ABSTRACT

**Purpose** Neglected tropical diseases (NTDs) represent a heterogeneous group of communicable diseases that are found within the poorest populations of the world. There are 23 NTDs that have been prioritized by the World Health Organization, which are endemic in 149 countries and affect more than 1.4 billion people, costing these developing economies billions of dollars annually. The NTDs result from four different causative pathogens: protozoa, bacteria, helminth and virus. The majority of the diseases lack effective treatments. Therefore, new therapeutics for NTDs are desperately needed.

**Methods** We describe various high throughput screening and computational approaches that have been performed in recent years. We have collated the molecules identified in these studies and calculated molecular properties.

**Results** Numerous global repurposing efforts have yielded some promising compounds for various neglected tropical diseases. These compounds when analyzed as one would expect appear drug-like. Several large datasets are also now in the public domain and this enables machine learning models to be constructed that then facilitate the discovery of new molecules for these pathogens.

**Conclusions** In the space of a few years many groups have either performed experimental or computational repurposing high throughput screens against neglected diseases. These have identified compounds which in many cases are already approved drugs. Such approaches perhaps offer a more efficient way to develop treatments which are generally not a focus for global pharmaceutical companies because of the economics or the lack of a viable market. Other diseases could perhaps benefit from these repurposing approaches.

**KEY WORDS** dengue · ebola · echinococcosis · elephantiasis · high throughput screening · kinetoplastids · lymphatic filariasis · neglected tropical diseases · onchocerciasis · repurposing · schistosoma · zika

INTRODUCTION

Since the 1980’s the pharmaceutical industry has taken advantage of the systematic advances in molecular biology/genetic engineering and replaced phenotypic and whole-cell High Throughput Screening (HTS) with target-based screening assays (1). While target-based screens were developed that utilized simple recombinant protein enzyme assays provided many advantages in terms of cost and scalability, they relied on the assumption that the selected target is in fact the best and most druggable target for a given disease. However, in the last decade, we have seen a clear shift back towards using phenotypic screens as an initial starting point for drug discovery, especially for neglected diseases for which the drug targets...
are generally poorly understood or where target-based approaches failed (1,2). An analysis of the origin of first-in-class small molecules proposed that phenotypic screens identified more novel inhibitors than other approaches between the years 1999 and 2008 (3). However, phenotypic screening has the disadvantage that it does not provide information about the target/s for a hit.

Neglected Tropical Diseases (NTDs) are a diverse group of 23 predominantly communicable diseases (Table I) that prevail in tropical and subtropical conditions, affecting 1.49 billion people in the developing world (4,5), and costing those global economies billions of dollars every year. These 23 NTDs disproportionately impact poorer regions and contribute to substantial morbidity, disability and poverty in many low- and middle-income countries (6). It is important to realize that in many cases these pathogens are eukaryotic parasites which have complex life cycles and varied mechanisms for evading their host immune system. In addition, some of these parasites are not genetically tractable in the laboratory which further complicates drug discovery efforts. The inability to translate many of these pathogens into a lab setting is one reason for the lack of research into these diseases. Perhaps more critical is the absence of a lucrative market for NTD treatments. These diseases generally lack market incentives to spur product development (7–9). As a result, NTDs receive relatively little research investment ($80M - $500M / year) from governments or pharmaceutical companies, compared with the billions of dollars invested in diseases such as cancer and heart disease or other blockbuster diseases (10). Although these neglected diseases continue to lack major economic incentives due to their financially unattractive affected population, (7–9) there is a tropical disease priority review voucher awarded to companies that successfully develop FDA-approved treatments for particular diseases (11–13) (Table II) some of which overlap with the WHO definitions of tropical neglected diseases (Table I). This is anticipated to assist companies to focus on developing treatments for these diseases. Another way to overcome the challenges of NTD drug development is to reposition or repurpose existing drugs (15–22). Utilizing compounds that have already been approved by the FDA, compounds with known physicochemical and absorption, distribution, metabolism, excretion and toxicology (ADMET) properties, can accelerate the development process of an old drug through financial and regulatory hurdles to market. The efficiency of a repurposing approach is especially critical for NTDs which typically do not have viable commercial products already approved or treatment paths that are economically feasible. In addition to the potential monetary benefits, the development of a repositioned product can shorten the timeframe for drug discovery from 10-17 years to 3-12 years which is especially important in cases where the spread of disease is rapid and costly (23,24).

Despite the benefits of drug repurposing, pharmaceutical companies do not seem to be making this standard practice as yet. ‘Use patents’ are not as valuable as ‘synthesis patents’ and since repurposing is almost by definition exclusively limiting to ‘use patents’, it is more beneficial for pharmaceutical companies to invest their financial resources in their own research and development of new compounds, potentially overlooking effective drugs that already exist. For small drug companies and biotech firms, it is more urgent to build valuable IP in order to compete with larger companies and so they are even less likely to find value in repurposing drugs. Due to the poor economic tradeoffs, a lot of repurposing efforts are being championed by academia. However, there are many recent examples of successful collaborations between industry, government, and academia, where large libraries of compounds are made available for high throughput screening including by Novo Nordisk (25), Novartis (26), GlaxoSmith Kline (27), Pfizer (28), and other companies as parts of consortia (e.g. More Medicines for TB (29,30), TB Drug Accelerator, NIH NCATS etc).

