glutamate in the patch-pipette. The rectangular current steps representing the opening and closing of GluCl channels can be seen. Each current step is  $2 \text{ pA}$  ( $10^{-12} \text{ A}$ ). The probability of the channels being open ( $p_{\text{Open}}$ ) is 0.08, that is, each channel was open for 8% of the time. The lower trace shows the effect of adding  $1 \mu\text{M}$  ivermectin to the bath, and after a few minutes for ivermectin to move into and within the membrane, the number of GluCl opening increased as did the probability of the channel being open. It increased to 0.14, or 14% of the time. The opening of many ion channels together allows more  $\text{Cl}^-$  current to flow through the membranes and to increase the conductance of the membrane.



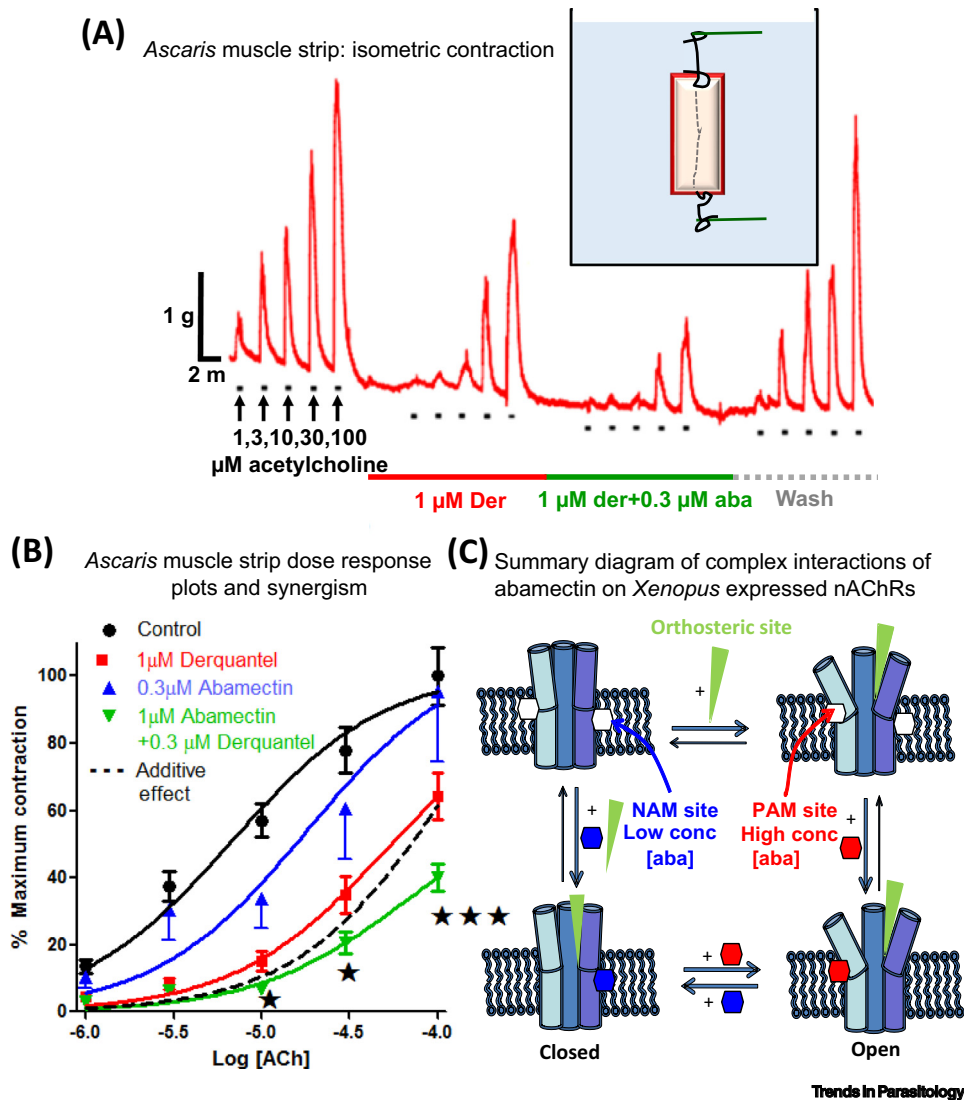


Figure 4. Interactions of Abamectin and Derquantel on *Ascaris* Muscle Strip Contraction and Summary Diagram of Complex Effects of Abamectin on Nicotinic Acetylcholine Receptors (nAChRs). (A) Isometric contraction of *Ascaris suum* muscle strips produced by application of increasing concentrations of acetylcholine and antagonism by 1  $\mu\text{M}$  derquantel (red bar), 1  $\mu\text{M}$  derquantel + 0.3  $\mu\text{M}$  abamectin (green bar), and wash (gray bar). Note that derquantel decreases the responses to acetylcholine and that the addition of abamectin increases the inhibition. (B) An *A. suum* muscle strip concentration–contraction–response plot of acetylcholine showing mean  $\pm$  S.E. bars ( $n = 11$ ). Control (black); in the presence of 1  $\mu\text{M}$  derquantel (red); 1  $\mu\text{M}$  derquantel + 0.3  $\mu\text{M}$  abamectin (green); and wash (blue). Note that abamectin increases the inhibition produced by derquantel. The inhibitory effect of abamectin and derquantel is greater than an additive effect, dotted line [37]. (C) Model of ligand sites of action showing complex effects on heteromeric ion channels. The cholinergic anthelmintic agonists bind to the orthosteric sites, opening the channel. Low concentrations of abamectin (0.03 and 0.1  $\mu\text{M}$ ) bind to a negative allosteric site (NAM) in the lipid phase of the channel, inhibiting opening. Higher concentrations of abamectin (0.3, 1, and 10  $\mu\text{M}$ ) bind to a positive allosteric site (PAM), increasing opening [35].

