CellPress

Trends in Parasitology

Opinion

High-content approaches to anthelmintic drug screening

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Most anthelmintics were discovered through *in vivo* screens using animal models of infection. Developing *in vitro* assays for parasitic worms presents several challenges. The lack of *in vitro* life cycle culture protocols requires harvesting worms from vertebrate hosts or vectors, limiting assay throughput. Once worms are removed from the host environment, established anthelmintics often show no obvious phenotype – raising concerns about the predictive value of many *in vitro* assays. However, with recent progress in understanding how anthelmintics subvert host–parasite interactions, and breakthroughs in high-content imaging and machine learning, *in vitro* assays have the potential to discern subtle cryptic parasite phenotypes. These may prove better endpoints than conventional *in vitro* viability assays.

Highlights

Many current anthelmintics were discovered by screening relatively low-throughput animal models of infection. Several frontline anthelmintics lack obvious *in vitro* phenotypes and would not be detected using common *in vitro* screens.

Anthelmintics that lack overt phenotypes may have 'cryptic phenotypes', disrupting subtle processes that are nonetheless crucial to parasite survival within the host.

Provision of parasite material may be an inherent limit on assay throughput, but improved profiling of existing anthelmintics and expanding the scope of phenotypes observed can maximize the productivity of parasite screening.

Advances in high-content imaging allow for in-depth profiling of anthelmintic phenotypes, and advances in parasite culture can more closely approximate the *in vivo* environment.

A need for new approaches to screening?

Most **anthelmintics** (see Glossary) in current use were discovered between the 1950s and the 1980s through low-throughput *in vivo* screens in small-animal models of infection (Figure 1). While advances in genomics and breakthroughs in molecular tools are generating large amounts of data on parasite biology, this has not yet translated into the discovery of new anthelmintic classes. Anthelmintics recently approved for human use (moxidectin, triclabendazole) and macrofilaricidal leads in clinical development (oxfendazole, emodepside) belong to classes that have been in veterinary use for decades [1]. Resistance is common to classes of broad-spectrum anthelmintics in agricultural settings [2], and there is a concern that mass drug administration could produce a similar outcome for human disease. Much has been written about the scarcity of new leads in the anthelmintic development pipeline [3,4]. We are interested in why this earlier era of anthelmintic drug discovery was more successful and how *in vitro* assays can be developed to best recapitulate past successful approaches.

While *in vivo* screens of infected animals capture any hit, regardless of the compound's **mechanism of action**, *in vitro* screens are more likely to miss compounds without overt phenotypes. There is a recognition that many anthelmintics require both a significant host and parasite component to their mechanism of action. An anthelmintic could conceivably (i) act on molecular targets in the parasite but require a host component for clearance, (ii) have polypharmacological effects on both parasite and host targets, or (iii) act directly on host targets and have negligible direct antiparasitic action.

A growing body of evidence reveals that anthelmintics essential to nematode and flatworm control may fall within the middle of this spectrum (Figure 2A, Key figure). For example, praziquantel acts directly on schistosomes, causing contractile paralysis and tegument damage which is followed by immune recognition and parasite clearance [5–7]. Praziquantel may also act on host vasculature [8] and immune cells [9]. While exhibiting potent *in vitro* effects against gastrointestinal (GI) nematodes, the microfilaricide ivermectin does not have obvious effects on

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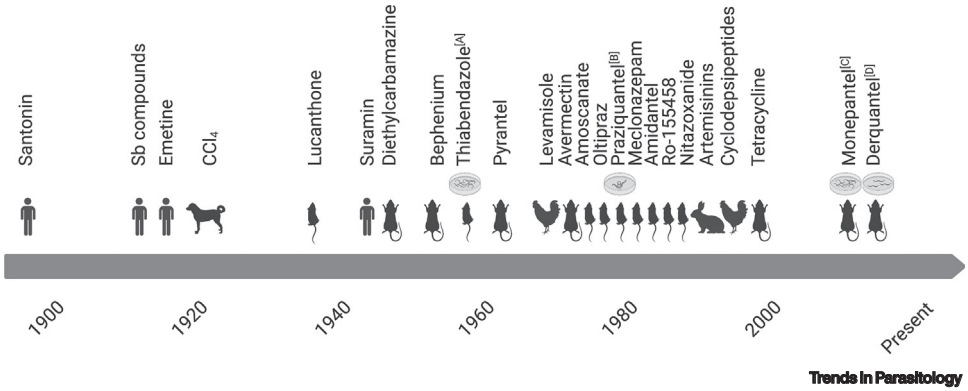


