New approaches for understanding mechanisms of drug resistance in schistosomes

ROBERT M. GREENBERG

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA

(*Received 2 January 2013; revised 11 February 2013; accepted 13 February 2013; first published online 3 April 2013*)

SUMMARY

Schistosomes are parasitic flatworms that cause schistosomiasis, a neglected tropical disease that affects hundreds of millions worldwide. Treatment and control of schistosomiasis relies almost entirely on the single drug praziquantel (PZQ), making the prospect of emerging drug resistance particularly worrisome. This review will survey reports of PZQ (and other drug) resistance in schistosomes and other platyhelminths, and explore mechanisms by which drug resistance might develop. Newer genomic and post-genomic strategies that offer the promise of better understanding of how drug resistance might arise in these organisms will be discussed. These approaches could also lead to insights into the mode of action of these drugs and potentially provide markers for monitoring the emergence of resistance.

Key words: schistosomiasis, drug resistance, multidrug transporters, praziquantel.

INTRODUCTION

Parasitic flatworms of the genus *Schistosoma* cause schistosomiasis, a neglected tropical disease that affects hundreds of millions of people worldwide (van der Werf *et al*. 2003; King, 2010). Schistosome infections can result in permanent damage to various organs, major morbidity, devastating effects on childhood development and adult productivity and, in some cases, death. The global health burden of schistosomiasis is now considered, in some analyses, to be similar to that of malaria or tuberculosis (Hotez and Fenwick, 2009; King, 2010).

Although there is no vaccine, the disease can be treated and controlled with praziquantel (PZQ), a drug developed in the 1970s (Gonnert and Andrews, 1977) and shortly thereafter identified as the treatment of choice by the World Health Organization (Andrews *et al*. 1983). Though new lead antischistosomal compounds have been identified (Sayed *et al*. 2008), no new drugs [other than repositioned antimalarials such as artemisinins (Keiser and Utzinger, 2012)] have entered the market since the development of PZQ. Furthermore, due to the success of PZQ, other antischistosomal drugs are no longer available in most parts of the world. Thus, treatment and control of this hugely prevalent disease relies almost entirely on a single drug.

Why has PZQ supplanted other, older drugs that have been used in the past? Probably the most important advantage of PZQ is that it is effective against all human schistosome species, while oxamniquine (which is still in limited use against *Schistosoma mansoni* infections) and the related drug hycanthone are not (reviewed by Cioli *et al*. 1995). PZQ also has relatively mild side effects, is inexpensive, and has proven its value in large-scale schistosomiasis control efforts in a variety of countries (Vennervald *et al*. 2005; Xianyi *et al*. 2005; Toure *et al*. 2008).

Reliance on a single drug for any disease of this magnitude is dangerous, as there are few if any alternatives should resistance arise. Moreover, PZQ has limitations which make this situation particularly precarious. Thus, even though PZQ has overall proved successful in treatment and control programmes, reported failure rates in the field nonetheless may reach as high as 30% (Behbehani and Savioli, 1998; Day and Botros, 2006; Mutapi *et al*. 2011), and this value could be optimistic, as the standardly-used Kato-Katz technique for measuring egg counts can underestimate levels of infection and has problems with reliability (Kongs *et al*. 2008; Lin *et al*. 2008). Additionally, liver-stage juvenile schistosomes ($\sim$ 28 days post infection) are refractory to PZQ, a major concern in regions with high reinfection rates. Worms become fully susceptible only when egg production begins approximately 6 weeks following infection of the mammalian host (Xiao *et al*. 1985; Sabah *et al*. 1986; Pica-Mattoccia and Cioli, 2004; Aragon *et al*. 2009). Furthermore, the molecular target of PZQ has not been rigorously defined. Thus, though substantial evidence suggests that PZQ interacts with schistosome voltage-gated Ca^{2+} channels, other molecular targets have also been

Corresponding author: Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia, PA 19104, USA. Tel: 215-898-5678. Fax: 215-898-5301. E-mail: rgree@vet.upenn.edu

Parasitology (2013), **140**, 1534–1546.
doi:10.1017/S0031182013000231

proposed (Redman *et al*. 1996; Greenberg, 2005; Doenhoff *et al*. 2008). Even if the molecular target of PZQ were known however, it is clear that other downstream factors contribute to PZQ action. For example, the juvenile worms that are refractory to PZQ still undergo a Ca^{2+}-dependent contraction and paralysis similar to that observed in adult worms (Pica-Mattoccia *et al*. 2008). Unlike adults, however, juveniles recover and survive, indicating that though the initial target is likely similar, adaptive responses that allow parasite survival come into play in the immature, but not mature, worms (Hines-Kay *et al*. 2012).

Finally, though there is as of yet no indication of widespread drug resistance, researchers have identified field and laboratory isolates that exhibit significantly reduced susceptibility to PZQ (Day and Botros, 2006; Doenhoff and Pica-Mattoccia, 2006; Melman *et al*. 2009; Couto *et al*. 2011), perhaps a forerunner for emergence of more widespread drug resistance. Several excellent reviews that survey and discuss evidence for PZQ and other drug resistance in schistosomes have been published over the past few years (Cioli, 2000; Day and Botros, 2006; Doenhoff *et al*. 2008, 2009*a*, *b*; Wang *et al*. 2012*b*). As such, this review will only briefly summarize that work, and will concentrate more on new approaches for understanding potential mechanisms underlying drug resistance in schistosomes, including recent work on the possible role of multidrug transporters in drug resistance and drug action.

DRUG RESISTANCE IN SCHISTOSOMES

In any discussion of drug resistance in schistosomes, it is necessary to define clearly what drug resistance is and to distinguish it from other explanations of sub-optimal drug activity (see discussion in Day and Botros, 2006). As opposed to tolerance, which represents an innate lack of susceptibility that is not in response to prior drug exposure, resistance is a heritable increase in the frequency of individuals in a population able to tolerate doses of a compound following exposure of that population to the drug (Prichard *et al*. 1980; Coles and Kinoti, 1997). Drug resistance therefore depends on the selective pressure of drug exposure, and is heritable. Thus, to distinguish properly between resistance and native tolerance, there needs to be some knowledge of the endogenous susceptibility of a particular population prior to drug administration; typically, such information is not available for schistosomiasis treatment programmes. Indeed, historically, it has been unusual to find any regular monitoring of susceptibility for these programmes. To complicate matters further, as noted above, there is already a significant background failure rate for a drug such as PZQ, as well as a range of factors other than resistance that can increase the incidence of drug failure (e.g. compromised health and immunocompetency of the host), as enumerated in Day and Botros (2006). One of the factors suggested to account for persistence of infections following PZQ treatment is the reduced susceptibility of juvenile parasites to the drug (Cioli and Pica-Mattoccia, 2003). Recent infections will contain a significant portion of PZQ-refractory juvenile worms, leading to less than optimal cure rates, and population genetic evidence from Brazil supports this idea. Thus, worms that persist following PZQ treatment were shown to have genotypes that do not differ significantly from susceptible worms, and therefore do not appear to represent a sub-population selected for PZQ resistance (Blanton *et al*. 2011). Despite these caveats, there are nonetheless several reports of possible PZQ resistance in schistosomes, both in the laboratory and in the field (reviewed by Fallon *et al*. 1996; Cioli, 2000; Day and Botros, 2006; Doenhoff *et al*. 2008; Wang *et al*. 2012*b*), including those using newer strategies for experimentally inducing and screening for PZQ-resistant schistosomes.

EXPERIMENTALLY-INDUCED PZQ RESISTANCE

Fallon and Doenhoff (1994) exploited an approach similar to that used to induce oxamniquine/hycanthone resistance in the laboratory (Cioli *et al*. 1993) to select for resistance to PZQ in *S. mansoni*. Thus, sub-curative, but increasing PZQ doses were administered to *S. mansoni*-infected mice over seven passages through the life cycle (a separate group of worms was also selected for resistance to oxamniquine in this study). By the seventh life cycle passage, this PZQ drug pressure produced a population of schistosomes in which 93% of the worms survived a PZQ dose that killed 89% of control, unselected worms. Interestingly, PZQ- and oxamniquine-resistant worms showed no cross-resistance to the other drug, indicating that resistance to the two drugs arises via different mechanisms. More recently, experimentally-induced PZQ resistance has been reported in *Schistosoma japonicum* using a similar approach (Liang *et al*. 2011).

A notable recent advance in obtaining PZQ-resistant schistosomes uses drug selection on the asexual stages of the life cycle in the snail host (Couto *et al*. 2011). The technique derives from observations showing that treating *S. mansoni*-infected *Biomphalaria glabrata* snails with 1000 mg/kg PZQ interrupts almost 90% of cercarial shedding (Mattos *et al*. 2007). Based on that finding, Couto *et al*. (2011) used successive treatments of *B. glabrata* infected with *S. mansoni* (LE strain) with the far lower dose of 100 mg/kg PZQ to select for cercariae that, upon infection of mice, developed into adult worms with a significantly reduced susceptibility to PZQ. The ED50 of PZQ for these LE-PZQ worms in mice was approximately five-fold higher than that

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

for the parental LE strain (362 mg/kg for LE-PZQ vs 68 mg/kg for LE). Following PZQ, LE-PZQ worms were also less contracted than LE worms and, as assayed by fluorescent probes, showed less severe tegumental damage and, unlike LE worms, appeared to retain a functional excretory system (Couto *et al*. 2010). The ability to use this approach to select for drug resistance at the snail stage is far less costly and labour intensive than previous strategies of applying drug pressure through multiple intramammalian-stage passages. It holds the promise of much more readily providing new drug-resistant isolates that will be useful for studying the mechanisms of PZQ resistance and perhaps lend insights into the mode of action of PZQ.

FIELD ISOLATES

There have been several reports of schistosome field isolates exhibiting reduced PZQ susceptibility. Many of these reports have been thoroughly reviewed and discussed by others, and will therefore be described only briefly here. More recent reports will also be included.

In Northern Senegal, lower than expected cure rates were initially reported in the 1990s for *S. mansoni* infections treated with PZQ. Alarmingly, cure rates were reported to be as low as 18% (Gryseels *et al*. 1994; Stelma *et al*. 1995). Subsequent follow-up studies and analysis of the data (Fallon, 1998; Gryseels *et al*. 2001; Danso-Appiah and De Vlas, 2002) suggested that some portion (though not all) of this drug failure could be attributed to factors other than drug resistance, including high-intensity infection, rapid reinfection and transmission, presence of PZQ-refractory juvenile worms, variations in methodology for analysis of efficacy, and perhaps native tolerance of these schistosomes. Interestingly, in patients relocated to urban areas (which do not have ongoing transmission), cure rates rose to near-normal levels (Gryseels *et al*. 2001). Furthermore, despite the reduced cure rates in the area of interest, PZQ treatment nonetheless dramatically lowered the infection intensity and curbed morbidity in treated individuals (reviewed by Fallon, 1998). On the other hand, worms from the Senegalese isolate exhibiting reduced cure rates are also less susceptible to PZQ when grown in experimentally-infected mice (Fallon, 1995; Fallon *et al*. 1997), suggesting loss of PZQ sensitivity is an endogenous trait of the worms themselves. Furthermore, in both human and mouse infections, these worms were susceptible to oxamniquine, which, like PZQ, is not effective against immature worms, casting some doubt on arguments suggesting low cure rates in this area may reflect large numbers of PZQ-refractory juvenile worms due to high rates of transmission (Fallon *et al*. 1997; Stelma *et al*. 1997).