### Ion Channels in the Mammalian Central Nervous System (CNS) Are Also Affected by Ivermectin

Although relatively free from toxicity, ivermectin – when large overdoses are administered – may cross the blood–brain barrier, producing depressant effects on the CNS [38–43]. Ivermectin may also enter the brain when there are mutant multiple-drug-resistance transporters in the

blood–brain barrier that fail to exclude from the brain drugs that are present in the plasma. In the brain, ivermectin targets the mammalian **glycine receptors (GlyRs)**, GABA receptors, and nAChRs [44–46]. Ivermectin acts as a PAM at a lower concentration (0.03  $\mu\text{M}$ ) and as an irreversible agonist at higher concentration on the mammalian GlyRs [44]. At nM concentrations, ivermectin potentiates GABA-induced  $\text{Cl}^-$  currents in mouse hippocampal neurons [47].

Ivermectin also has a significant effect on other CNS ion channels [48]: (i) it modulates the adenine channel P2X4 receptor [49]; (ii) it is an inhibitor of the ether-a-go-go-related gene (**hERG**)  $\text{K}^+$  channels [50]; (iii) it increases  $\text{Ca}^{2+}$  release from sarcoplasmic reticulum (SR) vesicles by activating the **ryanodine receptor (RyR)** channels [51]; and (iv) it acts as an agonist of **G-protein-coupled inwardly rectifying potassium (GIRK) channels**. A better understanding of the modulatory effects of ivermectin and different ivermectin analogs on various ion channels could advance development of future CNS therapeutics.

### The Spiroketal Groups of Ivermectin Analogs Affect Binding to the Allosteric Site of Ion Channels

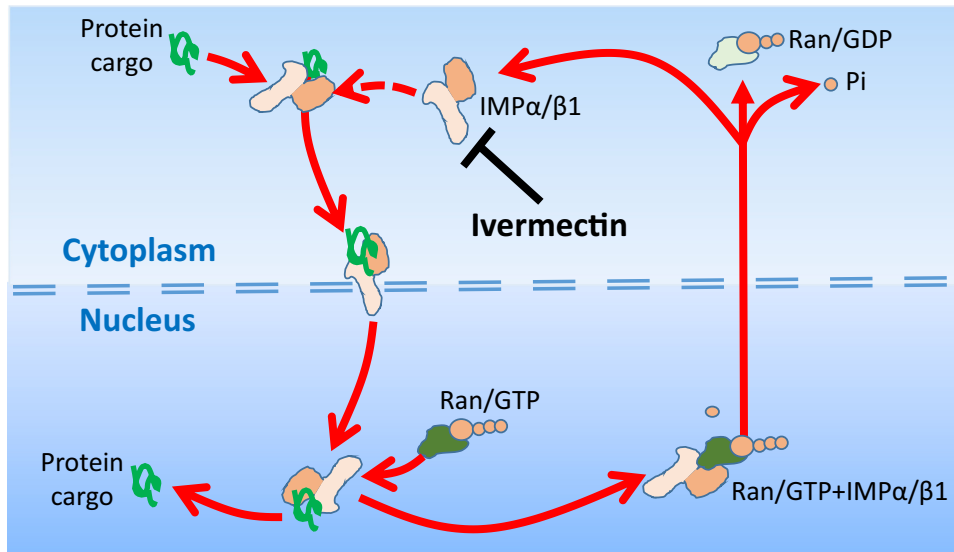
Abamectin, doramectin, eprinomectin, emamectin, milbemycin, and moxidectin have a common pharmacophore: a 16-membered macrocyclic lactone ring fused with benzofuran, spiroketal, and disaccharide groups (except that there is no saccharide for moxidectin or milbemycin) (Figure 1). Binding to the ion channels in the transmembrane region involves the benzofuran group, which is inserted deeply into the transmembrane regions, reaching nearly to the pore of the channel. The spiroketal group interacts with the M1, and the disaccharide group is left on the outside of the channels [26]. The size of the spiroketal groups of the ivermectin analogs affects their ability to bind within the channels because of the limited room in the binding pocket. Abamectin is most like ivermectin, with a difference only at the C22 and C23 (R3, Figure 1). The potency of effects of abamectin is therefore similar to that of ivermectin on various ion channels [48]. Doramectin has a large spiroketal group due to the presence of the cyclohexyl moiety (R2, Figure 1) and is less potent. Moxidectin lacks the disaccharide and has a bigger R3  $-\text{C}=\text{C}(\text{NOCH}_3)-$  in the spiroketal group and has less effect on host GABA receptors, and reduced CNS toxicity [52], but is more potent on some nematode parasite GluCl channels [53].

### Antiviral Effects of Ivermectin

#### Ivermectin Inhibits NS3 Helicase and Selected Protein Import through Nuclear Pores

The most potent antiviral effects of ivermectin reported appears to be its inhibitory effects, with an  $\text{EC}_{50}$  of 3–6 nM concentrations, in virus yield reduction assays with cultures of yellow fever virus. The effect is mediated by inhibition of viral NS3 helicase [54]. Ivermectin also inhibits, at higher concentrations, other flaviviruses, such as Japanese encephalitis virus and tick-borne encephalitis virus, with inhibition of replication  $\text{EC}_{50}$ s of 200–300 nM concentrations.

Ivermectin also inhibits the nuclear import of selected cytoplasmic proteins. Figure 5 illustrates how ivermectin inhibits by binding to the heterodimer protein importin (IMP)  $\alpha/\beta 1$ , and inhibits the binding of cargo proteins that are carried through the nuclear pore by IMP $\alpha/\beta 1$  into the nucleus. If IMP binding were not inhibited by ivermectin, IMP $\alpha/\beta 1$  + cargo protein would be able to pass through the nuclear pore, where the cargo protein is released into the nucleus when RanGTP binds to IMP $\alpha/\beta 1$  [55]. Viral protein cargos known to bind to IMP $\alpha/\beta 1$  include: (i) HIV-1 integrase, needed for HIV-1 propagation and incorporation into the host genome; (ii) DENV N55, a dengue virus nonstructural protein-5; and (iii) the simian virus SV40 large tumor antigen [56,57]. **IC<sub>50</sub>s** in the 1–4  $\mu\text{M}$  range have been found to limit growth of the following