Figure 1. How were existing anthelmintics discovered? Timeline for the discovery of a selection of anthelmintics used over the past century. Early antiparasitic compounds, already known to be efficacious against protozoa, were used in humans harboring helminths. Drug screens were later performed on animal models of infection – either rodents (mice, rats, jirds) or agricultural animals (poultry). The animal symbol denotes *in vivo* models used in primary screens. A Petri dish indicates that an *in vitro* assay was employed. Many of these discoveries spawned numerous derivatives but we have restricted our summary to the first representative of the chemical series. [A]The initial benzimidazole hit was discovered using an *in vitro* trichostrongylid assay and an *in vivo* rodent model of *Heligmosomoides* infection. [B]In the case of praziquantel, an *in vitro* screen on *Schistosoma mansoni* complemented two *in vivo* murine models of flatworm infection. [C]The initial amino-acetonitrile derivative hit that led to monepantel was discovered using an *in vitro Haemonchus contortus* larval development assay, and this series was optimized using the jird model. [D]Derquantel was developed from paraherquamide, which was discovered in rodent screens, but also incorporated structure–activity relationship data from a related hit serendipitously discovered in a *Caenorhabditis elegans* screen. For a complete list of these compounds, and references, see Table S1 in the supplemental information online.

Glossary

Anthelmintic: a medication for human or veterinary use to treat infection with parasitic worms.
Deep learning: a class of machine learning algorithms that rely on multilayer neural networks that are particularly well suited for learning from large and unstructured datasets.
High-content imaging: automated microscopy of drug–parasite interactions to enable the collection of complex spatial and morphological readouts of cell and organismal health.
Machine learning: algorithms capable of learning from structured data to make determinations and predictions without explicit programming.
Mechanism of action: how a compound interacts with its target and, in the case of anthelmintics, triggers parasite elimination from the host.
Supervised learning: a machine learning technique that relies on the use of labeled data to train a predictive model.
Unsupervised learning: a machine learning technique for deducing structures present in unlabeled data.