Another site for intense study of potential PZQ resistance has been in Egypt, where schistosomes were isolated from several *S. mansoni*-infected patients (1·6% of those screened) who continued to pass viable eggs following three successive doses of PZQ (Ismail *et al*. 1996). The schistosomes isolated from these patients were subsequently propagated in mice, where they showed 3–5-fold lower sensitivity to PZQ, as measured by ED50 (Ismail *et al*. 1996, 1999). Tests of known responses of worms to PZQ *in vitro* (i.e. in the absence of any confounding host factors) showed that at least some of these isolates were less susceptible to the drug (Ismail *et al*. 1999; William *et al*. 2001*a*). Indeed, *in vitro* measures of PZQ susceptibility correlated well in some cases with ED50 determinations in murine infections (William and Botros, 2004), further indicating that factors in the worms themselves were responsible for the reduced PZQ susceptibility of these isolates. Interestingly, approximately half of the isolates tested retained their lower response to PZQ even after multiple passages through the life cycle in the absence of drug pressure, while others reverted. Indeed, application of drug pressure does not appear to be required for maintenance of the PZQ insusceptibility trait (Sabra and Botros, 2008), and ED50 differences, though reproducible, are relatively small (2–3-fold), and certainly not indicative of 'super-resistant' worms (Cioli and Pica-Mattoccia, 2005). On the other hand, those isolates that did retain the trait often exhibited evidence of compromised biological fitness such as reduced cercarial production by infected snails (William *et al*. 2001*b*). This observation, as well as those of others (Liang *et al*. 2001) suggest that there are costs to schistosomes associated with lessening PZQ susceptibility. These costs may serve to limit the spread of PZQ resistance. Indeed, 10 years after the initial Egyptian studies, the same villages in Egypt in which the original PZQ failures were followed up and revealed no evidence of uncured patients despite a decade of drug pressure (Botros *et al*. 2005). The presence of large refugia in endemic areas may also limit the spread of resistance; indeed, PZQ-refractory immature schistosomes may act as a refugia (Webster *et al*. 2008).

Further evidence for isolates showing PZQ insusceptibility has been found in Kenya. Researchers used an *in vitro* assay on miracidia hatched from eggs excreted by *S. mansoni*-infected Kenyan car washers to screen for *S. mansoni* exhibiting decreased susceptibility to PZQ (Melman *et al*. 2009). Different patients produced eggs that hatched into miracidia with variable PZQ sensitivity (as measured by miracidial killing); miracidia from previously-treated patients showed significantly lower sensitivity to the drug. Further characterization of an isolate from a patient who was never fully cured by PZQ (KCW) revealed that adult worms derived from these eggs were less sensitive to PZQ, both *in vivo*, in murine infections and *in vitro*, as assayed by schistosome length. Interestingly, the reduced susceptibility

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

of one sub-isolate of KCW was heritable and persisted through at least 6 life cycle passages in the absence of drug pressure. However, a second KCW sub-isolate had reverted to a PZQ-susceptible state when retested after 8 generations. This now-susceptible sub-isolate survived; the sub-isolate that retained PZQ tolerance eventually perished (Melman *et al*. 2009). Thus, as with the Egyptian isolates, there appears to be variability in the stability of this trait, as well as a biological cost associated with PZQ insusceptibility.

There have also been attempts to assess the status of PZQ resistance in other species of schistosomes that infect humans (*S. japonicum, S. haematobium*). China has for many years relied on PZQ-based chemotherapy in its programme against *S. japonicum* infections. Wang *et al*. (2012*b*) recently reviewed several studies that monitored different endemic areas of China for evidence of PZQ insusceptibility. These studies found little if any evidence for emerging PZQ resistance, and suggest that, despite decades of intense chemotherapy, PZQ continues to be effective in treating schistosomiasis japonicum in China (Yu *et al*. 2001; Wang *et al*. 2010, 2012*a*; Seto *et al*. 2011). Isolated incidents of failure of PZQ to cure *S. haematobium* infections have been reported, including a notable case in which PZQ failed to cure Brazilian soldiers returning from Africa (Silva *et al*. 2005), though there is currently no evidence for heritable resistance (Herwaldt *et al*. 1995; Alonso *et al*. 2006).

Evidence for drug failure has also been found in other trematodes. Of particular interest is the liver fluke *Fasciola hepatica*. Though not particularly susceptible to PZQ, *F. hepatica* can be treated quite effectively with other compounds such as the benzimidazoles, which target β-tubulin, and are most frequently used as anti-nematodals. The benzimidazole triclabendazole (TCBZ) is effective against both immature and mature flukes (Boray *et al*. 1983) and has seen widespread use since its introduction. Recent reports have suggested the localized emergence of TCBZ-resistant fluke isolates (Moll *et al*. 2000; Fairweather, 2011), and work described below has focused on defining the underlying source of resistance in one of these TCBZ-resistant isolates.

MECHANISMS OF RESISTANCE

Resistance to a single class of drugs can arise via several mechanisms. The most obvious is target modification. For example, benzimidazoles such as albendazole act to inhibit microtubule polymerization; in nematodes and fungi, resistance has been mapped to a F200Y point mutation in β-tubulin (Kwa *et al*. 1995). Similarly, simultaneous point mutations in three glutamate-gated chloride channel α-type subunits in *Caenorhabditis elegans* confer resistance ($\sim$4000-fold) to the antiparasitic drug ivermectin (IVM) in these worms (Dent *et al*. 2000). Interestingly, mutations in the Dyf (dye filling defective) class of genes, which appear to affect cuticle permeability, produce moderate IVM resistance (2–5-fold) and act additively to increase resistance of the channel mutations. This effect speaks to yet another mechanism for generation of resistance, namely heritable alterations that reduce drug availability or activity. These changes can be in uptake/permeability, activation/metabolism or drug efflux.

One of the more instructive cases of such non-target-dependent development of resistance comes from studies on schistosome resistance to oxamniquine (reviewed by Cioli *et al*. 1995). As noted above, oxaminiquine is highly effective against *S. mansoni*, but lacks activity against other human schistosomes such as *S. haematobium* and *S. japonicum* (hycanthone, a related antischistosomal compound, is active against *S. mansoni* and *S. haematobium*, but not *S. japonicum*). In a series of elegant and challenging experiments using genetic crosses of drug-sensitive and drug-resistant schistosomes, Donato Cioli and colleagues showed that oxamniquine/hycanthone resistance in these worms was controlled by a single autosomal recessive gene. They also showed that the antischistosomal activity of the drug requires biotransformation to an active form by a parasite sulfotransferase. When activated, the drug is thought to act as an alkylating agent of schistosome DNA and other macromolecules, interfering with nucleic acid synthesis (Cioli and Pica-Mattoccia, 1984; Cioli *et al*. 1992, 1993). The drug is inactive against schistosome species that lack this sulfotransferase activity and drug resistance can arise when this activity is lost in species that normally express it (Pica-Mattoccia *et al*. 1992, 1997). More recent similar genetic studies on worms showing reduced sensitivity to PZQ suggest either dominant (Liang *et al*. 2003) or partially dominant (Pica-Mattoccia *et al*. 2009) inheritance of the trait. Other examples of non-target-based mechanisms involved in anthelmintic drug action and development of resistance have recently been reviewed (Cvilink *et al*. 2009; James *et al*. 2009).

With regard to PZQ failure, the fact that the PZQ target has not been rigorously defined makes the search for differences more problematic. However, no clear changes in candidate targets have been found to date. Thus, voltage-gated Ca^{2+} (Ca_v) channel β subunits have been implicated in PZQ action (Greenberg, 2005; Nogi *et al*. 2009), but an examination of Ca_v channel β subunits in different isolates showing reduced PZQ susceptibility revealed no meaningful sequence differences or changes in expression levels (Valle *et al*. 2003; Kohn and Greenberg, unpublished data). On the other hand, reducing Ca_v channel subunit levels in the planarian *Dugesia japonica* confers resistance to these free-living platyhelminths against PZQ-elicited dramatic disruptions of normal regeneration patterns

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

(Nogi *et al*. 2009; Zhang *et al*. 2011). The relationship between PZQ effects on planarian regeneration vs. its antischistosomal activity is not clear. Interestingly, however, pre-treating worms with the actin depolymerizing agent cytochalasin D renders *S. mansoni* refractory to PZQ, suggesting that changes in cytoskeletal dynamics can alter susceptibility to PZQ (Pica-Mattoccia *et al*. 2007).

There are also several non-target-based changes that could alter PZQ effectiveness. For example, as noted above, juvenile schistosomes are refractory to PZQ. Additionally, adult female schistosomes, though still PZQ-sensitive, are more tolerant of the drug than adult males (Pica-Mattoccia and Cioli, 2004). Thus, changes in worm maturation rates (Fallon *et al*. 1997) or sex ratios could influence the effectiveness of PZQ. Since PZQ-induced killing of *S. mansoni* within the mammalian host appears to be immune dependent (Brindley and Sher, 1987; Doenhoff *et al*. 1987; Brindley, 1994), another possibility is that loss or modulation of schistosome antigens that become exposed following PZQ treatment could lead to reduced antischistosomal activity. Interestingly, recent evidence indicates that two other platyhelminths (the trematode *Dicrocoelium dendriticum* and the cestode *Hymenolepis nana*) are not capable of enzymatically metabolizing PZQ (Vokřál *et al*. 2012). Acknowledging the caveat that schistosomes may differ from these other platyhelminths, these results nonetheless suggest that development of more efficient PZQ metabolism by the parasite is not a particularly likely scenario for acquisition of PZQ resistance.