## Trends in Parasitology

**Figure 5. Ivermectin Inhibits Nuclear Import of Selected Proteins, Including Some Virus Proteins.** Illustrations of how ivermectin binds to the heterodimer protein importin, IMP $\alpha$ / $\beta$ 1, to inhibit the binding of cargo proteins that are carried through the nuclear pore by IMP $\alpha$ / $\beta$ 1 into the nucleus. The IMP $\alpha$ / $\beta$ 1 protein recognises a cargo by its nuclear localization signal (NLS), short sequences of basic amino acids either alone or separated by a linker region of 10–12 amino acids. The import process is energy-dependent, involving GTP and its hydrolysis. Once through the nuclear pore, the protein is released when RanGTP binds to IMP $\alpha$ / $\beta$ 1 to allow release of the cargo into the nucleus. The empty IMP $\alpha$ / $\beta$ 1 is then shuttled back out through the nuclear pore to continue the cycle. GTP: Guanosine triphosphate. GDP: Guanosine diphosphate. Ran: a small 25-kDa nuclear protein that is involved in transport into and out of the nucleus.

RNA viruses in tissue culture: dengue virus, West Nile virus, and Venezuelan equine encephalitis virus (VEEV) [58] (Table 1). This broad-spectrum activity of ivermectin may be due to the reliance on IMP $\alpha$ / $\beta$ 1 for RNA virus protein transport during infection [56,59].

At higher concentrations, ivermectin also exhibits activity against the DNA virus pseudorabies virus (PRV) *in vitro* and *in vivo*, with ivermectin treatment increasing the survival of pseudorabies virus-infected mice [60]. Ivermectin, however, has not been reported to be effective against Zika virus (ZIKV) in mice [61]. We point out that the antiviral effects of ivermectin have only been observed in tissue culture or at very high doses in mice, underlining the fact that normal therapeutic anthelmintic doses are not anticipated to show significant antiviral activity, except, perhaps, for yellow fever virus.

#### Covid-19

Caly *et al.* [107] tested the antiviral activity of 5  $\mu$ M ivermectin for 48 h on SARS-CoV-2-infected Vero/hSLAM cell cultures and observed a 5000-fold reduction in viral RNA with an IC<sub>50</sub> of 5  $\mu$ M (4375 ng/ml). This concentration is ~100-fold more than the peak plasma concentration ( $C_{max}$ ) from a single 150  $\mu$ g/kg dose that is used for normal human anthelmintic therapy [1,62]. Although 2000  $\mu$ g/kg doses, which are greater than those approved by the Food and Drug Administration (FDA), have been tested on humans (producing a  $C_{max}$  of 250 ng/ml, and appearing to be safe [63]), the  $C_{max}$  was still well below the effective concentration of 4375 ng/ml used in the cell-culture experiments. These observations are not encouraging for ivermectin clinical trials for the treatment of Covid-19. A phase III trial in Thailand, using ivermectin at a dose of 400  $\mu$ g/kg for 3 days, was found to be safe and showed a modest effect against dengue virus (DENV) [64]. These observations are scientifically interesting, but ivermectin is not yet proven to be clinically relevant for Covid-19 treatment. Further research, and the development of more potent analogs, are required for an anti-Covid-

19 action. Because of concerns that some individuals have access to veterinary formulations, and that they may consider these formulations for their own use against Covid-19, on 10 April 2020 the FDA issued guidance not to use ivermectin intended for animals as a treatment for Covid-19 in humans<sup>ii</sup>.

### Antimalarial Effects of Ivermectin

In addition to controlling filariasis, the **mass drug administration (MDA)** of ivermectin has also had effects limiting the spread of malaria. Although this is likely due to an inhibitory effect on the mosquito vectors of malaria, there are reports of a direct inhibitory effect of ivermectin on the liver stages of malarial parasites [65,66]. Singh *et al.* [67] tested a number of novel ivermectin analogs and reported that ivermectin inhibits *Plasmodium falciparum* erythrocyte stages *in vitro*, with an  $IC_{50}$  near 0.5  $\mu$ M, and that an improved novel compound (analog-19) had a better  $IC_{50}$  of 0.05  $\mu$ M. Mendes *et al.* [66] also describe the inhibitory effects of ivermectin on the development of the parasite inside liver cells in infected mice. The liver-stage inhibition reduced the subsequent blood-stage parasitemia, reduced the clinical effects, and enhanced the survival of the infected mice. The *in vivo* mice experiments did, however, use a very high (10 mg/kg) ivermectin dose to show the effect and used *Plasmodium berghei* in the liver of the mice [66].

The mechanism of this antimalarial effect involves **signal recognition particles (SRPs)** which are universal eukaryote ribonucleoprotein complexes that target proteins to the endoplasmic reticulum (ER). The SRPs are present in both the host and the malaria parasite. Micromolar concentrations of ivermectin inhibit SRP nuclear import in *P. falciparum* by blocking the movement of the heterodimer carrier IMP $\alpha/\beta$  that carries *P. falciparum* SRP polypeptides into the nucleus. The effect in tissue culture is destruction of *P. falciparum* [65].

The clinical benefits of these antimalarial effects have not been established. Experiments by the Mendes group [68] reported that 5 mg/kg ivermectin in mice did not result in inhibition of *P. berghei* transmission, and ivermectin inhibition arises only in the mosquito stage of the transmission cycle. They interpreted their observations to suggest that the gamete/zygote to the ookinete stage is resistant to ivermectin analogs (epinomectin, abamectin, ivermectin, moxidectin, doramectin, and emamectin), and the oocyst is the most vulnerable stage of the parasite's sporogonic cycle. The published literature suggests that, at this stage, there is an *in vitro* effect with high concentrations of ivermectin but the repositioning of ivermectin as an effective antimalarial has not been established.