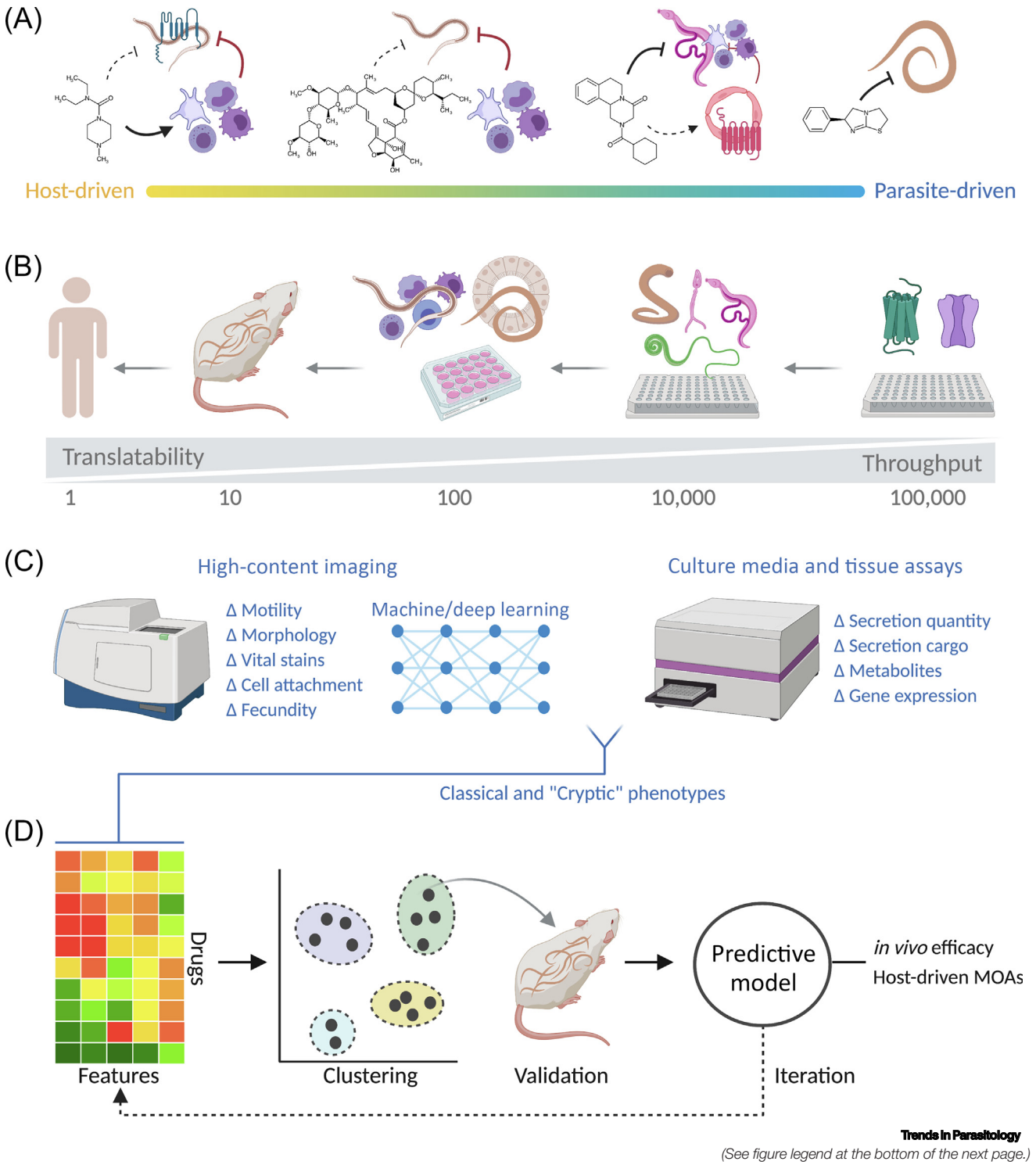
filarial worms at concentrations that are therapeutic *in vivo* and likely acts through dysregulation of parasite immune evasion [10–12]. Diethylcarbamazine also has subtle effects on parasites [13,14] and exhibits *in vitro* filaricidal activity only at high millimolar concentrations [15]. However, diethylcarbamazine is lethal to parasites *in vitro* if microfilariae are cocultured with donor blood, and its *in vivo* activity in an animal model is eliminated by pretreatment with an immunosuppressant [16,17]. So, while the mechanisms of many anthelmintics remain poorly understood, the host immune system can clearly play an important role in drug action.

In vivo screening: historically productive, but limited throughput

Screening using animal models of infection has the advantage that no prior knowledge of drug mechanism is required. Most frontline drugs were discovered by screening infected animals (Figure 1), and even those that are broad-spectrum may or may not evoke obvious phenotypes depending on the parasite's species and the developmental stage being assayed. For example, there are notable differences between filarial and GI parasites (Box 1). Would narrow-spectrum diethylcarbamazine have been identified had it not been screened *in vivo*, given its lack of *in vitro* potency against the parasites it effectively treats? Identifying the broad-spectrum macrocyclic lactones would also have required the fortuitous screening of a parasite species and developmental stage exhibiting an obvious *in vitro* phenotype. Similarly, artemisinins can kill schistosomes *in vitro*, but only following prolonged exposure to high micromolar concentrations that do not resemble *in vivo* conditions [18]. Other antischistosomal hits that show no obvious phenotype *in vitro* include Ro 15-5458 [19] and Ro 13-1978 [20]. And even when compounds exhibit *in vitro* movement or morphology phenotypes they may not be relevant to the drug's mechanism of action. Hycanthone impairs worm movement due to action at acetylcholinesterases, separate from its therapeutic mechanism of action involving DNA binding [21]. The striking contractile phenotype of praziquantel *in vitro* does not strictly equate to drug efficacy *in vivo*; liver-stage worms display the exact same contractile response to drug as adults even