Molecular differences associated with reduced PZQ susceptibility in schistosomes would provide useful markers to monitor emergence of resistance in drug administration programmes. They could also serve as entrées into understanding how resistance develops and provide insights into the mechanism of drug action. There have been a handful of attempts to define such molecular correlates of PZQ resistance. For example, subtractive PCR and cloning of differentially-expressed RNAs revealed higher levels of an RNA encoding subunit 1 of mitochondrial cytochrome *c*-oxidase (SCOX1) in schistosomes selected for reduced PZQ susceptibility (Pereira *et al*. 1998). Analysis by semi-quantitative RT-PCR confirmed that the SCOX1 RNA was expressed at 5–10-fold higher levels in the resistant worms than in a PZQ-sensitive strain. Interestingly, no differences were found in expression of RNAs encoding SMDR2, a schistosome multidrug transporter (see below), nor NADH dehydrogenase subunit 5, another mitochondrial gene. Surprisingly, however, the enzymatic activity of cytochrome c-oxidase showed an expression pattern opposite to that found for the SCOX1 RNA. Thus, cytochrome c-oxidase activity in resistant worms was approximately 4-fold lower than in the PZQ-susceptible worms, an unexpected result given the 5–10-fold higher levels of SCOX1 RNA found in the resistant worms.

Another group (Tsai *et al*. 2000) used random amplified polymorphic DNA (RAPD) PCR to test for markers of PZQ resistance. They found that an Egyptian isolate with reduced PZQ susceptibility (SO5) had 2 major differences in banding pattern from several PZQ-sensitive strains from the same endemic area of Egypt. Whether this difference can serve as a marker is unclear, as are any potential functional implications.

One of the more common mechanisms for development of drug resistance is through increased drug efflux, often mediated by multidrug transporters. Multidrug transporters underlie multidrug resistance (MDR), a phenomenon in which resistance to a single drug is accompanied by unexpected cross-resistance to several structurally unrelated compounds. Multidrug transporters have broad substrate specificity and actively remove xenobiotics and toxic compounds, including drugs, from cells and tissues, though non-transport-related MDR can also occur (Pommier *et al*. 1999, 2004). Genes for multidrug transporters are found in all living cells (Blackmore *et al*. 2001), and are classified into five basic families (Paulsen, 2003; Higgins, 2007). The crystal structure of at least one representative of each of these families has been solved (van Veen, 2010). Broadly speaking, these different transporter types fit into one of two major classes, the primary-active transporters and the secondary-active transporters (Ventner *et al*. 2005). The primary-active transporters couple translocation of substrate directly to the hydrolysis of ATP, while transport in the secondary-active transporters utilizes chemiosmotic energy derived from the electrochemical gradient of proton/sodium ions across the cytoplasmic membrane.

Members of the ATP-binding cassette (ABC) superfamily of transporters are primary-active transporters that comprise one of the largest groups of transmembrane proteins found in living cells (Dassa and Bouige, 2001; Borst and Elferink, 2002). ABC transporters are found in organisms from all living kingdoms. They bind and hydrolyze ATP and use the resultant energy to translocate compounds across the membrane. ABC importers transport compounds into the cell, and are found in prokaryotes; ABC exporters are efflux transporters found in both prokaryotes and eukaryotes (Dassa and Bouige, 2001; Saier and Paulsen, 2001).

All ABC transporters share at least one highly conserved ATPase domain containing the Walker_{A} and Walker_{B} motifs typically found in ATPases as well as a specific signature motif. Full ABC transporters contain two of these cytoplasmic ATP-binding cassettes that alternate with two membrane-spanning domains; half transporters contain one of each of these structural features (Ambudkar *et al*.

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

2003; Szakacs *et al*. 2006). Vertebrates have on the order of 50 ABC transporter genes that define seven distinct sub-families (designated ABCA to ABCG) based on phylogenetic analysis (Dean *et al*. 2001; Dean and Annilo, 2005). Subsets of these ABC transporters are associated with MDR (Szakacs *et al*. 2006). The *S. mansoni* genome appears to contain approximately 25 genes for ABC transporters, including several potentially involved in MDR (Kasinathan and Greenberg, 2012).

Largely because of its role in MDR in cancer chemotherapy, P-glycoprotein (Pgp; ABCB1) is the most thoroughly studied of the eukaryotic multidrug transporters, and mammalian and *C. elegans* Pgp have recently been crystallized and their structures solved (Aller *et al*. 2009; Jin *et al*. 2012). MDR is linked to gene amplification, overexpression or mutation of Pgp or other multidrug transporters, resulting in increased drug efflux (reviewed by Borst and Elferink, 2002; Ambudkar *et al*. 2003; Szakacs *et al*. 2006). In addition to Pgp, known ABC proteins involved in MDR include the multidrug resistance-associated proteins (MRPs; ABCCs), breast cancer resistance protein (BCRP; ABCG2), as well as others (Szakacs *et al*. 2006).

Pgp and other multidrug transporters such as MRP1 transport a broad spectrum of compounds including several anticancer and other drugs (Kartner *et al*. 1983; Higgins, 2007). Though the substrate specificities of the transporters show some overlap, there are clear preferences. Thus, Pgp shows selectivity for neutral and cationic hydrophobic compounds, while MRP1 preferentially transports organic anions, drugs and other compounds such as glutathione and other biotransformed conjugates, and signaling molecules such as the immunomodulator leukotriene C4 (reviewed by Ambudkar *et al*. 2003; Gimenez-Bonafe *et al*. 2008). Members of the ABC transporter family also show selectivity for biologically significant compounds such as lipids, steroids, cyclic nucleotides and peptides, indicative of their important roles in cellular and organismal physiology (Mizutani *et al*. 2008; van de Ven *et al*. 2009). In addition to the broad selection of substrates that interact with these transporters, there are also a host of inhibitors that can reverse MDR by blocking multidrug transporter-mediated drug efflux (reviewed by Gimenez-Bonafe *et al*. 2008). Many of these inhibitors are inexpensive and safe compounds in wide clinical use (e.g. verapamil). Indeed, PZQ is an inhibitor of both mammalian and *S. mansoni* Pgp (Hayeshi *et al*. 2006; Kasinathan *et al*. 2010*a*).

MULTIDRUG TRANSPORTERS IN SCHISTOSOMES AND OTHER TREMATODES

Could changes in multidrug transporter expression or structure be contributing to drug resistance in schistosomes? There are precedents in the literature for such an association, as ABC multidrug transporters such as Pgp have been implicated in drug resistance in other parasites, including parasitic helminths (reviewed by Kerboeuf *et al*. 2003; Jones and George, 2005; James *et al*. 2009; Leprohon *et al*. 2011; Lespine *et al*. 2012). For example, the macrocyclic lactone ivermectin is an anthelmintic that is both a substrate and inhibitor of Pgp. Indeed, the excellent safety profile of ivermectin is due in large part to Pgp in the blood-brain barrier excluding the drug from the host central nervous system; defects in Pgp in the blood-brain barrier result in hypersensitivity to ivermectin neurotoxicity (Schinkel *et al*. 1994; Mealey *et al*. 2001). Ivermectin also likely interacts with nematode multidrug transporters, and resistance to it and other macrocyclic lactones is associated with changes in Pgp alleles or expression levels. Notably, several studies show that co-administration of MDR reversing agents (e.g. Pgp inhibitors such as verapamil) can increase the efficacy of macrocyclic lactones in drug-resistant and -sensitive nematodes (Xu *et al*. 1998; Molento and Prichard, 1999; Bartley *et al*. 2009; Tompkins *et al*. 2011; Ardelli and Prichard, 2013).

There have also been reports suggesting a role for ABC multidrug transporters in other platyhelminths. As noted above, certain *F. hepatica* isolates exhibit reduced susceptibility to TCBZ. Recent preliminary evidence indicates that an amino acid substitution in a critical region of *F. hepatica* Pgp is associated with TCBZ resistance (Wilkinson *et al*. 2012). Work on another liver fluke, *F. gigantica*, provided evidence for expression of four ABC multidrug transporters in this worm, with expression of two of them increased in the presence of TCBZ in isolated fluke cells. Furthermore, efflux of rhodamine from these fluke cells could be inhibited by a MDR reversing agent (Kumkate *et al*. 2008).

Work on schistosome Pgp and other multidrug transporters essentially began in 1994, when cDNAs encoding Pgp (SMDR2) and an ABC half transporter (SMDR1) were cloned and sequenced (Bosch *et al*. 1994). Two different oxamniquine/hycanthone-resistant isolates showed no evidence for amplification or overexpression of SMDR2. As noted above, the subsequent availability of the sequenced genome revealed ~25 ABC transporter-like sequences in *S. mansoni*, including other Pgp-like genes and representatives of other ABC transporter sub-families (Kasinathan and Greenberg, 2012).

Fluorescent substrates of mammalian Pgp and MRP have been used to localize these substrates to the excretory system of schistosomes (Sato *et al*. 2002, 2004). PZQ dramatically disrupts the distribution of the Pgp substrate in PZQ-susceptible worms (Kusel *et al*. 2006; Oliveira *et al*. 2006), but not in the recently-derived *S. mansoni* isolate selected at the snail stage for reduced PZQ susceptibility (Couto *et al*. 2010). These results suggest a role for

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

ABC multidrug transporters in schistosome excretory activity, and may be providing hints of a role for Pgp and other ABC transporters in PZQ resistance.

There are further indications that multidrug transporters may be involved in modulating levels of PZQ susceptibility in schistosomes. PZQ is both an inhibitor and a substrate of recombinant SMDR2 (Kasinathan *et al*. 2010*a*), and chronic exposure of worms to sub-lethal concentrations of PZQ results in up-regulation of SMDR2 and schistosome MRP1 (SmMRP1), and changes the distribution of anti-Pgp immunoreactivity in the worm (Messerli *et al*. 2009; Kasinathan *et al*. 2010*b*). Importantly, higher levels of schistosome SMDR2 and SmMRP1 are associated with reduced PZQ susceptibility (Messerli *et al*. 2009; Kasinathan *et al*. 2010*b*). Indeed, EE2, an Egyptian isolate with reduced PZQ susceptibility, expresses dramatically higher levels of SMDR2 RNA and protein (Messerli *et al*. 2009); interestingly, SmMRP1 does not appear to be expressed at a higher level in EE2 (Kasinathan *et al*. 2010*b*). At this juncture, only an association between reduced drug susceptibility and ABC multidrug transporters has been demonstrated. Further pharmacological and genetic experiments will be required to establish a causal relationship. However, we have recently used both of these approaches to implicate *S. mansoni* ABC multidrug transporters in schistosome reproduction (Kasinathan *et al*. 2011).

NEW POST-GENOMIC APPROACHES TO DEFINE THE MECHANISMS OF DRUG RESISTANCE IN SCHISTOSOMES

For a variety of reasons, most notably that they are obligate parasites, schistosomes are notoriously difficult systems for experimental analysis. In recent years, however, tools that are feasible in other organisms have been, or are being, adapted to schistosomes. These new approaches, some of which are listed below, hold the promise of providing major advances in our knowledge about schistosome biology and physiology, including the underlying basis for anti-schistosomal drug action and drug resistance.