In addition to industrial libraries, repurposing studies benefit from any compound libraries that are accessible. These include NCATS (31) /NIH (32,33), Microsource (34–36), LOPAC (37), Prestwick (38), Broad (39), Calibr (40,41), bioactive libraries (42), small molecule libraries (43), collections of natural products (42), and others. The library that arguably

| Table I | Neglected Tropical Diseases as Defined by the WHO |
|---------|-----------------------------------------------|
| Buruli ulcer | Leprosy (Hansen’s disease) |
| Chagas disease | Lymphatic filariasis |
| Dengue and Chikungunya | Mycetoma, chromoblastomycosis and other deep mycoses |
| Dracunculiasis (guinea-worm disease) | Onchocerciasis (river blindness) |
| Echinococcosis | Rabies |
| Foodborne trematodiases | Scabies and other ectoparasites |
| Human African trypanosomiasis (sleeping sickness) | Schistosomiasis |
| Leishmaniasis | Soil-transmitted helminthiasis |
| Snakebite envenoming | Trachoma |
| Taeniasis/Cysticercosis | Yaws (Endemic treponematoses) |
provides the best benefit is the FDA approved library (44–48). Because this list seems to be updated sporadically and obtained from different sources, studies that refer to the library of FDA approved drugs describe a library ranging from 774 (45), to 1,012 (48) to 1,766 compounds (49). Johansen et al. extended this library to screen a combined 2,600 US and “non-US” approved drugs in one study (50), and a combination of FDA approved drugs and “ex-US-approved drugs” in another (51). Although diseases occurring throughout the globe can be impacted by these types of repurposing efforts, there are no studies identified that have screened libraries compiled by regulatory bodies of other countries with the intent of repurposing drugs for a neglected disease to our knowledge. While these regulatory bodies exist, they seem to be overlooked by most researchers trying to repurpose drugs for NTD’s and the majority of “approved” compound screening uses the US FDA library.

**DATA MINING APPROACH**

While tremendous progress has been made in neglected disease drug discovery over the last few years (52), there are still many gaps to be addressed (53). These diseases and variations of their associated terminology were searched in the literature using multiple search engines along with “high-throughput screen” and “identified” to find those papers in which screening was performed with some success. The ~7000 rare diseases often discussed alongside neglected diseases (54) were not considered for this study. The use of the Medicines for Malaria Ventures (MMV) box has been extensive and only a handful of those studies are presented here. Van Voorhis et al. have a more complete compilation of that work (55). There are also considerable efforts in the development of assays (56–61), lead optimization (62,63), mechanism of action elucidation (64), and smaller scale repurposing studies for neglected diseases (65–71), but these were outside the scope of this investigation. Due to the number of people living with these neglected diseases and the urgency needed to respond to their emergence, studies involving high throughput screening for Dengue, Zika, and Ebola viruses were included as recent examples. In addition to presenting some of the work that has been done in the NTD repurposing field, we have compiled a database of over 1,200 compounds suggested for repurposing by the authors developed across the many studies summarized herein.

**CHAGAS DISEASE**

Chagas disease is a NTD that is known to be caused by the kinetoplastid parasite *Trypanosoma cruzi* (72). The disease is a problem in Latin America where it is endemic, but it is also increasingly found in North America and Europe, which it reaches through immigration (73–75). The clinical signs of Chagas diseases are manifested in different phases. In all cases, the disease ultimately leads to death and the only available contingency plans rely on organ transplantation. The spread of Chagas disease to North America and beyond is highlighting the need for novel, safe, and effective therapeutics to treat *T. cruzi* infection. Both target-based and phenotypic-based studies have been performed in recent years to address this.

De Rycker et al. employed a novel screening cascade to screen two libraries, the SelleckChem, which is comprised of 421 FDA-approved drugs and the NIH Clinical Collection, which consists of 727 compounds tested in clinical trials ((76), (77)). The drug concentrations were 5 and 15 μM. The cascade was made up of an initial single-point primary screen, that was followed by a high content screening assay to determine potency, a static-cidality and rate-of-kill assessment, as well as an *in vitro* CYP51 activity determination (to measure the inhibition of agonist-induced calcium flux). Inhibitors that were found to interfere with calcium homeostasis were shown to have moderately slow killing profiles despite their high potency. The remaining hits were classified as central nervous system (CNS) targeting lipophilic amines. Based on potency, selectivity and cidal profiles, the most interesting drugs were determined to be ifenprodil, ziprasidone, clemastine, clofibrate and azelastine. All compounds have reasonable bioavailability but are currently limited by toxicity. Future work would have to be performed to eliminate the CNS penetration or H1 antagonism to make the compounds more suitable for treatment of *T. cruzi* in human targets (77). During the development of the above mentioned CYP51 assay, Riley et al., identified several hits mentioned briefly in a separate paper (78). They used the CYP51 enzyme of the Chagas parasite expressed in *E. coli* as a way to distinguish compounds with similar mechanisms of actions from those used by current