### Antimetabolic Effect of Ivermectin as a Partial Agonist of the Nuclear Farnesoid Receptor (FXR)

FXR is a nuclear receptor, involved in metabolic regulation, that binds to ivermectin with an  $EC_{50}$  in the 200 nM range [69,70]. It is normally bound by bile acids (as the physiologic agonist) and is expressed highly in the liver, small intestine, kidney, and adrenals [71,72]. Following the binding of bile acid, the FXR translocates to the nucleus to target DNA-regulating genes that are involved in the metabolism of bile acids, lipid, and glucose. Although ivermectin has the potential to treat metabolic syndromes, such as nonalcoholic fatty liver disease [72], it is pointed out that it is not proven to do so at antiparasitic concentrations. Nonetheless, ivermectin has been found to be more selective for the FXR, with a higher affinity than bile acids, and the crystal structure of the ligand-binding domain of ivermectin to the FXR has been characterized [69,73]. Ivermectin has a potent partial agonist action on the FXR receptor and has antidiabetic and cholesterol-reducing effects in mice. Because ivermectin is a widely used clinical drug, these observations provide a useful chemical skeleton for the design of novel FXR ligands that have antimetabolic effects [69].

### Anticancer Effects of Ivermectin

To overcome the cancer therapeutic bottleneck, repurposing of a number of drugs, including anthelmintics, has been suggested [74]. Juarez *et al.* [75] and Antoszczak *et al.* [76] have reviewed the anticancer effects of ivermectin at high concentrations (0.1–100  $\mu$ M or doses of 3–40 mg/kg) in tissue culture and in mice. The modes of action of ivermectin on different types of cancer are proposed to involve different mechanisms: (i) the multidrug-resistance transporter protein [77]; (ii) Akt/mTOR [78] and WNT-TCF pathways [79]; (iii) purinergic receptors [80]; (iv) PAK-1 [81]; (v) the cancer-related epigenetic deregulators SIN3A and SIN3B, RNA helicase; (vi) effects on mammalian tubulin polymerization and depolymerization dynamics [82]; and (vii) preferential targeting of cancer stem-cell-like populations [83]. The potential for repurposing ivermectin for cancer treatment remains to be established, as does the mechanisms of action.

### Mycobacterium

Ivermectin is not recognized as an effective antibacterial compound. Despite this, there have been some reports of very high concentrations (3–24  $\mu$ g/ml) of ivermectin having effects on *Mycobacterium tuberculosis* [84], but these observations have been challenged because of the methods used [85]. A subsequent publication has suggested that ivermectin be considered for the treatment of *Mycobacterium ulcerans*, which causes Buruli ulcer [86]. No mode of action for this effect of ivermectin has been proposed, and no further publications were found to date.

### Asthma

Ivermectin has been reported to exhibit anti-inflammatory properties by affecting cellular and humoral responses [87], and inhibiting lipopolysaccharide (LPS)-induced nitric oxide and prostaglandin  $E_2$  production [88]. Yan *et al.* [89] investigated the therapeutic potential of 2 mg/kg ivermectin for the treatment of allergic asthma in mice and found that it reduced the symptoms in mice by curtailing the recruitment of inflammatory cells and reducing the hypersecretion of mucus. These anti-inflammatory effects were likely due to decreased production of the bronchoalveolar lavage fluid cytokines, and reduced secretion of ovalbumin-specific IgE and IgG1. Ivermectin induces immunogenic cell death by a variety of mechanisms, including the mechanisms described in the previous text associated with its anticancer action and ATP-dependent immune responses [75]. The use of ivermectin 1% cream for the inflammatory lesions of rosacea is recognized, but this may relate to its antiparasitic effect on the *Demodex* mite.

### Resistance to Ivermectin: Candidate Genes, Pgps, and NHR-8

Resistance to ivermectin and its analogs in parasitic nematodes is of increasing concern; the mechanisms of this resistance has not been explained satisfactorily. We do have information on ivermectin resistance in the model nematode, *C. elegans*, where it appears that simultaneous mutations of several genes are required for very high levels (>100 $\times$ ) of resistance. Dent *et al.* [13] observed that three GluCl channel genes – *glc-1*, *avr-14*, and *avr-15* – were required to produce strong ivermectin resistance, and if one of these genes were not mutated then the level of resistance was lower. The resistance phenotype was modified by mutations in the innexin genes, which code for proteins that coordinate electrical coupling between cells via gap junctions (*unc-7* and *unc-9*), and genes of sensory head amphid neurons associated with dye uptake (*osm-1*, *osm-5*, *dyf-7*, *dyf-11*, and *che-3*) [90]. These high levels of resistance are less likely to be required for ivermectin resistance in nematode parasites because the maximum concentrations that the parasite in the host is exposed to during therapy is related to the maximum plasma concentrations, which are less than the concentrations applied to *C. elegans* in the Petri dish.



These genes of *C. elegans* have been pursued in parasitic nematodes as candidate genes of ivermectin resistance. Freeman *et al.* [91] reported that amphidial neurons were abnormal in an ivermectin-resistant isolate of *H. contortus* in comparison to ivermectin-sensitive isolates. However, to date, no readily identified mutant candidate gene in the parasitic nematodes has been associated with field resistance. Still, it is worth noting that a broad major **quantitative trait locus (QTL)** on chromosome V for ivermectin resistance has been identified in *H. contortus* by backcrossing experiments [92,93], so a molecular test for ivermectin resistance may emerge from these studies.

Increased excretion of ivermectin analogs by **P-glycoproteins (Pgps)** has also been a focus of much research as a source of ivermectin resistance [94,95]. Pgps belong to the superfamily of ATP-binding cassette (ABC) transporters and form the ABCB subfamily [96]. The xenobiotic transporters are only modestly selective for their substrates, often preferring lipophilic or neutral drugs for transport [97,98].

The mechanism of anthelmintic drug resistance due to Pgps has been linked to a relatively modest increase in the expression of some specific candidate Pgps in parasitic nematodes: Pgp-2 and Pgp-9 in *H. contortus* [99], Pgp-9 in *Teladorsagia circumcincta* [100], Pgp-11 in *P. univalens* [101] and *T. circumcincta* [102]. Ivermectin analogs with the disaccharide moiety are better substrates for the Pgps than the aglycones like moxidectin [103]. This difference appears to contribute to the ability of moxidectin to overcome low levels of ivermectin resistance and become 'tolerant'. We point out, however, that the increase in Pgps in parasitic nematodes as the explanation for resistance remains relatively weak given the large number of differentially expressed genes between isolates. This difficulty is perhaps understandable given the process of gene selection under drug pressure and the polymorphisms of the genes.