GENE MAPPING

As noted, traditional genetic experiments provided important insights into the mechanism of oxamniquine/hycanthone drug resistance and mode of action (Cioli *et al*. 1993). Genetic crosses showed that oxamniquine/hycanthone resistance is linked to inheritance of a single autosomal recessive gene, while reduced PZQ susceptibility appears to be either a dominant (Liang *et al*. 2003) or partially dominant (Pica-Mattoccia *et al*. 2009) trait. Newer, post-genomic approaches have the potential for much greater power. By combining the ability to conduct genetic crosses with the availability of a high resolution genetic linkage map (Criscione *et al*. 2009) and an increasingly well-assembled and annotated genome sequence for *S. mansoni* (Protasio *et al*. 2012), linkage mapping holds the promise to become a feasible approach for locating genes that underlie drug resistance. A particular appeal of this approach is that it surveys all of the genome, and is therefore not based on preconceived ideas about mechanism or possible candidate genes.

TRANSCRIPTOMICS AND OTHER '-OMICS'

Examination of global changes in gene expression following drug treatment or between isolates showing differential drug susceptibility may provide an entrée into identification of genes underlying drug action or resistance. Microarray studies of schistosomes following low-dose PZQ treatment *ex vivo* have revealed molecular pathways that appear to be activated by PZQ, including expression of multidrug transporters and Ca^{2+} regulatory-, stress-, and apoptosis-related pathways (Aragon *et al*. 2009; Hines-Kay *et al*. 2012). Comparison of the gene expression patterns of PZQ-refractory juvenile and PZQ-susceptible adult schistosomes following sub-lethal PZQ showed that the juvenile worms exhibited a greater transcriptomic flexibility that may allow them to respond to and survive exposure to PZQ.

Although microarray studies can be enlightening, the greater power of next-generation sequencing technologies such as RNAseq, along with robust bioinformatics algorithms (Cantacessi *et al*. 2012), may provide a higher-resolution analysis of meaningful gene expression changes in schistosomes following exposure to PZQ, as well as new drug targets. Furthermore, use of these approaches on worms treated with PZQ within the host should provide a better approximation of real-world responses to treatment. Examination of differences in microRNAs, which could produce reduced drug susceptibility by repressing expression of the drug target (or associated co-factors), could provide novel pathways to development of resistance (Devaney *et al*. 2010). Other '-omics' analyses of parasite responses to drug treatment should of course be explored; proteomic, glycomic, lipidomic and metabolomic approaches will likely also prove enlightening. Powerful 'chemogenomics' and comparative genomics approaches have the potential to provide new drug targets and perhaps insights into drug resistance (Caffrey *et al*. 2009; Swain *et al*. 2011). Finally, in order to obtain a better understanding of mechanisms underlying resistance, these same tools should be brought to bear to compare drug-sensitive worms vs isolates with reduced susceptibility.

RNA INTERFERENCE (RNAI)

RNAi is gene silencing (or suppression) triggered by exogenous double-stranded (ds) RNA.

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

Knockdown of genes using RNAi has proven to be an especially powerful molecular tool for analysis of a variety of gene functions. Though many parasitic nematodes appear to be refractory to RNAi (Britton *et al*. 2012; Selkirk *et al*. 2012), the methodology has proven relatively robust in schistosomes, and has provided important insights into schistosome biology (reviewed by Krautz-Peterson *et al*. 2010). Both small interfering ds RNAs (siRNAs) and larger dsRNAs are effective. Analysis of phenotypes following knockdown of potential drug targets or of components of pathways hypothesized to modulate drug activity could provide important clues regarding drug action and mechanisms underlying resistance. However, there are several caveats for the use of this approach in schistosomes. For example, there can be tremendous variability in knockdown efficiency depending on the target (Mourao *et al*. 2009; Stefanic *et al*. 2010). To a first approximation, targets expressed in the intestine and on the tegument appear to be most amenable to knockdown. Off-target effects, in which dsRNA directs knockdown of transcripts other than those intended, have been reported in other systems and are an ever-present concern (Sioud, 2011). Knockdown is furthermore often partial even when successful, with perhaps 30–50% of transcripts remaining; a 50% level of expression might be sufficient to maintain function and mask any detectable phenotypes. Redundancy of genes can also often be a confounding factor. For example, as noted above, there are several predicted Pgp-like genes in *S. mansoni*, and suppression of one might be compensated for by the others, again masking a phenotype. Defining phenotypes other than those that are obvious (death, paralysis, egg production) can be challenging, even *ex vivo*, and particularly *in vivo*, within the host. Finally, it is currently technically difficult to perform these types of experiments *in vivo*, within the mammalian host. As knockdown of genes in infectious cercariae has not been reported, experiments are instead typically done by performing knockdown in schistosomules produced *in vitro* from cercariae. These schistosomules are then injected into the host, which is a far less efficient means of infection than using cercariae. Though this approach has been successful (see, for example, Bhardwaj *et al*. 2011), there is low and variable recovery of the adults that develop from these injected schistosomules, confounding data analysis. Reports of knockdown of parasite genes by injecting the infected host with siRNA have appeared (Pereira *et al*. 2008; Cheng *et al*. 2009; Yang *et al*. 2012), though it remains to be seen if this approach will be incorporated more generally in the field.

TRANSGENESIS

Although RNAi is an extraordinarily powerful tool for analyzing gene function, a more complete armamentarium for functional genomics in schistosomes will require the availability of somatic and germline transgenesis. In conjunction with the completion of draft genomes for all three major human schistosome species and many years of concerted efforts, relatively efficient gene insertion and knockout strategies may be coming to fruition for schistosomes (Tchoubrieva and Kalinna, 2010; Beckmann and Grevelding, 2012; Suttiprapa *et al*. 2012). Of particular note is an exciting recent report of murine leukaemia virus-mediated germ-line transgenesis and insertional mutagenesis in *S. mansoni* (Rinaldi *et al*. 2012*a*). Other technical advances have also been steadily appearing, including antibiotic selection of transgenic worms (Rinaldi *et al*. 2012*b*) and vector-mediated RNAi (Tchoubrieva *et al*. 2010; Duvoisin *et al*. 2012). These types of advances offer the promise of more feasible transgenesis in schistosomes, with more widespread adoption, and hold the promise of new strategies for studies of drug action and resistance. A model for the type of power that could be brought to bear on these questions was recently provided in studies using transgenesis of *C. elegans* resistant to the anthelmintic emodepside. These *C. elegans* were transformed with genes from other nematodes or mammals to investigate the role of Ca^{2+}-activated potassium (SLO-1) channels in the selectivity and mode of action of this drug (Crisford *et al*. 2011; Welz *et al*. 2011).

OTHER ADVANCES

One of the challenges of working with schistosomes is that they are obligate parasites and, currently, only one developmental stage (schistosomules) can be archived by freezing for re-establishment of the life cycle (Cooper *et al*. 1989). Though primary *S. mansoni* cells such as muscle fibres have been used to study drug and neurotransmitter action (Novozhilova *et al*. 2010), the establishment of cell lines in schistosomes would be a huge boon for the field, and could provide high-throughput screening opportunities to investigate drug targets and test hypotheses regarding mechanisms of resistance. To date, no such cell lines have been successfully established, but newer approaches for immortalization of cells are promising (Quack *et al*. 2010), and advances in the study and culture of multipotent cells (neoblasts) from Schistosomes and other platyhelminths (Eisenhoffer *et al*. 2008; Brehm, 2010; Collins, *et al*. 2013) may eventually prove adaptable to schistosomes.

High- and medium-throughput systems for screening schistosome phenotypes such as paralysis or contraction will also be useful. These include video analysis systems (Caffrey *et al*. 2009) and a novel adoption of the xCelligence (Roche) system for measuring electrical impedance across micro-electrodes interdigitated on the bottom of tissue culture plates (Smout *et al*. 2010). New and powerful

https://doi.org/10.1017/S0031182013000231 Published online by Cambridge University Press

markers that have been developed for *S. mansoni* organs and developmental stages (Collins *et al*. 2011) should also aid in these types of analyses.

CONCLUDING REMARKS

The prospect of schistosomes developing widespread resistance to PZQ is an alarming one, and understanding mechanisms by which resistance might emerge could provide markers for monitoring response to mass treatment programmes, as well as possible strategies for reversing drug resistance. It is somewhat heartening that worms exhibiting apparent PZQ resistance often appear to be compromised, and furthermore, exhibit relatively small (2–5-fold) changes in drug susceptibility, as measured by ED50s. Indeed, typical treatment with PZQ uses an ED90 dose, which should be sufficient to eliminate worms showing 2–3-fold reductions in susceptibility (Cioli and Pica-Mattoccia, 2005). Nonetheless, complacency is clearly unwarranted, as in both Egypt and Kenya (Ismail *et al*. 1999; Melman *et al*. 2009), isolates were derived from patients who simply were not cured by such doses. These patients continued to pass eggs (presumably carrying a PZQ-tolerant trait), suggesting that clinically-significant treatment failures potentially indicative of emerging resistance can occur in the field. One hopes that some of the newer technologies described in this review may lead to development of tools to deal with such resistance before disastrous consequences ensue.

ACKNOWLEDGEMENTS

I thank Ravi Kasinathan for helpful discussions and the reviewers of this manuscript for their very valuable suggestions.

FINANCIAL SUPPORT

RMG is supported by NIH grants R01 AI073660 and R21 AI082390.

REFERENCES

Aller, S. G., Yu, J., Ward, A., Weng, Y., Chittaboina, S., Zhuo, R., Harrell, P. M., Trinh, Y. T., Zhang, Q., Urbatsch, I. L. and Chang, G. (2009). Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **323**, 1718–1722. doi: 10.1126/science.1168750.

Alonso, D., Munoz, J., Gascon, J., Valls, M. E. and Corachan, M. (2006). Failure of standard treatment with praziquantel in two returned travelers with *Schistosoma haematobium* infection. *American Journal of Tropical Medicine and Hygiene* **74**, 342–344.

Ambudkar, S. V., Kimchi-Sarfaty, C., Sauna, Z. E. and Gottesman, M. M. (2003). P-glycoprotein: from genomics to mechanism. *Oncogene* **22**, 7468–7485.

Andrews, P., Thomas, H., Pohlke, R. and Seubert, J. (1983). Praziquantel. *Medicinal Research Reviews* **3**, 147–200.

Aragon, A. D., Imani, R. A., Blackburn, V. R., Cupit, P. M., Melman, S. D., Goronga, T., Webb, T., Loker, E. S. and Cunningham, C. (2009). Towards an understanding of the mechanism of action of praziquantel. *Molecular and Biochemical Parasitology* **164**, 57–65. doi: 10.1016/j.molbiopara.2008.11.007.

Ardelli, B. F. and Prichard, R. K. (2013). Inhibition of P-glycoprotein enhances sensitivity of *Caenorhabditis elegans* to ivermectin. *Veterinary Parasitology* **191**, 264–275. doi: 10.1016/j.vetpar.2012.09.021.