| Table II | Diseases that are Eligible for the FDA Tropical Disease Priority Review Voucher (14) |
|----------|--------------------------------------------------------------------------------------|
| Malaria  | Binding trachoma                                                                    |
| Buruli Ulcer | Cholera                                                                            |
| Dengue/Dengue haemorrhagic fever | Dracunculiasis (guinea-worm disease)                                                |
| Fascioliasis | Human African trypanosomiasis                                                         |
| Leishmaniasis | Leprosy                                                                            |
| Lymphatic filariasis | Onchocerciasis                                                                      |
| Schistosomiasis | Soil transmitted helminthias                                                          |
| Yaws     | Tuberculosis                                                                        |
| Cueva virus | Ebola virus                                                                        |
| Marburg virus | Zika virus                                                                       |
| Chagas   | Neurocysticercosis                                                                  |
| Chikungunya Virus Disease | Lassa fever                                                                       |
| Rabies   | Cryptococcal Meningitis                                                             |

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drugs where resistance is a growing problem. 200,000 compounds were initially screened for their activity and the resulting hit compounds were represented by 129 compound classes. Compounds were de-prioritized based on chemical similarity to known treatments and the remaining hits, represented by 5 singletons, were suggested for repurposing (78).

Keenan et al. utilized a luminescence cell-based HTS in order to identify inhibitors of *T. cruzi* replication (39). The selected compounds were screened based on their ability to inhibit intracellular trypanosomes from replicating inside the host cells. The surviving parasites were then assessed via detection of a luminescent reporter after cells were lysed. This group screened over 300,000 compounds, and over 4,000 were identified (hit rate ~1.4%). Compounds from this list were then prioritized based on the following characteristics: homology to known antifungal pharmacophores with anti-trypanosomal activity, compounds that contain a basic heterocycle and two additional lipophilic or aromatic moieties in a trigonal or tetrahedral arrangement around a central atom, synthetic tractability, novel structures to the field, and the extent to which structural domains influenced the activity. Fifteen lead hit structures were initially identified, and based on the mentioned criteria, seven were verified. In *vivo* activity studies against *Trypanosoma cruzi* and in *vivo* studies for toxicity further filtered the candidates down to CM100 (IC$_{50}$ = 0.169 nM) and CM74 (IC$_{50}$ = 0.075 μM) as promising therapeutics for Chagas disease (39).

In 2014 Planer et al. suggested two combination therapies for the treatment of Chagas disease; amiodipine and clemastine, both in combination with posaconazole (36). Most drugs screened did not have dramatic effect alone, with the exceptions of benznidazole and posaconazole. However, it should be noted that many compounds were not ineffective in *vivo* because investigators intentionally used low dosages in order to observe the effects on bloodstream parasitemia (36).

From the genome of *T. cruzi*, several enzymatic pathways were identified as potential targets and compounds with associations to those pathways were investigated in a Bayesian model that considered 300,000 small molecules from previous studies. After in *silico* screening, 97 compounds were screened in *vivo*, 11 were found to be active and several were found to be quite effective in *vivo* in mice, with one compound, pyronaridine (85% efficacy) selected for further investigation (79).

To find new inhibitors of the cruzain enzyme present in *Trypanosoma cruzi*, Wiggers et al. screened the ZINC database for compounds with lead-like properties and then performed consensus scoring using both target-based molecular docking, and ligand based similarity searching (80). This narrowed 8.5 million compounds to 8,600 compounds, which were then screened through Glide XP and HQSAR. The top 5% of compounds that passed both screens were visually inspected and 23 compounds were selected for in *vivo* testing. The top two compounds from this screen were structurally characterized by X-ray crystallography to understand their binding modes with cruzain (80).

Gunatilleke et al. screened over 100,000 compounds from multiple libraries and observed that several compounds that contain similar structural features were effective against Chagas disease using CYP51 as a permissive target for the small molecule assay (43). Some 185 of the top hits were nitrogen-containing aromatic heterocyclic pharmacophores. Of these, 92 compounds had the R group in the para position and the nitrogen in meta position within a ring. These compounds have been added to the ZINC database for future use, but no in *vivo* verification has been performed yet (43).

Unfortunately, due to the complexity of the disease, the various stages in parasitic lifecycle, and the inherent differences between human patients and *in vivo*, *in vitro*, and *in silico* studies, compounds which show great promise in pre-clinical studies do not necessarily translate to effective clinical approaches (81). For Chagas disease this was evident in the case of posaconazole which failed to maintain sustained efficacy over the course of a 60 day clinical trial (82). In contrast, benznidazole (a currently utilized treatment), was shown in a separate study to have 82% sustained response after a year (83). Unfortunately, benznidazole has known side effects which can limit its broad use (81). A major project by the DNDi to find superior treatment options has been updated to filter out compounds with CYP51 inhibitors like posaconazole and to better understand rate of kill to predict slow acting versus fast acting compounds (81).