If we return to recent studies on the model *C. elegans* and the development of ivermectin tolerance in *C. elegans* [104], it appears that a modest ivermectin resistance, comparable to that in parasitic nematodes, may be due to increased excretion and metabolism rather than to mutation of target genes like *glc-1*, *avr-14*, and *avr-15*. The increased removal of ivermectin is produced by: i) increased excretion associated with more Pgps transporters (*pgp-1*, *pgp-3*, *pgp-6*, *pgp-9*, *pgp-13*, and *mrp-6*); ii) increased, metabolism by the cytochrome oxidases, like CYP14, and; iii) increased metabolism by the glutathione S-transferases (GSTs), GST-4 and GST-10. These changes are driven by the nuclear hormone receptor NHR-8 [104]. Thus, an effect of ivermectin activating NHR-8 would lead to a catalog of changes in expressed genes, leading to enhanced removal of ivermectin and reduced sensitivity to ivermectin by a polygenic mechanism and resistance.

### Concluding Remarks

We have seen that ivermectin and analogs are remarkable anthelmintics and insecticides. Despite their wide spectrum of action on many species of parasite they have limited effects on adult filarial nematodes. Future research should seek to determine the explanation for this (see Outstanding Questions). If it is found that the GluCl channels of adult filaria are less sensitive to the existing macrocyclic lactones, a second generation of ivermectin analogs, or a combination with other anthelmintics, should be developed. The strongest commercial driver for the development of new ivermectin analogs may be the need to treat filarial heartworm in dogs that have become less sensitive to the current macrocyclic lactones. The development of a successful ivermectin analog for animals that is safe and effective against adult filaria could support the development of improved MDA control of onchocerciasis and lymphatic filariasis in humans.

### Outstanding Questions

Why does ivermectin have a limited effect on adult filaria? Can the limited efficacy be overcome with a second generation of ivermectin analogs?

GluCl channels are heterogeneous, varying in ivermectin sensitivity between the muscles, nerves, intestine, and reproductive tissue of individual nematodes and between the species of nematodes. What are the important locations of GluCl channels that are sensitive to ivermectin in important nematode parasites? How does activation of these GluCl channels affect the parasite?

What are the mechanisms of ivermectin resistance in nematode parasites, and can they be overcome?

Can ivermectin or its analogs be repositioned successfully for the treatment of viruses, malaria, cancer, and selected metabolic diseases such as diabetes?

Much is known about the mode of action of ivermectin as PAMs of GluCl channels. We need more research to characterize the heterogeneity of GluCl channels to determine how the sensitivity to ivermectin varies with the molecular structures of the different GluCl channels seen in the different parasite species and in their tissues. We also need more research to explain the slow and gradual effect of ivermectin on *Dirofilaria immitis* reproduction.

Another important area for future research is to determine the mechanisms of ivermectin resistance in parasitic nematodes. In this regard, the only substantial evidence is the large effect of the QTL on chromosome V in *H. contortus*. The mechanisms could include Pgps, increased metabolism, and NHR-8, but we still do not have an adequate cellular and molecular explanation for ivermectin resistance in parasitic nematodes.

Repurposing studies, using tissue culture and high concentrations of ivermectin, have revealed antiviral, antimalarial, antimetabolic, and anticancer actions, but the concentrations required to see effects in most tissue-culture studies are above the plasma concentrations seen in the usual therapeutic doses. New improved delivery systems for ivermectin that increase local concentrations without toxicity, or new analogs, should be studied for the treatment of viruses, malaria, cancer, or selected metabolic diseases such as diabetes.

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### Resources

<sup>i</sup><https://wormbase.org/>

<sup>ii</sup>[www.fda.gov/animal-veterinary/product-safety-information/fda-letter-stakeholders-do-not-use-ivermectin-intended-animals-treatment-covid-19-humans](http://www.fda.gov/animal-veterinary/product-safety-information/fda-letter-stakeholders-do-not-use-ivermectin-intended-animals-treatment-covid-19-humans).