Bartley, D. J., McAllister, H., Bartley, Y., Dupuy, J., Menez, C., Alvinerie, M., Jackson, F. and Lespine, A. (2009). P-glycoprotein interfering agents potentiate ivermectin susceptibility in ivermectin sensitive and resistant isolates of *Teladorsagia circumcincta* and *Haemonchus contortus*. *Parasitology* **136**, 1081–1088. doi: 10.1017/S0031182009990345.

Beckmann, S. and Grevelding, C. G. (2012). Paving the way for transgenic schistosomes. *Parasitology* **139**, 651–668. doi: 10.1017/S0031182011001466.

Behbehani, M. and Savioli, L. (1998). *Report of the WHO informal consultation on monitoring of drug efficacy in the control of schistosomiasis and intestinal nematodes*. WHO/CDS/CPC/SIP/99.1. World Health Organization, Geneva.

Bhardwaj, R., Krautz-Peterson, G., Da'dara, A., Tzipori, S. and Skelly, P. J. (2011). Tegumental phosphodiesterase SmNPP-5 is a virulence factor for schistosomes. *Infection and Immunity* **79**, 4276–4284. doi: 10.1128/IAI.05431-11.

Blackmore, C. G., McNaughton, P. A. and Van Veen, H. W. (2001). Multidrug transporters in prokaryotic and eukaryotic cells: physiological functions and transport mechanisms. *Molecular Membrane Biology* **18**, 97–103.

Blanton, R. E., Blank, W. A., Costa, J. M., Carmo, T. M., Reis, E. A., Silva, L. K., Barbosa, L. M., Test, M. R. and Reis, M. G. (2011). *Schistosoma mansoni* population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. *International Journal for Parasitology* **41**, 1093–1099. doi: 10.1016/j.ijpara.2011.06.002.

Boray, J. C., Crowfoot, P. D., Strong, M. B., Allison, J. R., Schellembaum, M., von Orelli, M. and Sarasin, G. (1983). Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Veterinary Record* **113**, 315–317.

Borst, P. and Elferink, R. O. (2002). Mammalian ABC transporters in health and disease. *Annual Review of Biochemistry* **71**, 537–592.

Bosch, I. B., Wang, Z. X., Tao, L. F. and Shoemaker, C. B. (1994). Two *Schistosoma mansoni* cDNAs encoding ATP-binding cassette (ABC) family proteins. *Molecular and Biochemical Parasitology* **65**, 351–356.

Botros, S., Sayed, H., Amer, N., El-Ghannam, M., Bennett, J. L. and Day, T. A. (2005). Current status of sensitivity to praziquantel in a focus of potential drug resistance in Egypt. *International Journal for Parasitology* **35**, 787–791.

Brehm, K. (2010). *Echinococcus multilocularis* as an experimental model in stem cell research and molecular host-parasite interaction. *Parasitology* **137**, 537–555. doi: 10.1017/S0031182009991727.

Brindley, P. J. (1994). Relationships between chemotherapy and immunity in schistosomiasis. *Advances in Parasitology* **34**, 133–161.

Brindley, P. J. and Sher, A. (1987). The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *Journal of Immunology* **139**, 215–220.

Britton, C., Samarasinghe, B. and Knox, D. P. (2012). Ups and downs of RNA interference in parasitic nematodes. *Experimental Parasitology* **132**, 56–61. doi: 10.1016/j.exppara.2011.08.002.

Caffrey, C. R., Rohwer, A., Oellien, F., Marhofer, R. J., Braschi, S., Oliveira, G., McKerrow, J. H. and Selzer, P. M. (2009). A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen, *Schistosoma mansoni*. *PLoS ONE* **4**, e4413. doi: 10.1371/journal.pone.0004413.

Cantacessi, C., Campbell, B. E., Jex, A. R., Young, N. D., Hall, R. S., Ranganathan, S. and Gasser, R. B. (2012). Bioinformatics meets parasitology. *Parasite Immunology* **34**, 265–275. doi: 10.1111/j.1365-3024.2011.01304.x.

Cheng, G., Fu, Z., Lin, J., Shi, Y., Zhou, Y., Jin, Y. and Cai, Y. (2009). *In vitro* and *in vivo* evaluation of small interference RNA-mediated gynaecophoral canal protein silencing in *Schistosoma japonicum*. *Journal of Gene Medicine* 11, 412–421. doi: 10.1002/jgm.1314.

Cioli, D. (2000). Praziquantel: is there real resistance and are there alternatives? *Current Opinion in Infectious Diseases* **13**, 659–663.

Cioli, D. and Pica-Mattoccia, L. (1984). Genetic analysis of hycanthone resistance in *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **33**, 80–88.

Cioli, D. and Pica-Mattoccia, L. (2003). Praziquantel. *Parasitology Research* **90**(Supp. 1), S3–S9.

Cioli, D. and Pica-Mattoccia, L. (2005). Current and future antischistosomal drugs. In *Schistosomiasis* (ed. Secor, W. E. and Colley, D. G.), pp. 191–206. Springer, New York, NY.

Cioli, D., Pica-Mattoccia, L. and Moroni, R. (1992). *Schistosoma mansoni*: hycanthone/oxamniquine resistance is controlled by a single autosomal recessive gene. *Experimental Parasitology* **75**, 425–432.

Cioli, D., Pica-Mattoccia, L. and Archer, S. (1993). Drug resistance in schistosomes. *Parasitology Today* **9**, 162–166.

Cioli, D., Pica-Mattoccia, L. and Archer, S. (1995). Antischistosomal drugs: past, present. . .and future? *Pharmacology and Therapeutics* **68**, 35–85.

Coles, G. C. and Kinoti, G. K. (1997). Defining resistance in *Schistosoma*. *Parasitology Today* **13**, 157–158.

Collins, J. J., King, R. S., Cogswell, A., Williams, D. L. and Newmark, P. A. (2011). An atlas for *Schistosoma mansoni* organs and life-cycle stages using cell type-specific markers and confocal microscopy. *PLoS Neglected Tropical Diseases* **8**, e1009. doi: 10.1371/journal.pntd.0001009.

Collins, J. J., Wang, B., Lambrus, B. G., Tharp, M. E., Iver, H. and Newmark, P. A. (2013). Adult somatic stem cells in the human parasite *Schistosoma Mansoni*. *Nature* **494**, 476–479. doi: 10.1038/nature11924.

Cooper, L. A., Lewis, F. A. and File-Emperador, S. (1989). Re-establishing a life cycle of *Schistosoma mansoni* from cryopreserved larvae. *Journal of Parasitology* **75**, 353–356.

Couto, F. F., Coelho, P. M., Araujo, N., Kusel, J. R., Katz, N., Jannotti-Passos, L. K. and Mattos, A. C. (2011). *Schistosoma mansoni*: a method for inducing resistance to praziquantel using infected *Biomphalaria glabrata* snails. *Memorias do Instituto Oswaldo Cruz* **106**, 153–157.

Couto, F. F., Coelho, P. M., Araujo, N., Kusel, J. R., Katz, N. and Mattos, A. C. (2010). Use of fluorescent probes as a useful tool to identify resistant *Schistosoma mansoni* isolates to praziquantel. *Parasitology* **137**, 1791–1797. doi: 10.1017/S003118201000065X.

Criscione, C. D., Valentim, C. L. L., Hirai, H., LoVerde, P. T. and Anderson, T. J. C. (2009). Genomic linkage map of the human blood fluke Schistosoma mansoni. *Genome Biology* **10**, R71. doi: 10.1186/gb-2009-10-6-r71.

Crisford, A., Murray, C., O'Connor, V., Edwards, R. J., Kruger, N., Welz, C., von Samson-Himmelstjerna, G., Harder, A., Walker, R. J. and Holden-Dye, L. (2011). Selective toxicity of the anthelmintic emodepside revealed by heterologous expression of human KCNMA1 in *Caenorhabditis elegans*. *Molecular Pharmacology* **79**, 1031–1043. doi: 10.1124/mol.111.071043.

Cvilink, V., Lamka, J. and Skalova, L. (2009). Xenobiotic metabolizing enzymes and metabolism of anthelminthics in helminths. *Drug Metabolism Reviews* **41**, 8–26. doi: 10.1080/03602530802602880.

Danso-Appiah, A. and De Vlas, S. J. (2002). Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. *Trends in Parasitology* **18**, 125–129.

Dassa, E. and Bouige, P. (2001). The ABC of ABCs: a phylogenetic and functional classification of ABC systems in living organisms. *Research in Microbiology* **152**, 211–229.

Day, T. A. and Botros, S. (2006). Drug resistance in schistosomes. In *Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology* (ed. Maule, A. and Marks, N. J.), pp. 256–268. CAB International, Oxfordshire, UK.

Dean, M. and Annilo, T. (2005). Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annual Review of Genomics and Human Genetics* **6**, 123–142.

Dean, M., Rzhetsky, A. and Allikmets, R. (2001). The human ATP-binding cassette (ABC) transporter superfamily. *Genome Research* **11**, 1156–1166. doi: 10.1101/gr.184901.

Dent, J. A., McHardy, M. S., Vassilatis, D. M. and Avery, L. (2000). The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences, USA* **97**, 2674–2679.

Devaney, E., Winter, A. D. and Britton, C. (2010). microRNAs: a role in drug resistance in parasitic nematodes? *Trends in Parasitology* **26**, 428–433. doi: 10.1016/j.pt.2010.05.003.

Doenhoff, M. J., Cioli, D. and Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Current Opinion in Infectious Diseases* **21**, 659–667. doi: 10.1097/QCO.0b013e328318978f.

Doenhoff, M. J., Coles, G. C., Pica-Mattoccia, L. and Wheatcroft-Francklow, K. (2009*a*). Chemotherapy and drug resistance in schistosomiasis, fascioliasis and tapeworm infections. In *Antimicrobial Drug Resistance, Vol. 1: Mechanisms of Drug Resistance*, Vol. 1 (ed. Mayers, D. L.), pp. 629–646. Humana Press, Totowa, NJ.

Doenhoff, M. J., Hagan, P., Cioli, D., Southgate, V., Pica-Mattoccia, L., Botros, S., Coles, G., Tchuem Tchuente, L. A., Mbaye, A. and Engels, D. (2009*b*). Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. *Parasitology* **136**, 1825–1835. doi: 10.1017/S0031182009000493.

Doenhoff, M. J. and Pica-Mattoccia, L. (2006). Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. *Expert Review of Anti-infective Therapy* **4**, 199–210.

Doenhoff, M. J., Sabah, A. A., Fletcher, C., Webbe, G. and Bain, J. (1987). Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 947–951.