**HUMAN AFRICAN TRYPAanosOMIASIS (HAT)**

Human African Trypanosomiasis (HAT) is another kinetoplastid disease that is found in sub-Saharan Africa, where 70 million people are at risk of this disease and an estimated 30,000 are currently infected (84). HAT is known to be caused by two different subspecies of the protozoan parasite *Trypanosoma brucei*. While there are treatments for this disease HAT drugs have historically had limitations relating to their administration, toxicity or cost. There are relatively few alternative drugs or validated drug targets which is driving research to identify parasite-specific antitrypanosomal lead compounds. Hence there have been efforts to develop a fluorometric viability assay for HTS (85–98). In a successful example of a partnership between industry and academia, 42,444 compounds were provided by GlaxoSmithKline and screened by Diaz et al., for growth inhibition of *Trypanosoma brucei* (27). 797 compounds showed activity against this sleeping sickness parasite while also not presenting signs of toxicity to human cells (27). Further analysis was performed to distinguish cell death vs. cell growth inhibition and 129
compounds that cause parasite death were grouped into clusters based on structural similarity (27).

Blazer et al., investigated a library of compound fragments that efficiently covered chemical space as defined by both complexity and number of rings (99). Beginning with 1,040 compounds, they screened the compounds in vitro against a phosphodiesterase (PDE) present in T. brucei and an ortholog from humans. The fragments displayed activity in each assay with only 7 fragments showing selectivity (99). The authors focused on 4 fragments with structural similarities to known drug scaffolds and 32 associated analogs in further phenotypic assays for parasites causing sleeping sickness, Chagas, leishmaniasis, and malaria, as well as human cells for selectivity consideration (99).

In 2011, Piclamilast was identified by Bland et al. as a phosphodiesterase (PDE) inhibitor potentially useful against HAT (100). Their approach involved an interesting method in which they aimed to repurpose knowledge about phosphodiesterase inhibitors from human-focused medicinal chemistry and to leverage this understanding into a treatment for the HAT parasite. They identified piclamilast (a known PDE inhibitor in humans) as a relevant starting point for optimization. This was followed by a comparison of piclamilast docking into the human and parasitic binding pockets as well as an SAR study of analogs.

LEISHMANIASIS

This parasite is transmitted by the sand-fly and can cause very different clinical manifestations depending on the infecting species and the host immune system (101). The disease is endemic in 88 countries and each year 1.6 million people are infected while approximately 350 million are at risk. There are at least four treatment options currently available including antimonials, amphotericin B, miltefosine and paromomycin, none of which are adapted to the field. The main drawbacks to these treatments are toxicity, long treatment courses, need for hospitalization, high costs, and resistance. This parasite is transmitted by the sand-fly and can cause very different clinical manifestations depending on the infecting species and the host immune system (101). The disease is endemic in 88 countries and each year 1.6 million people are infected while approximately 350 million are at risk.

Several studies have considered multiple kinetoplastids, such as the extensive work performed by Pena et al. (117). In order to identify potential new drugs, whole-cell phenotypic assays against Leishmania donovani, Trypanosoma cruzi, and Trypanosoma brucei were used to screen the GlaxoSmithKline HTS diversity set of 1.8 million compounds (117). For each of the three screens used, an orthogonal assay was conducted to ensure compounds with genuine activity were identified and enable compounds that interfered with the assays to be ruled out. The selection of candidates started first with the most potent, specific, and non-cytotoxic compounds in the dose-response outputs of each screen that was also filtered for lead-like compounds. The final boxes contained 592 compound: 192 of which were active against Leishmania (71)

De Rycker’s 2013 description of their assay optimization for Leishmania also resulted in hit compounds from two parallel studies. These authors performed an amastigote axenic assay that was more readily adaptable for high throughput but it did not differentiate between compounds that killed the parasite vs those that slow their growth (77). The authors screened almost 16,000 compounds and those that showed activity toward the amastigotes failed to perform at all or to the same extent in the more physiologically relevant intramacrophage assay the authors developed. 6 hits were selective for Leishmania as compared to human lung cells and exhibited dose-response relationships for intracellular Leishmania (77).

Sanderson et al. used both in vitro and in vivo methodologies and found several kinase inhibitors as well as other compounds (sorafenib, sunitinib, lapatinib, PP2, amphotericin and miltefosine) used for cancer therapy to also be effective against Leishmania (71).
Some work has also been performed for multiple kinetoplastids using the MMV malaria box (55). Van Voorhis et al., reviewed 236 screens performed by many different groups that used the malaria box for a variety of repurposing efforts (55). Kaiser et al. performed a handful of these studies. They screened 400 compounds from the malaria box against bloodstream *T. b. rhodesiense* and *T. brucei* and against intracellular amastigotes of *T. cruzi* and *L. infantum* (118). Out of 400 compounds, 4 passed initial in vitro screening, but failed in in vivo testing (118). The same year, Kaiser et al took 100 drugs with a high likelihood of repurposing and tested them against several neglected tropical disease parasites. They tested some of the more potent compounds in mouse models for the various diseases with mixed results (33). For activity against *T. b. rhodesiense* they show the antidepressants sertraline and paroxetine to be good repurposing candidates. Several were reported for the first time for having moderate anti-Chagas efficacy such as tadalafil, an erectile dysfunction drug, and mebeverine, an antispasmodic (33). Promazine and nortriptyline were also identified in this study as anti-malarial compounds alongside several tricyclic antidepressants (33).