Key figure

Developing *in vitro* assay endpoints with improved *in vivo* predictive value



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though they are completely refractory to praziquantel treatment [7]. Many anthelmintics with overt *in vitro* phenotypes may act *in vivo* by damaging the parasite to facilitate immune recognition and clearance of worms (Figure 2A). *In vitro* approaches to screening that do not reproduce *in vivo* conditions will be less capable of identifying hit compounds.

While past animal screens were conducted on hundreds to thousands of compounds, the current emphasis on a reduction in animal use (replacement, reduction, and refinement) makes this impractical as a primary screen. Animal screens also require synthesis of larger amounts of test compound than miniaturized *in vitro* screens. For example, one hit criteria for antischistosomal screening is drug efficacy clearing infections in mice dosed at 100 mg/kg over 5 days [22] – this screening paradigm would require nearly 100 mg of the test compound for a cohort of five mice. Therefore, *in vitro* screening approaches are needed, even if one limitation is that they have the potential to miss many 'true positive' hit compounds. Here, we discuss two aspects of assay development that can improve *in vitro* screening approaches.

(i) Approach 1. Making assays more '*in vivo* like' by incorporating aspects of the host immune system.
(ii) Approach 2. Employing more subtle *in vitro* phenotypic endpoints that are nevertheless important predictors of *in vivo* anthelmintic efficacy.

Employing *in vitro* assays that better approximate *in vivo* conditions

Challenges working with parasitic worms put an inherent limit on assay throughput. Two obvious barriers to high-throughput parasite screening are sourcing parasitic worms in large quantities and the complexity of *in vitro* culture conditions that recapitulate the host environment.

If the goal of a high-throughput screen is viewed as simply obtaining an enriched set of bioactive compounds from a large chemically diverse library, compromises to simplify and scale an assay are probably necessary. There is an enormous chemical space available for screening, both theoretically (166 billion organic compounds in the GDB-17 database) and available for purchase (>20 million unique compounds in the Enamine Diverse *REAL* drug-like set). The choice of what to screen is crucial to the success of the assay (Box 2). Since most compounds will typically be inactive, it may be economical to use free-living worms that can easily be scaled to enrich for chemicals with anthelmintic activity [23]. A crude readout of viability or development may be suitable for triaging inactive compounds. The active dataset may then be explored using lower throughput assays interrogating phenotypes in more detail, as has been done with the 'wactive' worm bio-active compounds [24,25]. The low historical success rate of *Caenorhabditis elegans* as an anthelmintic screening model [26] is likely linked to high false-negative rates and the unique biology of parasites belonging to different clades [27]. In some parasite species, juvenile worms

Figure 2. (A) Anthelmintic mechanisms of action can require varying degrees of host involvement. Examples range from (left to right) diethylcarbamazine and ivermectin, which show little activity on filarial worms *in vitro* (broken lines) but obvious immune-dependent effects *in vivo* (bold lines), to praziquantel (shown with schistosomes) and levamisole (shown with *Ascaris*), which have pronounced phenotypes *in vitro* indicating more parasite-driven mechanisms. (B) Drug screening similarly falls along a spectrum. *In vivo* assays are agnostic to worm phenotype or drug target but have limited throughput. More complex *in vitro* culture conditions can reproduce aspects of the host environment (coculture with host cell lines or organoids, inclusion of immune cells), although this complexity can limit throughput. Simple culture conditions are easier to scale reproducibly, especially if only one endpoint is measured in an assay, with target-based screens providing maximum throughput. This comes at the expense of host assay components and an increased likelihood of overlooking 'true positive' compounds that would be active *in vivo*. (C) High-content assays measure a greater phenotypic space and can be incorporated into machine-learning pipelines for classification of drug effects. Image-derived phenotypes can be multiplexed with assays on culture media, providing additional features to define *in vitro* drug phenotypes. (D) Features from multivariate phenotyping can be clustered to identify distinct drug-response patterns. Drugs from clusters exhibiting similar biological effects may be tested for efficacy *in vivo*. These data can be iteratively improved upon to identify *in vitro* phenotypic profiles with optimal *in vivo* anthelmintic predictive value. Abbreviation: MOA, mechanism of action.