Duvoisin, R., Ayuk, M. A., Rinaldi, G., Suttiprapa, S., Mann, V. H., Lee, C. M., Harris, N. and Brindley, P. J. (2012). Human U6 promoter drives stronger shRNA activity than its schistosome orthologue in *Schistosoma mansoni* and human fibrosarcoma cells. *Transgenic Research* **21**, 511–521. doi: 10.1007/s11248-011-9548-0.

Eisenhoffer, G. T., Kang, H. and Sanchez Alvarado, A. (2008). Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian *Schmidtea mediterranea*. *Cell Stem Cell* **3**, 327–339. doi: 10.1016/j.stem.2008.07.002.

Fairweather, I. (2011). Liver fluke isolates: a question of provenance. *Veterinary Parasitology* **176**, 1–8. doi: 10.1016/j.vetpar.2010.12.011.

Fallon, P. G. (1995). Short report: diminished susceptibility to praziquantel in a senegal isolate of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **53**, 61–62.

Fallon, P. G. (1998). Schistosome resistance to praziquantel. *Drug Resistance Updates* **1**, 236–241.

Fallon, P. G. and Doenhoff, M. J. (1994). Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. *American Journal of Tropical Medicine and Hygiene* **51**, 83–88.

Fallon, P. G., Mubarak, J. S., Fookes, R. E., Niang, M., Butterworth, A. E., Sturrock, R. F. and Doenhoff, M. J. (1997). *Schistosoma mansoni*: maturation rate and drug susceptibility of different geographic isolates. *Experimental Parasitology* **86**, 29–36.

Fallon, P. G., Tao, L. F., Ismail, M. M. and Bennett, J. L. (1996). Schistosome resistance to praziquantel: Fact or artifact? *Parasitology Today* **12**, 316–320.

Gimenez-Bonafe, P., Guillen Canovas, A., Ambrosio, S., Tortosa, A. and Perez-Tomas, R. (2008). *Drugs modulating MDR*. (ed. Colabufo, N. A.), pp. 63–99. Research Signpost, Kerala, India.

Gonnert, R. and Andrews, P. (1977). Praziquantel, a new broad-spectrum antischistosomal agent. *Zeitschrift für Parasitenkunde* **52**, 129–150.

Greenberg, R. M. (2005). Are Ca^{2+} channels targets of praziquantel action? *International Journal for Parasitology* **35**, 1–9.

Gryseels, B., Mbaye, A., De Vlas, S. J., Stelma, F. F., Guisse, F., Van Lieshout, L., Faye, D., Diop, M., Ly, A., Tchuem-Tchuente, L. A., Engels, D. and Polman, K. (2001). Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Tropical Medicine and International Health* **6**, 864–873.

Gryseels, B., Stelma, F. F., Talla, I., van Dam, G. J., Polman, K., Sow, S., Diaw, M., Sturrock, R. F., Doehring-Schwerdtfeger, E., Kardorff, R., Decam, C., Niang, M. and Deelder, A. M. (1994). Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. *Tropical and Geographical Medicine* **46**, 209–219.

Hayeshi, R., Masimirembwa, C., Mukanganyama, S. and Ungell, A. L. (2006). The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated efflux. *European Journal of Pharmaceutical Sciences* **29**, 70–81.

Herwaldt, B. L., Tao, L. F., van Pelt, W., Tsang, V. C. and Bruce, J. I. (1995). Persistence of *Schistosoma haematobium* infection despite multiple courses of therapy with praziquantel. *Clinical Infectious Diseases* **20**, 309–315.

Higgins, C. F. (2007). Multiple molecular mechanisms for multidrug resistance transporters. *Nature* **446**, 749–757.

Hines-Kay, J., Cupit, P. M., Sanchez, M. C., Rosenberg, G. H., Hanelt, B. and Cunningham, C. (2012). Transcriptional analysis of *Schistosoma mansoni* treated with praziquantel *in vitro*. *Molecular and Biochemical Parasitology* **186**, 87–94. doi: 10.1016/j.molbiopara.2012.09.006.

Hotez, P. J. and Fenwick, A. (2009). Schistosomiasis in Africa: an emerging tragedy in our new global health decade. *PLoS Neglected Tropical Diseases* **3**, e485. doi: 10.1371/journal.pntd.0000485.

Ismail, M., Botros, S., Metwally, A., William, S., Farghally, A., Tao, L. F., Day, T. A. and Bennett, J. L. (1999). Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. *American Journal of Tropical Medicine and Hygiene* **60**, 932–935.

Ismail, M., Metwally, A., Farghaly, A., Bruce, J., Tao, L. F. and Bennett, J. L. (1996). Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. *American Journal of Tropical Medicine and Hygiene* **55**, 214–218.

James, C. E., Hudson, A. L. and Davey, M. W. (2009). Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends in Parasitology* **25**, 328–335. doi: 10.1016/j.pt.2009.04.004.

Jin, M. S., Oldham, M. L., Zhang, Q. and Chen, J. (2012). Crystal structure of the multidrug transporter P-glycoprotein from *Caenorhabditis elegans*. *Nature* **490**, 566–569. doi: 10.1038/nature11448.

Jones, P. M. and George, A. M. (2005). Multidrug resistance in parasites: ABC transporters, P-glycoproteins and molecular modelling. *International Journal for Parasitology* **35**, 555–566.

Kartner, N., Riordan, J. R. and Ling, V. (1983). Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science* **221**, 1285–1288.

Kasinathan, R. S., Goronga, T., Messerli, S. M., Webb, T. R. and Greenberg, R. M. (2010*a*). Modulation of a *Schistosoma mansoni* multidrug transporter by the antischistosomal drug praziquantel. *FASEB Journal* **24**, 128–135. doi: 10.1096/fj.09-137091.

Kasinathan, R. S. and Greenberg, R. M. (2012). Pharmacology and potential physiological significance of schistosome multidrug resistance transporters. *Experimental Parasitology* **132**, 2–6. doi: 10.1016/j.exppara.2011.03.004.

Kasinathan, R. S., Morgan, W. M. and Greenberg, R. M. (2010*b*). *Schistosoma mansoni* express higher levels of multidrug resistance-associated protein 1 (SmMRP1) in juvenile worms and in response to praziquantel. *Molecular and Biochemical Parasitology* **173**, 25–31. doi: 10.1016/j.molbiopara.2010.05.003.

Kasinathan, R. S., Morgan, W. M. and Greenberg, R. M. (2011). Genetic knockdown and pharmacological inhibition of parasite multidrug resistance transporters disrupts egg production in *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases* **5**, e1425. doi: 10.1371/journal.pntd.0001425.

Keiser, J. and Utzinger, J. (2012). Antimalarials in the treatment of schistosomiasis. *Current Pharmaceutical Design* **18**, 3531–3538.

Kerboeuf, D., Blackhall, W., Kaminsky, R. and von Samson-Himmelstjerna, G. (2003). P-glycoprotein in helminths: function and perspectives for anthelmintic treatment and reversal of resistance. *International Journal of Antimicrobial Agents* **22**, 332–346.

King, C. H. (2010). Parasites and poverty: the case of schistosomiasis. *Acta Tropica* **113**, 95–104. doi: 10.1016/j.actatropica.2009.11.012.

Kongs, A., Marks, G., Verle, P. and Van der Stuyft, P. (2008). The unreliability of the Kato-Katz technique limits its usefulness for evaluating *S. mansoni* infections. *Tropical Medicine and International Health* **6**, 163–169. doi: 10.1046/j.1365-3156.2001.00687.x.

Krautz-Peterson, G., Bhardwaj, R., Faghiri, Z., Tararam, C. A. and Skelly, P. J. (2010). RNA interference in schistosomes: machinery and methodology. *Parasitology* **137**, 485–495. doi: 10.1017/S0031182009991168.

Kumkate, S., Chunchob, S. and Janvilisri, T. (2008). Expression of ATP-binding cassette multidrug transporters in the giant liver fluke *Fasciola gigantica* and their possible involvement in the transport of bile salts and anthelmintics. *Molecular and Cellular Biochemistry* **317**, 77–84. doi: 10.1007/s11010-008-9833-2.

Kusel, J. R., Oliveira, F. A., Todd, M., Ronketti, F., Lima, S. F., Mattos, A. C., Reis, K. T., Coelho, P. M., Thornhill, J. A. and Ribeiro, F. (2006). The effects of drugs, ions, and poly-l-lysine on the excretory system of *Schistosoma mansoni*. *Memorias do Instituto Oswaldo Cruz* **101**, 293–298.

Kwa, M. S. G., Veenstra, J. G., Dijk, M. V. and Roos, M. H. (1995). β-tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. *Journal of Molecular Biology* **246**, 500–510.

Leprohon, P., Legare, D. and Ouellette, M. (2011). ABC transporters involved in drug resistance in human parasites. *Essays in Biochemistry* **50**, 121–144. doi: 10.1042/bse0500121.

Lespine, A., Menez, C., Bourguinat, C. and Prichard, R. K. (2012). P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: prospects for reversing transport-dependent anthelmintic resistance. *International Journal for Parasitology: Drugs and Drug Resistance* **2**, 58–75. doi: 10.1016/j.ijpddr.2011.10.001.

Liang, Y. S., Coles, G. C., Dai, J. R., Zhu, Y. C. and Doenhoff, M. J. (2001). Biological characteristics of praziquantel-resistant and -susceptible isolates of *Schistosoma mansoni*. *Annals of Tropical Medicine and Parasitology* **95**, 715–723.

Liang, Y. S., Dai, J. R., Zhu, Y. C., Coles, G. C. and Doenhoff, M. J. (2003). Genetic analysis of praziquantel resistance in *Schistosoma mansoni*. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**, 274–280.

Liang, Y. S., Li, H. J., Dai, J. R., Wang, W., Qu, G. L., Tao, Y. H., Xing, Y. T., Li, Y. Z., Qian, K. and Wei, J. Y. (2011). Studies on resistance of *Schistosoma* to praziquantel XIII: resistance of *Schistosoma japonicum* to praziquantel is experimentally induced in laboratory. *Chinese Journal of Schistosomiasis Control* **23**, 605–610.

Lin, D. D., Liu, J. X., Liu, Y. M., Hu, F., Zhang, Y. Y., Xu, J. M., Li, J. Y., Ji, M. J., Bergquist, R., Wu, G. L. and Wu, H. W. (2008). Routine Kato-Katz technique underestimates the prevalence of *Schistosoma japonicum*: a case study in an endemic area of the People's Republic of China. *Parasitology International* **57**, 281–286. doi: 10.1016/j.parint.2008.04.005.

Mattos, A. C., Pereira, G. C., Jannotti-Passos, L. K., Kusel, J. R. and Coelho, P. M. (2007). Evaluation of the effect of oxamniquine, praziquantel and a combination of both drugs on the intramolluscan phase of *Schistosoma mansoni*. *Acta Tropica* **102**, 84–91.