**SCHISTOSOMIASIS**

Schistosomiasis is caused by a blood fluke (flatworm) and infects approximately 350 million people worldwide (119). The parasite is found in freshwater harboring the appropriate vector snails, and can actively invade skin. Children who play in water are particularly at risk (120). Once mature, the male and female flukes mate and lay eggs. These elicit a variety of immuno-inflammatory responses that can lead to a lifetime of pain and malaise (121), which interfere with school attendance and the ability to work, and consequently negatively impact both personal well-being and community development (122–124). Schistosomiasis also increases the risk of acquiring HIV infection (125–127).

Treatment and control of schistosomiasis relies on mass drug administration (MDA) with just one drug, praziquantel, which was developed 40 years ago (128–131). Apart from the obvious worry of drug resistance should PZQ fail, particularly as its use will be dramatically expanded in the next decade, the drug has a number of pharmaceutical and pharmacological weaknesses that encourage the search for a better chemotherapy (132). However, unlike the situation for malaria, other protozoal diseases and even the soil-transmitted helminthes (STHs) discussed here, there is no trans-nationally organized discovery or development program to find a better drug and little evidence that this situation will change anytime soon. Therefore, much if not all of the effort to identify new anti-schistosomal drugs occurs in the academic setting.

In the search for new chemical matter, the last decade has witnessed a number of advances in whole-organism (phenotypic) screening paradigms, which include the use viability- or vitality-based single-metric assays (reviewed in (133)), and partially or fully-quantitative image analysis approaches (134–138). Regardless of format, most of the assays have focused on post-infective larvae (schistosomula), which are a relatively abundant stage of the parasite (*Schistosoma mansoni* being the ‘model’ schistosome) allowing for a reasonable screening throughput. The use of larval parasites is rationalized on the basis that the target product profile (TPP) for new anti-schistosomal drugs should encompass activity against immature schistosomes and not just mature parasites, as is the case with PZQ (139–144). Hits identified against schistosomula are then screened in vitro for activity against the more limiting adult parasites (which must be harvested from small animals such as mice or hamsters) before being then considered for in vivo efficacy studies (134,145). This staged or tiered approach has involved the screening of both chemically diverse and focused chemical collections and has identified a variety of anti-schistosomal chemistries, including antibiotics (e.g., doramectin and clodazone (34)), neuroactives (134), anti-cancer agents (e.g., trametinib and vandetanib (108)) protease inhibitors (134), thiosemicarbazones (146) and statin drugs used for hypercholesterolemia (147). A few studies may be highlighted.

Mansour et al. obtained compounds from multiple libraries, i.e., from Pfizer and GSK, and performed high-throughput screening of 300,000 compounds on schistosomula followed by hit-screening against juvenile and mature parasites (28). They eventually prioritized seven compounds for future study, none of these were already approved drugs.

Ingram-Sieber et al. studied the antischistosomal properties of the MMV malaria box comprising 200 diverse drug-like and 200 probe-like compounds with confirmed in vitro activity against *Plasmodium falciparum* (malaria) (145). Five compounds were identified as lead compounds, and compound 2, a N,N9-diarylurea (IC50 = 0.83 μM), and compound 17, a 2,3-dianilinoquinoxaline (IC50 = 0.83 μM) were the most promising compounds to focus on for future antischistosomal studies. In vivo studies with mice infected with *Schistosoma mansoni* showed that a single oral dose of 400 mg/kg of the two drugs reduced worm burden by 52.5% (N,N9-diarylurea) and 40.8% (2,3-dianilinoquinoxaline). These two drugs represent a useful scaffold for preparing future small molecule inhibitors of *S. mansoni* (145). Apart from whole-organism screening, target-based approaches to defining novel anti-schistosomals have been employed. Liu et al. identified multiple compounds for repurposing for schistosomiasis by performing a high throughput docking screen of 14,000 molecules against an enzyme (3-oxoacyl-ACP reductase) involved in fatty acid synthesis. Compounds were tested directly for inhibition in a worm study, and for cell toxicity, resulting in 2 final
compounds that both impacted mortality rates while exhibiting low cytotoxicity in human cells (40).

HDAC8, an enzyme involved in the epigenetic functions of *Schistosoma mansoni* was selected as a target for high throughput screening in another study (148). Through homology modeling Kannan et al. were able to perform docking of ~5000 compounds from classes with known zinc binding capabilities. From this screen, 75 compounds were selected for *in vitro* testing. 8 compounds showed good toxicity against the parasitic enzyme and are considered to be lead compounds (148).