### References

- Canga, A.G. *et al.* (2008) The pharmacokinetics and interactions of ivermectin in humans – A mini-review. *AAPS J.* 10, 42–46
- González Canga, A. *et al.* (2009) The pharmacokinetics and metabolism of ivermectin in domestic animal species. *Vet. J.* 179, 25–37
- Merck (2019) *Stomectol safety data sheet*. p. 7
- Bauch, S. (1987) Ivermectin toxicity in small animals. *Can. Vet. J.* 28, 563–564
- Campbell, W.C. *et al.*, eds (1989) *Ivermectin and Abamectin*, Springer-Verlag
- Vercruyse, J. *et al.*, eds (2002) *Macrocyclic Lactones in Antiparasitic Therapy*, CABI
- Li, B.W. *et al.* (2014) 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 Drug Resist.* 4, 71–76
- Ballesteros, C. *et al.* (2016) The effects of ivermectin on *Brugia malayi* females *in vitro*: a transcriptomic approach. *PLoS Negl. Trop. Dis.* 10, e0004929
- Cully, D.F. *et al.* (1994) Cloning of an ivermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature* 371, 707–711
- Cully, D.F. *et al.* (1996) Molecular biology and electrophysiology of glutamate-gated chloride channels of invertebrates. *Parasitology* 113, S191–S200
- Vassiliatis, D.K. *et al.* (1997) Genetic and biochemical evidence for a novel ivermectin-sensitive chloride channel in *Caenorhabditis elegans*. Isolation and characterization. *J. Biol. Chem.* 272, 33167–33174
- Dent, J.A. *et al.* (1997) *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.* 16, 5867–5879
- Dent, J.A. *et al.* (2000) The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2674–2679
- Horoszkó, L. *et al.* (2001) GLC-3: a novel fipronil and BIDN-sensitive, but picrotoxinin-insensitive, L-glutamate-gated chloride channel subunit from *Caenorhabditis elegans*. *Br. J. Pharmacol.* 132, 1247–1254
- Glendinning, S.K. *et al.* (2011) Glutamate-gated chloride channels of *Haemonchus contortus* restore drug sensitivity to ivermectin resistant *Caenorhabditis elegans*. *PLoS One* 6, e22390
- Richards, J.C. *et al.* (1995) *In vitro* studies on the relative sensitivity to ivermectin of *Necator americanus* and *Ancylostoma ceylanicum*. *Int. J. Parasitol.* 25, 1185–1191
- Behnke, J.M. *et al.* (1993) Sensitivity to ivermectin and pyrantel of *Ancylostoma ceylanicum* and *Necator americanus*. *Int. J. Parasitol.* 23, 945–952
- Semenov, E.P. and Pak, W.L. (1999) Diversification of *Drosophila* chloride channel gene by multiple posttranscriptional mRNA modifications. *J. Neurochem.* 72, 66–72
- Jones, A.K. *et al.* (2010) The cys-loop ligand-gated ion channel gene superfamily of the parasitoid wasp, *Nasonia vitripennis*. *Heredity (Edinb)* 104, 247–259
- Jones, A.K. and Sattelle, D.B. (2007) The cys-loop ligand-gated ion channel gene superfamily of the red flour beetle, *Tribolium castaneum*. *BMC Genom.* 8, 327
- Wendel, K. and Rompalo, A. (2002) Scabies and pediculosis pubis: an update of treatment regimens and general review. *Clin. Infect. Dis.* 35, S146–S151