Box 1. Targeting gastrointestinal versus tissue-dwelling helminths

Chemotherapy of worms that reside within host tissues may require different considerations than targeting worms within the gastrointestinal (GI) tract.

Drug absorption and distribution

Worms located within tissues present pharmacokinetic challenges of drug absorption and distribution that GI parasites do not. While GI parasites are endoparasites, the alimentary canal is technically 'outside' the body. A drug does not necessarily need to be absorbed by the host to be efficacious if it is present in the lumen and is taken up by parasites via the trans-cuticle or trans-tegumental route. For example, while praziquantel has near complete GI absorption, and works against most tissue-dwelling and GI flatworms, the nearly identical compound epsiprantel has poor GI absorption and is effective only against cestodes. Furthermore, tissue-dwelling parasites, such as adult *Onchocerca* residing in nodules or larval tapeworms that cause cysticercosis, may be more difficult to target *in vivo* with exposure to sustained therapeutic concentrations of drug. Finally, certain GI helminths may also present challenges similar to tissue-dwelling parasites. Some have tissue-dwelling life-cycle stages (e.g., larval hookworms can enter an encysted hypobiotic state), or as adults they may be exposed to drug from both the intestinal lumen and epithelial tissue. For example, whipworm benzimidazole absorption correlates most strongly with concentrations of drug in blood plasma rather than the intestinal lumen [71].

Expulsion versus elimination

Anthelmintics may drive clearance of GI helminths, perhaps by causing parasite paralysis, resulting in expulsion. However, transient paralysis of tissue-dwelling helminths may not lead to parasite death. Worms in close proximity to rapidly absorbed drugs, such as schistosomes living in the mesenteric vasculature, can still recover after drug clearance and migrate back to their preferred locations in the body [7]. Similarly, adult filarial worms that are damaged by drug treatment can recover, and microfilaria loads can rebound. Some tissue-dwelling parasites may require prolonged *in vivo* exposure to drug, which can be difficult to achieve in a convenient dosing form. Rather than killing these long-lived adult worms directly, drugs may trigger changes that promote immune-mediated clearance [45]. Therefore, a broad-spectrum drug may impact various parasite species differently. Ivermectin may acutely disrupt neuromuscular function of a GI nematode [12] while causing longer effects on fecundity of tissue-dwelling nematodes. Praziquantel may cause contraction of a tapeworm scolex leading to expulsion from the GI tract, while clearance of schistosomes involves host immune cells. For these reasons, different sets of *in vitro* phenotypes are likely to serve as reliable predictors of *in vivo* efficacy across tissue-dwelling and GI parasites.

offer comparable screening throughput and improved predictive value against disease-relevant life stages [27–29].

When smaller numbers of compounds are screened in medium- or low-throughput assays, it may be feasible to use adult parasites and incorporate aspects of the host immune system in culture conditions. This may be the case with pharmacophore-based screens centering on a small number of interesting chemical series. This approach, rather than high-throughput screening, has historically produced many of our existing anthelmintics and consists of screening a limited number (~several hundred) of structurally related compounds that display interesting bioactivity. At this stage, *in vitro* assays that more closely reflect the disease state should better predict *in vivo* efficacy as the assay endpoint is a better approximation of the disease endpoint [30].

Culture protocols have been developed that mimic the host environment in attempts to derive adult parasites from juvenile stages. Whole-blood culture systems promote the *in vitro* development of schistosomes from juvenile schistosomula [5]. Coculture systems with human leukocytes and endothelial cells [31] or 3D skin models [32] promote the *in vitro* development of *Onchocerca volvulus*, which has no laboratory-animal screening model to generate adult worms. And larval hookworms cocultured with human intestinal epithelial cells exhibit feeding behavior and gene expression profiles that more closely match similar-stage parasites harvested from animal hosts [33]. Conceivably, worm coculture within organoids may eventually provide a route to study host–parasite interactions [34].