Li et al. implemented a high throughput screen against 59,360 synthetic compounds targeting *Schistosoma mansoni* thioredoxin glutathione reductase (SmTGR), which is a well-characterized drug target for *Schistosoma mansoni* (25,149). Of the compounds, 74 showed a concentration-dependent inhibitory trend against thioredoxin glutathione reductase. Testing in cultured larvae showed that 39 compounds had cidal activity within 48 hours, while three of the compounds killed adult worms *ex vivo* at concentrations between 5 μM and 10 μM (25).

Targeting the same enzyme, using an integrated approach, Neves et al. developed QSAR models for SmTGR and applied them to virtual screen a commercial database of 150,000 compounds (150). The computational approach allowed for the selection of 29 virtual hits, which were tested using high content screening assays against schistosomula and adult worms. Six compounds were active against schistosomula and three active against adult worms (hit rate of 20.6%). Among them, 2-[2-(3-methyl-4-nitro-5-isoxazolyl)vinyl]pyridine and 2-(benzylsulfonyl)-1,3-benzothiazole, two compounds representing new chemical scaffolds, showed inhibitory effect equivalent to PZQ, with EC50 values around 2.50 μM against both schistosomula and adult worms, representing promising new antischistosomal hits for further hit-to-lead optimization (150).

A composite approach, involving both whole-organism and target-based strategies, was recently employed to discover and validate a phosphodiesterase 4 (PDE4) as a schistosome drug target using benzoaxaborole inhibitors of human PDE4 (151). Notably, the study employed a transgenic line of *C. elegans* that expressed the schistosome PDE4 in order to functionally characterize and validate the target.

Finally, chemogenomics and cheminformatic strategies have been employed to identify possible novel targets from anti-schistosomal drugs discovery. Neves et al. considered 2,114 proteins with the concept that "similar targets have similar drugs" and used each protein to search through 3 drug databases to find drugs with activities against *Schistosoma* flatworms (152). In addition to finding several compounds with confirmed positive activities, they suggested 115 untested compounds for potential repurposing (152). In the following study, the same authors (153) tested some of the suggested drugs in the previous study, in schistosomula and adult *S. mansoni* worms using a spectrophotometric assay and an automated image-based assay to accurately measure parasite viability and motility, respectively. They found that paroxetine, an antidepressant drug, showed a pronounced effect on schistosomula viability ([IC50] =2.5 μM) after 72 h of incubation and EC50 values for motility reduction in male and female worms were 5.1 μM and 9.9 μM after 24 h of exposure, respectively (153).

**Nematodes**

Burns et al. used *C. elegans* as a model system for parasitic nematodes that are otherwise difficult to screen and identified 275 compounds that were lethal (154). They re-screened hits on two parasitic nematodes that infect livestock and two vertebrate models as a way of determining a degree of selectivity. Interestingly, they noted that nematicidal compounds have a higher logP (octanol-water partition coefficient) than the rest of the 67,012 compounds screened (154).

Mathew et al., took a similar approach screening 25,986 compounds from multiple libraries initially identifying 137 compounds active against *C. elegans* and focused on 14 compounds that impact both growth and fecundity (155). They grouped the compounds according to Tanimoto similarity. They then identified 9 compounds that fell into 2 clusters from the previously described Burns (154) study and 5 compounds with structural features not belonging to previously suggested nematicides. They compared activity of these compounds on the representative nematode to mammalian and yeast cells. Structure-activity relationships (SAR) and comparative genome sequencing were considered to gain an understanding of potential risk for resistance development. They described the drug discovery process as being inherently difficult to find compounds that are effective and present variety in mechanism of action which is important for combating resistance but in turn requires large libraries to be screened (155).

Crowther et al. also recognized the need for large screens for parasitic nematodes like *Brugia malayi*, the causative agent of lymphatic filariasis (156). Lymphatic filariasis and River blindness are two related diseases that together affect over 100 million people and are associated with debilitating symptoms (156). These neglected diseases stand to benefit from broad-spectrum nematicide screens described above and/or more target specific screens. Unfortunately, after screening almost 400,000 compounds through a *C. elegans* containing workflow they identified only 2 compounds effective against *Brugia malayi* but not at particularly low concentrations, which resulted in a discussion of the druggability of protein targets (156). The paradox discussed was over the need for a quality protein target worth the expenditure of a high throughput screen, and the difficulty in determining a protein’s promise
before resources are spent. For this reason, some groups prefer to use smaller, enriched libraries of compounds.

Bilsland et al. developed an assay expressing genes for target proteins (both the worm and human counterpart) of the *Brugia malayi* parasite in yeast (157). They were then able to screen 400 compounds from the malaria box and found several selective compounds that were then tested in vitro. Several compounds killed the adult parasites while others impacted growth or motility in a significant manner. This is important since the majority of the symptoms of the disease are associated with the parasite in its adult form, there is a lack of drugs that function in this stage, and the initial screen improves the likelihood that these compounds are able to be taken up into a living organism (157).