22. Kobylinski, K.C. *et al.* (2010) The effect of oral anthelmintics on the survivorship and re-feeding frequency of anthropophilic mosquito disease vectors. *Acta Trop.* 116, 119–126
23. Ashour, D.S. (2019) Ivermectin: From theory to clinical application. *Int. J. Antimicrob. Agents* 54, 134–142
24. Degani-Katzav, N. *et al.* (2016) Subunit stoichiometry and arrangement in a heteromeric glutamate-gated chloride channel. *Proc. Natl. Acad. Sci. U. S. A.* 113, E644–E653
25. Martin, R.J. and Kusel, J.R. (1992) On the distribution of a fluorescent ivermectin probe (4'' 5,7-dimethyl-bodipy propionylivermectin) in *Ascaris* membranes. *Parasitology* 104, 549–555
26. Hibbs, R.E. and Gouaux, E. (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature* 474, 54–60
27. Martin, R.J. and Pennington, A.J. (1989) A patch-clamp study of effects of dihydroavermectin on *Ascaris* muscle. *Br. J. Pharmacol.* 98, 747–756
28. Atif, M. *et al.* (2017) Effects of glutamate and ivermectin on single glutamate-gated chloride channels of the parasitic nematode *H. contortus*. *PLoS Pathog.* 13, e1006663
29. King, I.L. and Li, Y. (2018) Host–parasite interactions promote disease tolerance to intestinal helminth infection. *Front. Immunol.* 9, 2128
30. Maizels, R.M. (2013) *Toxocara canis*: molecular basis of immune recognition and evasion. *Vet. Parasitol.* 193, 365–374
31. Moreno, Y. *et al.* (2010) Ivermectin disrupts the function of the excretory–secretory apparatus in microfilariae of *Brugia malayi*. *Proc. Natl. Acad. Sci. U. S. A.* 107, 20120–20125
32. Vatta, A.F. *et al.* (2014) Ivermectin-dependent attachment of neutrophils and peripheral blood mononuclear cells to *Dirofilaria immitis* microfilariae *in vitro*. *Vet. Parasitol.* 206, 38–42
33. Zheng, Y. *et al.* (2002) Identification of two novel *Drosophila melanogaster* histamine-gated chloride channel subunits expressed in the eye. *J. Biol. Chem.* 277, 2000–2005
34. Mounsey, K.E. *et al.* (2007) Molecular characterisation of a pH-gated chloride channel from *Sarcoptes scabiei*. *Invertebr. Neurosci.* 7, 149–156
35. Abongwa, M. *et al.* (2016) Curiouser and curiouser: the macrocyclic lactone, abamectin, is also a potent inhibitor of pyrantel/tribendimidine nicotinic acetylcholine receptors of gastrointestinal worms. *PLoS One* 11, e0146854
36. Martin, R.J. *et al.* (2015) The Conqueror Worm: recent advances with cholinergic anthelmintics and techniques excite research for better therapeutic drugs. *J. Helminthol.* 89, 387–397
37. Puttachary, S. *et al.* (2013) Derquantel and abamectin: effects and interactions on isolated tissues of *Ascaris suum*. *Mol. Biochem. Parasitol.* 188, 79–86
38. Geyer, J. and Janko, C. (2012) Treatment of MDR1 mutant dogs with macrocyclic lactones. *Curr. Pharm. Biotechnol.* 13, 969–986
39. Janko, C. and Geyer, J. (2013) Moxidectin has a lower neurotoxic potential but comparable brain penetration in P-glycoprotein-deficient CF-1 mice compared to ivermectin. *J. Vet. Pharmacol. Ther.* 36, 275–284
40. Geyer, J. *et al.* (2009) Brain penetration of ivermectin and selamectin in mdr1a,b P-glycoprotein- and bcrcp- deficient knockout mice. *J. Vet. Pharmacol. Ther.* 32, 87–96
41. Schinkel, A.H. *et al.* (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 77, 491–502
42. Mealey, K.L. *et al.* (2002) Frequency of the mutant MDR1 allele associated with ivermectin sensitivity in a sample population of collies from the northwestern United States. *Am. J. Vet. Res.* 63, 479–481
43. Roulet, A. *et al.* (2003) MDR1-deficient genotype in Collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. *Eur. J. Pharmacol.* 460, 85–91
44. Shan, Q. *et al.* (2001) Ivermectin, an unconventional agonist of the glycine receptor chloride channel. *J. Biol. Chem.* 276, 12556–12564
45. Adelsberger, H. *et al.* (2000) Activation of rat recombinant alpha(1)beta(2)gamma(2S) GABA(A) receptor by the insecticide ivermectin. *Eur. J. Pharmacol.* 394, 163–170
46. Krause, R.M. *et al.* (1998) Ivermectin: a positive allosteric effector of the alpha7 neuronal nicotinic acetylcholine receptor. *Mol. Pharmacol.* 53, 283–294
47. Krušák, J. and Zemková, H. (1994) Effect of ivermectin on  $\gamma$ -aminobutyric acid-induced chloride currents in mouse hippocampal embryonic neurones. *Eur. J. Pharmacol.* 259, 121–128
48. Chen, I.S. and Kubo, Y. (2018) Ivermectin and its target molecules: shared and unique modulation mechanisms of ion channels and receptors by ivermectin. *J. Physiol.* 596, 1833–1845
49. Silberberg, S.D. *et al.* (2007) Ivermectin Interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. *Neuron* 54, 263–274
50. Kauthale, R.R. *et al.* (2015) Assessment of temperature-induced hERG channel blockade variation by drugs. *J. Appl. Toxicol.* 35, 799–805
51. Ahern, G.P. *et al.* (1999) Effects of ivermectin and midcamycin on ryanodine receptors and the Ca<sup>2+</sup>-ATPase in sarcoplasmic reticulum of rabbit and rat skeletal muscle. *J. Physiol.* 514, 313–326
52. Ménez, C. *et al.* (2012) Relative neurotoxicity of ivermectin and moxidectin in Mdr1ab (-/-) mice and effects on mammalian GABA(A) channel activity. *PLoS Negl. Trop. Dis.* 6, e1883
53. Njue, A.I. *et al.* (2004) Mutations in the extracellular domains of glutamate-gated chloride channel alpha3 and beta subunits from ivermectin-resistant *Cooperia oncophora* affect agonist sensitivity. *J. Neurochem.* 89, 1137–1147
54. Mastrangelo, E. *et al.* (2012) Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J. Antimicrob. Chemother.* 67, 1884–1894
55. Wagstaff, K.M. *et al.* (2011) An AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import. *J. Biomol. Screen.* 16, 192–200
56. Caly, L. *et al.* (2012) Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? *Antivir. Res.* 95, 202–206
57. Wagstaff, K.M. *et al.* (2012) Ivermectin is a specific inhibitor of importin  $\alpha/\beta$ -mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem. J.* 443, 851–856
58. Lundberg, L. *et al.* (2013) Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan equine encephalitis virus replication. *Antivir. Res.* 100, 662–672
59. Jans, D.A. *et al.* (2019) Inhibitors of nuclear transport. *Curr. Opin. Cell Biol.* 58, 50–60
60. Lv, C. *et al.* (2018) Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus *in vitro* and *in vivo*. *Antivir. Res.* 159, 55–62
61. Ketkar, H. *et al.* (2019) Lack of efficacy of ivermectin for prevention of a lethal Zika virus infection in a murine system. *Diagn. Microbiol. Infect. Dis.* 95, 38–40
62. Chaccour, C. *et al.* (2020) Ivermectin and COVID-19: keeping rigor in times of urgency. *Am. J. Trop. Med. Hyg.* 102, 1156–1157
63. Guzzo, C.A. *et al.* (2002) Safety, tolerability, and pharmacokinetics of escalating high doses of ivermectin in healthy adult subjects. *J. Clin. Pharmacol.* 42, 1122–1133
64. Eakkawit, Y. *et al.* (2018) Efficacy and Safety of Ivermectin against Dengue Infection: A Phase III, Randomized, Double-blind, Placebo-controlled Trial. In *The 34th Annual Meeting the Royal College of Physicians of Thailand: 'Internal Medicine and One Health'*
65. Panchal, M. *et al.* (2014) *Plasmodium falciparum* signal recognition particle components and anti-parasitic effect of ivermectin in blocking nucleo-cytoplasmic shuttling of SRP. *Cell Death Dis.* 5, e994
66. Mendes, A.M. *et al.* (2017) Inhibition of *Plasmodium* liver infection by ivermectin. *Antimicrob. Agents Chemother.* 61, 1–7 e02005-16
67. Singh, L. *et al.* (2020) Molecular design and synthesis of ivermectin hybrids targeting hepatic and erythrocytic stages of *Plasmodium* parasites. *J. Med. Chem.* 63, 1750–1762
68. Azevedo, R. *et al.* (2019) Inhibition of *Plasmodium* sporogonic stages by ivermectin and other avermectins. *Parasit. Vectors* 12, 549
69. Jin, L. *et al.* (2013) The antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. *Nat. Commun.* 4, 1937
70. Jin, L. *et al.* (2015) Selective targeting of nuclear receptor FXR by avermectin analogues with therapeutic effects on nonalcoholic fatty liver disease. *Sci. Rep.* 5, 17288