Mealey, K. L., Bentjen, S. A., Gay, J. M. and Cantor, G. H. (2001). Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics* **11**, 727–733.

Melman, S. D., Steinauer, M. L., Cunningham, C., Kubatko, L. S., Mwangi, I. N., Wynn, N. B., Mutuku, M. W., Karanja, D. M., Colley, D. G., Black, C. L., Secor, W. E., Mkoji, G. M. and Loker, E. S. (2009). Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases* **3**, e504. doi: 10.1371/journal.pntd.0000504.

Messerli, S. M., Kasinathan, R. S., Morgan, W., Spranger, S. and Greenberg, R. M. (2009). *Schistosoma mansoni* P-glycoprotein levels increase in response to praziquantel exposure and correlate with reduced praziquantel susceptibility. *Molecular and Biochemical Parasitology* **167**, 54–59. doi: 10.1016/j.molbiopara.2009.04.007.

Mizutani, T., Masuda, M., Nakai, E., Furumiya, K., Togawa, H., Nakamura, Y., Kawai, Y., Nakahira, K., Shinkai, S. and Takahashi, K. (2008). Genuine functions of P-glycoprotein (ABCB1). *Current Drug Metabolism* **9**, 167–174.

Molento, M. B. and Prichard, R. K. (1999). Effects of the multidrug-resistance-reversing agents verapamil and CL 347,099 on the efficacy of ivermectin or moxidectin against unselected and drug-selected strains of *Haemonchus contortus* in jirds (*Meriones unguiculatus*). *Parasitology Research* **85**, 1007–1011.

Moll, L., Gaasenbeek, C. P., Vellema, P. and Borgsteede, F. H. (2000). Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in The Netherlands. *Veterinary Parasitology* **91**, 153–158.

Mourao, M. M., Dinguirard, N., Franco, G. R. and Yoshino, T. P. (2009). Phenotypic screen of early-developing larvae of the blood fluke, *Schistosoma mansoni*, using RNA interference. *PLoS Neglected Tropical Diseases* **3**, e502. doi: 10.1371/journal.pntd.0000502.

Mutapi, F., Rujeni, N., Bourke, C., Mitchell, K., Appleby, L., Nausch, N., Midzi, N. and Mduluza, T. (2011). *Schistosoma haematobium* treatment in 1–5 year old children: safety and efficacy of the antihelminthic drug praziquantel. *PLoS Neglected Tropical Diseases* **5**, e1143. doi: 10.1371/journal.pntd.0001143.

Nogi, T., Zhang, D., Chan, J. D. and Marchant, J. S. (2009). A novel biological activity of praziquantel requiring voltage-operated Ca^{2+} channel β subunits: subversion of flatworm regenerative polarity. *PLoS Neglected Tropical Diseases* **3**, e464. doi: 10.1371/journal.pntd.0000464.

Novozhilova, E., Kimber, M. J., Qian, H., McVeigh, P., Robertson, A. P., Zamanian, M., Maule, A. G. and Day, T. A. (2010). FMRFamide-like peptides (FLPs) enhance voltage-gated calcium currents to elicit muscle contraction in the human parasite *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases* **4**, e790. doi: 10.1371/journal.pntd.0000790.

Oliveira, F. A., Kusel, J. R., Ribeiro, F. and Coelho, P. M. (2006). Responses of the surface membrane and excretory system of *Schistosoma mansoni* to damage and to treatment with praziquantel and other biomolecules. *Parasitology* **132**, 321–330.

Paulsen, I. T. (2003). Multidrug efflux pumps and resistance: regulation and evolution. *Current Opinion in Microbiology* **6**, 446–451.

Pereira, C., Fallon, P. G., Cornette, J., Capron, A., Doenhoff, M. J. and Pierce, R. J. (1998). Alterations in cytochrome-c oxidase expression between praziquantel-resistant and susceptible strains of *Schistosoma mansoni*. *Parasitology* **117**(Pt 1), 63–73.

Pereira, T. C., Pascoal, V. D., Marchesini, R. B., Maia, I. G., Magalhaes, L. A., Zanotti-Magalhaes, E. M. and Lopes-Cendes, I. (2008). *Schistosoma mansoni*: evaluation of an RNAi-based treatment targeting HGPRTase gene. *Experimental Parasitology* **118**, 619–623. doi: 10.1016/j.exppara.2007.11.017.

Pica-Mattoccia, L., Archer, S. and Cioli, D. (1992). Hycanthone resistance in schistosomes correlates with the lack of an enzymatic activity which produces the covalent binding of hycanthone to parasite macromolecules. *Molecular and Biochemical Parasitology* **55**, 167–175.

Pica-Mattoccia, L. and Cioli, D. (2004). Sex- and stage-related sensitivity of *Schistosoma mansoni* to *in vivo* and *in vitro* praziquantel treatment. *International Journal for Parasitology* **34**, 527–533.

Pica-Mattoccia, L., Doenhoff, M. J., Valle, C., Basso, A., Troiani, A. R., Liberti, P., Festucci, A., Guidi, A. and Cioli, D. (2009). Genetic analysis of decreased praziquantel sensitivity in a laboratory strain of *Schistosoma mansoni*. *Acta Tropica* **111**, 82–85. doi: 10.1016/j.actatropica.2009.01.012.

Pica-Mattoccia, L., Orsini, T., Basso, A., Festucci, A., Liberti, P., Guidi, A., Marcatto-Maggi, A. L., Nobre-Santana, S., Troiani, A. R., Cioli, D. and Valle, C. (2008). *Schistosoma mansoni*: lack of correlation between praziquantel-induced intra-worm calcium influx and parasite death. *Experimental Parasitology* **119**, 332–335. doi: 10.1016/j.exppara.2008.03.012.

Pica-Mattoccia, L., Novi, A. and Cioli, D. (1997). Enzymatic basis for the lack of oxamniquine activity in Schistosoma haematobium infections. *Parasitology Research* **83**, 687–689.

Pica-Mattoccia, L., Valle, C., Basso, A., Troiani, A. R., Vigorosi, F., Liberti, P., Festucci, A. and Cioli, D. (2007). Cytochalasin D abolishes the schistosomicidal activity of praziquantel. *Experimental Parasitology* **115**, 344–351.

Pommier, Y., Pourquier, P., Urasaki, Y., Wu, J. and Laco, G. S. (1999). Topoisomerase I inhibitors: selectivity and cellular resistance. *Drug Resistance Updates* **2**, 307–318.

Pommier, Y., Sordet, O., Antony, S., Hayward, R. L. and Kohn, K. W. (2004). Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. *Oncogene* **23**, 2934–2959.

Prichard, R. K., Hall, C. A., Kelly, J. D., Martin, I. C. and Donald, A. D. (1980). The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal* **56**, 239–251.

Protasio, A. V., Tsai, I. J., Babbage, A., Nichol, S., Hunt, M., Aslett, M. A., De Silva, N., Velarde, G. S., Anderson, T. J., Clark, R. C., Davidson, C., Dillon, G. P., Holroyd, N. E., LoVerde, P. T., Lloyd, C., McQuillan, J., Oliveira, G., Otto, T. D., Parker-Manuel, S. J., Quail, M. A., Wilson, R. A., Zerlotini, A., Dunne, D. W. and Berriman, M. (2012). A systematically improved high quality genome and transcriptome of the human blood fluke *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases* **6**, e1455. doi: 10.1371/journal.pntd.0001455.

Quack, T., Wippersteg, V. and Grevelding, C. G. (2010). Cell cultures for schistosomes – chances of success or wishful thinking? *International Journal for Parasitology* **40**, 991–1002. doi: 10.1016/j.ijpara.2010.04.013.

Redman, C. A., Robertson, A., Fallon, P. G., Modha, J., Kusel, J. R., Doenhoff, M. J. and Martin, R. J. (1996). Praziquantel: an urgent and exciting challenge. *Parasitology Today* **12**, 14–20.

Rinaldi, G., Eckert, S. E., Tsai, I. J., Suttiprapa, S., Kines, K. J., Tort, J. F., Mann, V. H., Turner, D. J., Berriman, M. and Brindley, P. J. (2012*a*). Germline transgenesis and insertional mutagenesis in *Schistosoma mansoni* mediated by murine leukemia virus. *PloS Pathogens* **8**, e1002820. doi: 10.1371/journal.ppat.1002820.

Rinaldi, G., Suttiprapa, S., Tort, J. F., Folley, A. E., Skinner, D. E. and Brindley, P. J. (2012*b*). An antibiotic selection marker for schistosome transgenesis. *International Journal for Parasitology* **42**, 123–130. doi: 10.1016/j.ijpara.2011.11.005.

Sabah, A. A., Fletcher, C., Webbe, G. and Doenhoff, M. J. (1986). *Schistosoma mansoni*: chemotherapy of infections of different ages. *Experimental Parasitology* **61**, 294–303.

Sabra, A. N. and Botros, S. S. (2008). Response of *Schistosoma mansoni* isolates having different drug sensitivity to praziquantel over several life cycle passages with and without therapeutic pressure. *Journal of Parasitology* **94**, 537–541. doi: 10.1645/GE-1297.1.

Saier, M. H. and Paulsen, I. T. (2001). Phylogeny of multidrug transporters. *Seminars in Cell and Developmental Biology* **12**, 205–213.

Sato, H., Kusel, J. R. and Thornhill, J. (2002). Functional visualization of the excretory system of adult *Schistosoma mansoni* by the fluorescent marker resorufin. *Parasitology* **125**, 527–535.

Sato, H., Kusel, J. R. and Thornhill, J. (2004). Excretion of fluorescent substrates of mammalian multidrug resistance-associated protein (MRP) in the *Schistosoma mansoni* excretory system. *Parasitology* **128**, 43–52.

Sayed, A. A., Simeonov, A., Thomas, C. J., Inglese, J., Austin, C. P. and Williams, D. L. (2008). Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nature Medicine* **14**, 407–412. doi: 10.1038/nm1737.

Schinkel, A. H., Smit, J. J., van Tellingen, O., Beijnen, J. H., Wagenaar, E., van Deemter, L., Mol, C. A., van der Valk, M. A., Robanus-Maandag, E. C., te Riele, H. P., Berns, A. J. M. and Borst, P. (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.

Selkirk, M. E., Huang, S. C., Knox, D. P. and Britton, C. (2012). The development of RNA interference (RNAi) in gastrointestinal nematodes. *Parasitology* **139**, 605–612. doi: 10.1017/S0031182011002332.

Seto, E. Y., Wong, B. K., Lu, D. and Zhong, B. (2011). Human schistosomiasis resistance to praziquantel in China: should we be worried? *American Journal of Tropical Medicine and Hygiene* **85**, 74–82. doi: 10.4269/ajtmh.2011.10-0542.