Box 2. What to screen? Properties of past successful anthelmintics

Aside from assay development ('how to screen'), there is also the problem of compound selection ('what to screen'). Several common features can be seen in past successful anthelmintics. For example, many were derived from natural products. They also often exhibit sustained activity, either through unique pharmacokinetic or pharmacodynamic properties.

Natural products

Natural products have a long history as anthelmintics. Infections were treated with plant-derived traditional medicines before modern pharmaceutical sciences. More recently, drugs have been developed from active compounds produced by bacteria (*Streptomyces* cultures yielding milbemycins and avermectins) and fungi (*Mycelia sterilia* cultures yielding cyclooctadepsipeptides and *Penicillium* and *Aspergillus* cultures producing paraherquamides). Natural products continue to be productive sources of new bioactive compounds to treat a range of drug-resistant pathogens [72,73]. In addition to bacterial and fungal cultures, natural products produced by microfauna that interact with schistosomes are a promising area of exploration [74].

Extended pharmacokinetics

The search for adulticidal antifilarial drugs illustrates the importance of pharmacokinetics. Suramin, although no longer clinically used, has one of the longest half-lives of any drug (>1 month). The adult worm lifespan can be shortened if continually exposed to drug (repeated ivermectin dosing against *Onchocerca* [75]), and slower killing dynamics may be useful in avoiding inflammatory responses (Mazzotti reaction).

Unusual pharmacodynamics

Drugs can also display prolonged action depending on their pharmacodynamic properties. Many drugs with *in vivo* efficacy against flatworms have a short half-life (several hours), but kill worms on a protracted timescale [artemisinin and oxamniquine ~3 days, oltipraz ~1 week (reviewed in [76])]. How to reconcile this discrepancy? One explanation may be the covalent action of these drugs on their targets, uncoupling pharmacodynamics and pharmacokinetics. Numerous covalently acting antischistosomal drugs have been used in humans. Oxamniquine is activated by a parasite sulfotransferase, forming a reactive electrophilic product that binds DNA and protein [77]. Oltipraz irreversibly binds thiol groups on schistosome glutathione-*S*-transferase [78]. Niridazole is metabolically activated by worms and covalently binds worm proteins [79]. Dichlorvos (metabolized from metrifonate) irreversibly binds cholinesterases. Heme-activated artemisinin covalently modifies >100 malaria proteins [80], so a similar mechanism may occur in blood-feeding schistosomes. Covalent ligands come with concerns (selectivity of the reactive electrophilic functionality, or host immune response to the drug–protein conjugate), but these compounds merit consideration given the precedent of past efficacy.

In addition to promoting parasite development, these host cell types are likely crucial for drug mechanism of action *in vivo* (Figure 2). Ivermectin and diethylcarbamazine are examples of anthelmintics that do not elicit obvious *in vitro* phenotypes in filarial worms at therapeutically relevant concentrations. Some of these drugs promote immune-cell adhesion to parasites *in vitro* [16,35], an outcome crucial for parasite killing. Of the antischistosomal drugs, artemisinins are relatively inactive on cultured schistosomes unless media are supplemented with hemin or red blood cells [5,18], and praziquantel-mediated killing likely has a large immune component [5–7]. Including blood cells in parasite cultures may make it easier to detect compounds whose mechanisms involve the host immune system, particularly for tissue-dwelling or blood-consuming stages of the life cycle.