Considering multiple filarial nematodes, Bulman et al. screened an FDA-approved library with adult worms of *Brugia spp.* (microfilariae) and *Onchocerca ochengi*, third-stage larvae (L3s) of *Onchocerca volvulus*, and the microfilariae of *Onchocerca ochengi* and *Loa loa* (47). Drug candidates were first selected by using the Worminator assay with adult female *Brugia*, which measures adult worm motility in response to drug. Compounds that inhibited motility by 75% were then screened against *O. volvulus* molting larvae and *O. ochengi* adult worms in MTT assay and motility assay. Bulman et al. found that auranofin, a gold containing compound, that was first developed to treat rheumatoid arthritis, was effective at killing adult *Brugia* and *O. ochengi* worms and in inhibiting the larval *O. volvulus* from molting from third-stage larvae to fourth-stage larvae (47). Auranofin was next tested in vitro in gerbils infected with *B. pahangi* L3 by measuring the burden of *Brugia* adult worms after treatment for 28 days at 5 mg/kg. Gold plasma levels were also measured in the gerbils to assess the circulation time of the drug. Based on previous studies, Bulman et al. hypothesized that the mechanism of auranofin inhibition might be due to auranofin targeting thioredoxin reductase and thioredoxin glutathione reductase (TGR) (47).

Stadelmann et al. evaluated the *in vitro* efficacy of the MMV malaria box, for the treatment of *Echinococcus multilocularis*, the parasitic worm that causes alveolar echinococcosis, the most deadly of helminth infections (158). The authors first utilized PGI assays, which measure the levels of enzyme phosphoglucone isomerase (PGI) release after damage, to narrow down the Malaria Box to 24 compounds that demonstrated physical damage in metacestodes (proliferative form of *Echinococcus multilocularis*). However, only seven of the twenty-four compounds were active at 1 μM (158). A dose-response assay demonstrated that only two of the seven compounds had EC50 values below 3 μM. The most active drugs from the PGI screen were also assessed in vitro for toxicity in human foreskin fibroblasts (HFF) and Reuber rat hepatoma (RH) cells. Cells were seeded in 96-well plates and 10 μM of each compound was added after 24 hours. After five days, the viability of the cells was assessed. The cascade of *in vitro* screening described led to the identification of compound MMV665807 as a potential treatment for *Echinococcus multilocularis* infections. However, the results could not be reproduced *in vivo* testing in mouse models (158).

Johnston et al. screened approximately 2,600 drugs approved for use in humans in an assay against *Wolbachia*, an endosymbiotic bacteria with filarial nematodes (42). Of the drugs screened, 69 hits were orally available, fifteen were selectively screened, and minocycline, methacycline, sparfloxacin (antibiotics), and rifapentine (anti-TB) were active in mouse models (42).

**PROTISTS AND BACTERIA**

Love et al. used a whole-cell phenotypic screening platform to test a library of 78,942 small molecules against *Cryptosporidium parvum* (41). This is a cause of diarrhea, which is associated with significant mortality in developing countries. Two libraries of compounds were used for this study namely a bioactive (10,000 compound) set assembled by the California Institute for Biomedical Research (Calibr) and the Global Health Chemical Diversity Library (GHCDL; 69,000) that was obtained from the University of Dundee Drug Discovery Unit. *In vitro* cultivation of *Cryptosporidium* has not yet been established, so *in vivo* oocyst propagation of *C. parvum* in calves was used in this study. The five most potent compounds against *C. parvum* were found to be Gö 6976 (EC50 = 2.5 nM), monensin (EC50 = 7 nM), clofazimine (EC50 = 15 nM), cyclosporine (EC50 = 48 nM), and MST-312 (EC50 = 61 nM) (41). Gö 6976 is known as a potent inhibitor of protein kinase C and of the tyrosine kinases JAK 2 and FLT3. MST-312 is also known as Telomerase Inhibitor IX and is a potent and reversible inhibitor of telomerase activity, arresting cells in the G0-G1 phase during the cell cycle. Cyclosporine is relatively old drug that is an inhibitor of the phosphatase activity of calcineurin and has well known potent immunosuppressive properties. Monensin is an ionophore antibiotic that is added to cattle feed, and finally clofazimine is an FDA-approved drug used for the treatment of leprosy that has recently also been evaluated for tuberculosis. However, only clofazimine showed a promising safety profile. Future work is still needed to establish whether clofazimine is targeting a parasitic pathway or modulating a host-mediated pathway essential to the parasite proliferation (41).

Several libraries of previously FDA-approved medications were assessed for their ability to inhibit the growth of the bacteria *Borrelia burgdorferi*, the causative agent of Lyme Disease in North America. From these studies, 150 compounds were identified and the top 20 further characterized for activity (159).

A standard phenotypic screen was performed using the Malaria box on two different protists (160). Toxoplasmosis is
a widely spread disease which can be fatal to developing fetuses and individuals with weakened immune systems (161). Entamoeba histolytica is a disease especially pronounced in sub-Saharan Africa with mortality rates near that of malaria. For toxoplasmosis, seven compounds were identified that exhibited IC₅₀ of < 3 μM and had a selectivity index of greater than 6. These compounds were compared to currently used anti-toxoplasmosis drugs, and it was determined that there was no overlap in scaffold type. This indicates the structural novelty of these compounds for this particular repurposing effort. This is beneficial for the cases in which drug-resistance has emerged. Two compounds were similarly identified for repurposing for Entamoeba histolytica (160).