Silva, I. M., Thiengo, R., Conceição, M. J., Rey, L., Lenzi, H. L., Perreira Filho, E. and Ribeiro, P. C. (2005). Therapeutic failure of praziquantel in the treatment of *Schistosoma haematobium* infection in Brazilians returning from Africa. *Memorias do Instituto Oswaldo Cruz* **100**, 445–449.

Sioud, M. (2011). Promises and challenges in developing RNAi as a research tool and therapy. *Methods in Molecular Biology* **703**, 173–187. doi: 10.1007/978-1-59745-248-9_12.

Smout, M. J., Kotze, A. C., McCarthy, J. S. and Loukas, A. (2010). A novel high throughput assay for anthelmintic drug screening and resistance diagnosis by real-time monitoring of parasite motility. *PLoS Neglected Tropical Diseases* **4**, e885. doi: 10.1371/journal.pntd.0000885.

Stefanic, S., Dvorak, J., Horn, M., Braschi, S., Sojka, D., Ruelas, D. S., Suzuki, B., Lim, K. C., Hopkins, S. D., McKerrow, J. H. and Caffrey, C. R. (2010). RNA interference in *Schistosoma mansoni* schistosomula: selectivity, sensitivity and operation for larger-scale screening. *PLoS Neglected Tropical Diseases* **4**, e850. doi: 10.1371/journal.pntd.0000850.

Stelma, F. F., Sall, S., Daff, B., Sow, S., Niang, M. and Gryseels, B. (1997). Oxamniquine cures *Schistosoma mansoni* infection in a focus in which cure rates with praziquantel are unusually low. *Journal of Infectious Diseases* **176**, 304–307.

Stelma, F. F., Talla, I., Sow, S., Kongs, A., Niang, M., Polman, K., Deelder, A. M. and Gryseels, B. (1995). Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **53**, 167–170.

Suttiprapa, S., Rinaldi, G. and Brindley, P. J. (2012). Genetic manipulation of schistosomes - Progress with integration competent vectors. *Parasitology* **139**, 641–650. doi: 10.1017/S003118201100134X.

Swain, M. T., Larkin, D. M., Caffrey, C. R., Davies, S. J., Loukas, A., Skelly, P. J. and Hoffmann, K. F. (2011). *Schistosoma* comparative genomics: integrating genome structure, parasite biology and anthelmintic discovery. *Trends in Parasitology* **27**, 555–564. doi: 10.1016/j.pt.2011.09.003.

Szakacs, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C. and Gottesman, M. M. (2006). Targeting multidrug resistance in cancer. *Nature Reviews: Drug Discovery* **5**, 219–234.

Tchoubrieva, E. B. and Kalinna, B. (2010). Advances in mRNA silencing and transgene expression: a gateway to functional genomics in schistosomes. *Biotechnology and Genetic Engineering Reviews* **26**, 261–280.

Tchoubrieva, E. B., Ong, P. C., Pike, R. N., Brindley, P. J. and Kalinna, B. H. (2010). Vector-based RNA interference of cathepsin B1 in *Schistosoma mansoni*. *Cellular and Molecular Life Sciences* **67**, 3739–3748. doi: 10.1007/s00018-010-0345-3.

Tompkins, J. B., Stitt, L. E., Morrissette, A. M. and Ardelli, B. F. (2011). The role of *Brugia malayi* ATP-binding cassette (ABC) transporters in potentiating drug sensitivity. *Parasitology Research* **109**, 1311–1322. doi: 10.1007/s00436-011-2378-4.

Toure, S., Zhang, Y., Bosque-Oliva, E., Ky, C., Ouedraogo, A., Koukounari, A., Gabrielli, A. F., Bertrand, S., Webster, J. P. and Fenwick, A. (2008). Two-year impact of single praziquantel treatment on infection in the national control programme on schistosomiasis in Burkina Faso. *Bulletin of the World Health Organization* **86**, 780–787.

Tsai, M. H., Marx, K. A., Ismail, M. M. and Tao, L. (2000). Randomly amplified polymorphic DNA (RAPD) polymerase chain reaction assay for identification of *Schistosoma mansoni* strains sensitive or tolerant to antischistosomal drugs. *Journal of Parasitology* **86**, 146–149.

Valle, C., Troiani, A. R., Festucci, A., Pica-Mattoccia, L., Liberti, P., Wolstenholme, A., Francklow, K., Doenhoff, M. J. and Cioli, D. (2003). Sequence and level of endogenous expression of calcium channel beta subunits in *Schistosoma mansoni* displaying different susceptibilities to praziquantel. *Molecular and Biochemical Parasitology* **130**, 111–115.

van de Ven, R., Oerlemans, R., van der Heijden, J. W., Scheffer, G. L., de Gruijl, T. D., Jansen, G. and Scheper, R. J. (2009). ABC drug transporters and immunity: novel therapeutic targets in autoimmunity and cancer. *Journal of Leukocyte Biology* **86**, 1075–1087. doi: 10.1189/jlb.0309147.

van der Werf, M. J., de Vlas, S. J., Brooker, S., Looman, C. W., Nagelkerke, N. J., Habbema, J. D. and Engels, D. (2003). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Tropica* **86**, 125–139.

van Veen, H. W. (2010). Structural biology: last of the multidrug transporters. *Nature* **467**, 926–927. doi: 10.1038/467926a.

Vennervald, B. J., Booth, M., Butterworth, A. E., Kariuki, H. C., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Kenty, L., Mwatha, J., Ouma, J. H. and Dunne, D. W. (2005). Regression of hepatosplenomegaly in Kenyan school-aged children after praziquantel treatment and three years of greatly reduced exposure to *Schistosoma mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 150–160.

Ventner, H., Shahi, S., Balakrishnan, L., Velamakanni, S., Bapna, A., Woebking, B. and van Veen, H. W. (2005). Similarities between ATP-dependent and ion-coupled multidrug transporters. *Biochemical Society Transactions* **33**, 1008–1011.

Vokřál, I., Jirásko, R., Jedličková, V., Bártíková, H., Skálová, L., Lamka, J., Holčapek, M. and Szotáková, B. (2012). The inability of tapeworm *Hymenolepis diminuta* and fluke *Dicrocoelium dendriticum* to metabolize praziquantel. *Veterinary Parasitology* **185**, 168–174. doi: 10.1016/j.vetpar.2011.09.026.

Wang, W., Dai, J. R., Li, H. J., Shen, X. H. and Liang, Y. S. (2010). Is there reduced susceptibility to praziquantel in *Schistosoma japonicum*? Evidence from China. *Parasitology* **137**, 1905–1912. doi: 10.1017/S0031182010001204.

Wang, W., Dai, J. R., Li, H. J., Shen, X. H. and Liang, Y. S. (2012*a*). The sensitivity of *Schistosoma japonicum* to praziquantel: a field evaluation in areas with low endemicity of China. *American Journal of Tropical Medicine and Hygiene* **86**, 834–836. doi: 10.4269/ajtmh.2012.11-0701.

Wang, W., Wang, L. and Liang, Y. S. (2012*b*). Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research* **111**, 1871–1877. doi: 10.1007/s00436-012-3151-z.

Webster, J. P., Gower, C. M. and Norton, A. J. (2008). Evolutionary concepts in predicting and evaluating the impact of mass chemotherapy schistosomiasis control programmes on parasites and their hosts. *Evolutionary Applications* **1**, 66–83. doi: 10.1111/j.1752-4571.2007.00012.x.

Welz, C., Kruger, N., Schniederjans, M., Miltsch, S. M., Krucken, J., Guest, M., Holden-Dye, L., Harder, A. and von Samson-Himmelstjerna, G. (2011). SLO-1-channels of parasitic nematodes reconstitute locomotor behaviour and emodepside sensitivity in *Caenorhabditis elegans slo-1* loss of function mutants. *PloS Pathogens* **7**, e1001330. doi: 10.1371/journal.ppat.1001330.

Wilkinson, R., Law, C. J., Hoey, E. M., Fairweather, I., Brennan, G. P. and Trudgett, A. (2012). An amino acid substitution in *Fasciola hepatica* P-glycoprotein from triclabendazole-resistant and triclabendazole-susceptible populations. *Molecular and Biochemical Parasitology* **186**, 69–72. doi: 10.1016/j.molbiopara.2012.08.008.

William, S. and Botros, S. (2004). Validation of sensitivity to praziquantel using *Schistosoma mansoni* worm muscle tension and Ca^{2+}-uptake as possible *in vitro* correlates to *in vivo* ED50 determination. *International Journal for Parasitology* **34**, 971–977.

William, S., Botros, S., Ismail, M., Farghally, A., Day, T. A. and Bennett, J. L. (2001*a*). Praziquantel-induced tegumental damage *in vitro* is diminished in schistosomes derived from praziquantel-resistant infections. *Parasitology* **122**, 63–66.

William, S., Sabra, A., Ramzy, F., Mousa, M., Demerdash, Z., Bennett, J. L., Day, T. A. and Botros, S. (2001*b*). Stability and reproductive fitness of *Schistosoma mansoni* isolates with decreased sensitivity to praziquantel. *International Journal for Parasitology* **31**, 1093–1100.

Xianyi, C., Liying, W., Jiming, C., Xiaonong, Z., Jiang, Z., Jiagang, G., Xiaohua, W., Engels, D. and Minggang, C. (2005). Schistosomiasis control in China: the impact of a 10-year World Bank Loan Project (1992–2001). *Bulletin of the World Health Organization* **83**, 43–48.

Xiao, S. H., Catto, B. A. and Webster, L. T., Jr. (1985). Effects of praziquantel on different developmental stages of *Schistosoma mansoni in vitro* and *in vivo*. *Journal of Infectious Diseases* **151**, 1130–1137.

Xu, M., Molento, M., Blackhall, W., Ribeiro, P., Beech, R. and Prichard, R. (1998). Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Molecular and Biochemical Parasitology* **91**, 327–335.

Yang, Y., Jin, Y., Liu, P., Shi, Y., Cao, Y., Liu, J., Shi, Y., Li, H. and Lin, J. (2012). RNAi silencing of type V collagen in *Schistosoma japonicum* affects parasite morphology, spawning, and hatching. *Parasitology Research* **111**, 1251–1257. doi: 10.1007/s00436-012-2959-x.

Yu, D. B., Li, Y., Sleigh, A. C., Yu, X. L., Li, Y. S., Wei, W. Y., Liang, Y. S. and McManus, D. P. (2001). Efficacy of praziquantel against *Schistosoma japonicum*: field evaluation in an area with repeated chemotherapy compared with a newly identified endemic focus in Hunan, China. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 537–541.

Zhang, D., Chan, J. D., Nogi, T. and Marchant, J. S. (2011). Opposing roles of voltage-gated Ca^{2+} channels in neuronal control of regenerative patterning. *Journal of Neuroscience* **31**, 15983–15995. doi: 10.1523/JNEUROSCI.3029-11.2011.