Identifying *in vitro* phenotypes with *in vivo* predictive value

Another strategy for improving *in vitro* assays is to develop methods for detecting subtle drug-evoked parasite changes that are nonetheless crucial to anthelmintic efficacy. Often, *in vitro* screens on roundworms [27,36,37] and flatworms [38–40] use changes in movement or morphology as a phenotypic readout, with the understandable assumption that dead worms do not move. But an immobile worm may not be a dead worm. Studies on *C. elegans* have shown that movement inhibition may be only transient and may vary based on the developmental stage being screened [41,42]. *Brugia* parasites show recovery after transient inhibition of movement in response to diethylcarbamazine and levamisole [14,43,44]. And while the premise that compounds which kill worms *in vitro* should be prioritized for *in vivo* screening seems reasonable,

it is possible that compounds that are efficacious *in vivo* will not cause obvious movement or morphology changes *in vitro*. However, this is not to say that drugs do not have nuanced phenotypes that may be important predictors of *in vivo* efficacy. For example, staining antigens on the schistosome surface after *in vitro* drug treatment reveals damage to the tegument that is likely crucial for immune recognition and parasite clearance [5], and drug-evoked changes in secretion of parasite-derived molecules may disrupt host–parasite signaling. As Moreno *et al*. note, adult parasites do not show obvious acute changes in mobility when treated with benzimidazoles or macrocyclic lactones, but these drugs do impact secretion of parasite-derived molecules that may well alter host–parasite cross-talk [45]. So what types of assays may be employed to achieve higher content and more informative phenotyping of test compounds?

High-content imaging is an approach using automated image acquisition and analysis to explore a larger phenotypic space than conventional high-throughput screens, which often look at just a single endpoint. Images of samples from various treatment conditions are analyzed to detect features, and combinations of features define a profile that distinguishes one phenotype from another. These assays could look at developmental outcomes (e.g., larval development or molting assays [46]), fecundity (e.g., adult female filarial release of microfilaria), or discern subtle changes in movement and morphology that segregate compounds based on mechanism of action [47,48]. The use of fluorescent dyes or molecular probes can also allow for multiplexing assays on numerous tissues or quantification of subtle tissue/cell type changes (Figure 2C), including observations of endosymbiotic *Wolbachia* [49]. Imaging may also be performed tracking worms across a time-course following drug treatment. Fixed timepoints can limit the mechanistic profile of the resulting hits, and protozoa screens have found that capturing both 'fast acting' and 'slow acting' hits increases the diversity of hit mechanisms [50,51]. **Machine learning** allows for phenotypes to be detected within these large datasets [47,48]. In **supervised learning** strategies, features collected from imaging data may be combined with manually annotated labels to generate training sets for future hit classification. While this allows screening of visually obvious phenotypes at increased scale, it is low-resolution in that it builds in our inability to distinguish subtle, cryptic phenotypes that may be valuable indicators of drug action. **Unsupervised learning** and **deep learning** approaches can be used to identify and discriminate new phenotypic categories from image-extracted features in an automated manner [52].

Multivariate phenotyping does not need to be limited to imaging data. This workflow can be multiplexed to include changes in gene expression, as well as biochemical endpoints measuring changes to assay media (Figure 2C). For example, while *in vitro* ivermectin treatment may cause only subtle changes in morphology or movement, it causes measurable changes in products (proteins and extracellular vesicles) secreted into the assay media [10,44,53]. Media can also be used to measure metabolic changes [54,55]. These assays do not always correspond to visual scoring of worm viability [45,56,57], indicating that there are interesting differences in underlying mechanisms.

Features from imaging and biochemical data can be used to define profiles that cluster based on compounds' mechanism of action. These clusters can then be screened in animal models to iteratively refine *in vitro* endpoints for optimal predictive value *in vivo* (Figure 2D). It is clear from other antiparasitic drug screening efforts that diverse assay endpoints are needed to ensure mechanistically diverse leads in the development pipeline [50,58], which is crucial given the widespread emergence of resistance to many anthelmintics [2].

Screening of new and existing compounds using quantitative endpoints may allow for combinatorial testing of drugs in a systematic way that can identify instances of synergy or additive effects.

For example, synergistic drug combinations may exhibit increased potency and allow for decreased dosing and off-target effects, and this can be investigated by generating isobolograms from plates consisting of a matrix of drug combinations across a series of concentrations. Additive effects can also be studied when multiplexing assay endpoints. Drug combinations that overlay independent phenotypes may indicate independent mechanisms of action, which is desirable for slowing the emergence of resistance. These studies would be useful not just in screening new libraries but also in gaining a better mechanistic understanding of existing anthelmintics.