71. Wang, Y.D. *et al.* (2008) Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 48, 1632–1643
72. Wang, Y.D. *et al.* (2008) FXR: a metabolic regulator and cell protector. *Cell Res.* 18, 1087–1095
73. Akwabi-Ameyaw, A. *et al.* (2009) FXR agonist activity of conformationally constrained analogs of GW 4064. *Bioorg. Med. Chem. Lett.* 19, 4733–4739
74. Zhang, Z. *et al.* (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. *Signal Transduct. Target Ther.* 5, 113
75. Juarez, M. *et al.* (2018) The multitargeted drug ivermectin: from an antiparasitic agent to a repositioned cancer drug. *Am. J. Cancer Res.* 8, 317–331
76. Antoszczak, M. *et al.* (2020) Old wine in new bottles: Drug repurposing in oncology. *Eur. J. Pharmacol.* 866, 172784
77. Zhang, X. *et al.* (2020) Inhibition of TMEM16A Ca. *Pharmacol. Res.* 156, 104763
78. Zhang, X. *et al.* (2020) Ivermectin augments the *in vitro* and *in vivo* efficacy of cisplatin in epithelial ovarian cancer by suppressing Akt/mTOR signaling. *Am J Med Sci* 359, 123–129
79. Melotti, A. *et al.* (2014) The river blindness drug ivermectin and related macrocyclic lactones inhibit WNT-TCF pathway responses in human cancer. *EMBO Mol. Med.* 6, 1263–1278
80. Draganov, D. *et al.* (2015) Modulation of P2X4/P2X7/Pannexin-1 sensitivity to extracellular ATP via ivermectin induces a non-apoptotic and inflammatory form of cancer cell death. *Sci. Rep.* 5, 16222
81. Chen, L. *et al.* (2020) Ivermectin suppresses tumour growth and metastasis through degradation of PAK1 in oesophageal squamous cell carcinoma. *J. Cell. Mol. Med.* 24, 5387–5401
82. Ashraf, S. and Prichard, R. (2016) Ivermectin exhibits potent anti-mitotic activity. *Vet. Parasitol.* 226, 1–4
83. Dominguez-Gomez, G. *et al.* (2018) Ivermectin as an inhibitor of cancer stem-like cells. *Mol. Med. Rep.* 17, 3397–3403
84. Lim, L.E. *et al.* (2013) Anthelmintic avermectins kill *Mycobacterium tuberculosis*, including multidrug-resistant clinical strains. *Antimicrob. Agents Chemother.* 57, 1040–1046
85. Muhammed Ameen, S. and Drancourt, M. (2014) Comment on: measurements of the *in vitro* anti-mycobacterial activity of ivermectin are method-dependent. *J. Antimicrob. Chemother.* 69, 1724–1725
86. Omansen, T.F. *et al.* (2015) *In-vitro* activity of avermectins against *Mycobacterium ulcerans*. *PLoS Negl. Trop. Dis.* 9, e0003549
87. Stankiewicz, M. *et al.* (1995) Influence of ivermectin on cellular and humoral immune responses of lambs. *Vet. Immunol. Immunopathol.* 44, 347–358
88. Zhang, X. *et al.* (2009) Inhibitory effects of ivermectin on nitric oxide and prostaglandin E2 production in LPS-stimulated RAW 264.7 macrophages. *Int. Immunopharmacol.* 9, 354–359
89. Yan, S. *et al.* (2011) Anti-inflammatory effects of ivermectin in mouse model of allergic asthma. *Inflamm. Res.* 60, 589–596
90. Urdaneta-Marquez, L. *et al.* (2014) A *dyf-7* haplotype causes sensory neuron defects and is associated with macrocyclic lactone resistance worldwide in the nematode parasite *Haemonchus contortus*. *Int. J. Parasitol.* 44, 1063–1071
91. Freeman, A.S. *et al.* (2003) Amphidial structure of ivermectin-resistant and susceptible laboratory and field strains of *Haemonchus contortus*. *Vet. Parasitol.* 110, 217–226
92. Sallé, G. *et al.* (2019) The global diversity of *Haemonchus contortus* is shaped by human intervention and climate. *Nat. Commun.* 10, 4811
93. Doyle, S.R. *et al.* (2019) Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, *Haemonchus contortus*. *BMC Genom.* 20, 218
94. Kotze, A. *et al.* (2014) Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. *Int. J. Parasitol. Drugs Drug Resist.* 4, 164–184
95. Kotze, A.C. and Prichard, R.K. (2016) Anthelmintic resistance in *Haemonchus contortus*: history, mechanisms and diagnosis. *Adv. Parasitol.* 93, 397–428
96. Jones, P.M. and George, A.M. (2004) The ABC transporter structure and mechanism: perspectives on recent research. *Cell. Mol. Life Sci.* 61, 682–699
97. Hodges, L.M. *et al.* (2011) Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet. Genom.* 21, 152–161
98. Hooiveld, G.J.E.J. *et al.* (2002) Stereoselective transport of hydrophilic quaternary drugs by human MDR1 and rat Mdr1b P-glycoproteins. *Br. J. Pharmacol.* 135, 1685–1694
99. Roulet, A. and Prichard, R.K. (2006) Ivermectin and moxidectin cause constitutive and induced over expression of different P-glycoproteins in resistant *Haemonchus contortus*. In *Annual Meeting of the American Association of Veterinary Parasitologists*, abstract no. 72, Honolulu, USA
100. Dicker, A.J. *et al.* (2011) Gene expression changes in a P-glycoprotein (Tci-pgp-9) putatively associated with ivermectin resistance in *Teladorsagia circumcincta*. *Int. J. Parasitol.* 41, 935–942
101. Janssen, I.J. *et al.* (2013) Genetic variants and increased expression of *Parascaris equorum* P-glycoprotein-11 in populations with decreased ivermectin susceptibility. *PLoS One* 8, e61635
102. Choi, Y.J. *et al.* (2017) Genomic introgression mapping of field-derived multiple-anthelmintic resistance in *Teladorsagia circumcincta*. *PLoS Genet.* 13, e1006857
103. David, M.A. *et al.* (2016) *In silico* manuscript analysis of the binding of anthelmintics to *Caenorhabditis elegans* P-glycoprotein 1. *Int. J. Parasitol. Drugs Drug Resist.* 6, 299–313
104. Ménez, C. *et al.* (2019) The transcription factor NHR-8: A new target to increase ivermectin efficacy in nematodes. *PLoS Pathog.* 15, e1007598
105. Nappi, L. *et al.* (2020) Ivermectin inhibits HSP27 and potentiates efficacy of oncogene targeting in tumor models. *J. Clin. Invest.* 130, 699–714
106. Sheriff, J.C. *et al.* (2002) Effects of macrocyclic lactone anthelmintics on feeding and pharyngeal pumping in *Trichostrongylus colubriformis* *in vitro*. *Parasitology* 125, 477–484
107. Caly, L. *et al.* (2020) The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 *in vitro*. *Antivir. Res.* 178