Identifying targets that underpin *in vitro* phenotypes

A complete understanding of how anthelmintics work will require insight into the molecular mechanisms underpinning clusters of phenotypic profiles. The therapeutic targets of many anthelmintics have yet to be deorphanized, although recent functional data have proposed candidates for praziquantel [59,60] and diethylcarbamazine [14]. This is also important since truly high-throughput assays may require target-based rather than phenotypic screens, and historically 'best in class' drugs have come from target-based screens on deorphanized receptors [61]. There is currently a wealth of parasite genomic information [62] which can inform screening by predicting essential drug targets and metabolic chokepoints [63]. However, tools for functionally annotating these genomes, such as CRISPR and transgenesis protocols [64–66], are not routinely employed in parasitic nematodes. Similarly, schistosome studies have reported editing of parasite eggs [67], but hatched miracidia need to survive propagation through snail and vertebrate hosts to allow routine interrogation of gene function in intramammalian parasite stages. Most species cannot be cryopreserved, and there is a lack of any *in vitro* 'egg-to-egg' parasite culture system – meaning that maintenance and propagation of edited lines will not be trivial for large-scale genomic screens. These obstacles will need to be addressed in order to fully understand the genetic basis for phenotypic profiles of either existing anthelmintics or novel leads.

Concluding remarks

Recent decades have seen a scarcity of new leads in the anthelmintic pipeline and few new drug classes, and resistance to many broad-spectrum anthelmintics is common in veterinary settings. Advances in genomics have allowed us to better understand the mechanisms of existing anthelmintics (often aided by resistant strains [68–70]), but progress in the search for drugs with new mechanisms has been slow. Over the past century, most anthelmintics were discovered using *in vivo* screens on animal models of infection. We argue that the efficiency of *in vitro* assays can be increased by designing phenotypic screens to better recapitulate the *in vivo* environment.

Drugs may conceivably act across a spectrum of mechanisms ranging from indirect antiparasitic action, subtly impacting the ability of worms to reside undetected within the host, to direct parasite killing. Assays looking only at obvious outcomes such as gross changes in movement or viability may neglect compounds that would be efficacious *in vivo* but lack obvious effects *in vitro*. In high-content imaging, an analogy has been made to the psychological phenomenon of inattentional blindness. In this case, a focus on an obvious outcome that we expect to see when looking for anthelmintics (perhaps changes in movement or viability) may actually distract us from subtle phenotypes that are nonetheless valuable for predicting antiparasitic effects.

Advances in automated imaging and the computational frameworks for analysis of these data have made it possible to profile worm phenotypes in unprecedented detail. By exploring this expanded phenotypic space and screening more subtle features of drug action we may not only improve upon existing anthelmintics but also have the opportunity to identify new classes of compounds. While resolving the exact mode of action for these compounds may require

Outstanding questions

Before embarking on screening new compounds, do we have a complete understanding of the phenotypic profile for existing frontline anthelmintics against target parasite species and stages?

Will high-content whole-organism screens using new multivariate and 'cryptic' endpoints reveal anthelmintics with novel mechanisms of action? Will deep phenotypic profiling better resolve anthelmintic interactions and inform combinatorial strategies to mitigate the spread of resistance?

How can we experimentally validate the *in vivo* efficacy of test compounds against parasites that are not amenable to animal models of infection? Are small-animal hosts made permissive through immunodeficiency less useful in capturing drug mechanisms of action that require a significant immune component?

How will the development of more routine parasite transgenesis and genome-editing tools alter *in vitro* and *in vivo* approaches to drug screening?

How can we associate specific genes to parasite drug response phenotypes, given sparse evidence-based genomic annotations and a lack of scalable functional genomic tools in many species?

advances in functional genomics, or the use of free-living models [70] (see Outstanding questions), leads that fall under distinct phenotypic clusters from existing anthelmintic classes would merit prioritization as potentially acting via unique mechanisms.

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Declaration of interests

The authors declare no competing interests.

Supplemental information

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