**VIRUSES**

**Dengue virus**

Dengue is a vector-borne disease that causes 20,000 deaths a year and places nearly half the world’s population at risk (162). The presence of multiple serotypes is a challenge for successful anti-viral strategies as it places our own immune strategies at a disadvantage. The disease can progress to dengue hemorrhagic fever, also called severe dengue, which is most likely to occur in secondary infections and can be potentially fatal (163). A 2013 review by Lim et al. summarized some important work concerning repurposing for dengue (164) and discusses two strategies used for dengue drug therapy namely, inhibiting viral targets or inhibiting host targets. In recent work by Cruz et al., cell-based screening of 4,000 small molecules was performed on 4 different serotypes of the dengue virus in host cells resulting in 157 active compounds with 4 general structural scaffolds (162). If compounds were toxic to host cells the virus would die and this test would not distinguish between the two effects. Several scaffolds of compounds were identified as being toxic to multiple sources of the dengue virus. With the use of confocal microscopy and time-of-addition assays further insight was gained into the potential mechanism of action of broad-spectrum hits. It was determined that 2-aminothiazole was not interfering with viral entry, but was likely blocking virus assembly. This was hypothesized based on structural similarity to the more-well studied dasatinib (162).

In a high throughput screen, Yang et al., screened 60,000 compounds at a single concentration of 10 μM, and identified a potential small-molecule inhibitor of dengue virus BP13944 (α quaternary ammonium salt) (165). BP13944 inhibited replication or viral RNA synthesis in all four serotypes but not Japanese encephalitis virus (JEV) without any apparent cytotoxicity (EC₅₀=1.03 μM). A stable reporter-DENV replicon cell line was used to screen the potency of inhibitors. They also demonstrated that E66G substitution in the NS3 region causes resistance of the virus to BP13944, suggesting that the inhibitor targets NS3 protease to inhibit viral replication and RNA synthesis. Future work is needed to understand the mechanism by which BP13944 interacts with NS3 protein to inhibit DENV replication (165).

Cheung et al. conducted screening of the US drug library collection (Microsource, Discovery Systems Inc., Gaylordsville, CT, USA), using an immunofluorescence based phenotypic screening assay (166). Lanatoside C, a cardiac glycoside, inhibited DENV-2 with an IC₅₀ of 0.19 μM and low cytotoxicity (CC₅₀ of 5.48 μM). The authors suggest that the compound inhibits the early processes of DENV replication cycle. Furthermore, after testing lanatoside C against the different DENV serotypes and other positive-strand RNA viruses, they found that it effectively inhibited all serotypes of DENV, flavivirus Kunjin, alphavirus Chikungunya and Sindbis virus as well as the human enterovirus (166).

A fluorescence-based high throughput assay, that tested DENV NS3 helicase activity, was used to screen a library of 1,600 compounds obtained from the Experimental Therapeutics Centre, Singapore (167). Among the compounds, suramin, a sleeping sickness drug, was shown to inhibit DENV helicase with an IC₅₀ of 0.4 μM. The compound presented a noncompetitive mode of inhibition, with a Kᵢ of 0.75 μM, indicating that it is able to bind to the apo form of the enzyme (167).

An immunofluorescence image-based assay identified the c-Src protein kinase inhibitors, dasatinib and AZD-0530 (saracatinib), as potent blockers of DENV (serotypes 1–4) and murine flavivirus Modoc (168). The Src-family kinases were hypothesized to have important roles in regulating DENV replication, based on the selectivity profiles of the compounds that exhibited anti-DENV activity in the primary and secondary screens. In this study they also screened a collection of 120 known inhibitors of mammalian Ser/Thr and Tyr kinases (168).

**Zika virus**

The Zika virus (ZIKV) is well known as an arthropod-borne flavivirus of the family Flaviviridae. It is phylogenetically close to dengue virus and yellow fever and is transmitted by Aedes mosquitoes, (169). It usually causes a mild dengue-like illness with possibly fever, joint pains, rash, and/or swollen lymph nodes (170). The virus has been associated in several studies with rare Guillain-Barré syndrome (171) and other severe neurological disorders (172). In 2015 ZIKV emerged due to an outbreak and was a major public health threat to the Americas (173). The World Health Organization deemed the cluster of microcephaly cases that were identified as well as the incidence of Guillain-Barré and other neurological disorders as all being associated with ZIKV in Latin America and the Caribbean. The WHO classified Zika as constituting a Public Health Emergency of International Concern (174).
Several approaches to repurposing drugs for the Zika virus have been attempted in recent years to respond to the recent outbreak. Barrows et al., screened 774 FDA approved drugs for anti-Zika activity and several were selected to extend the screening to multiple types of human tissue relevant to Zika survival and transmission (45). Importantly, the authors also considered the relative risk of these identified compounds if they were to be used during pregnancy. Of the 13 candidate drugs only three fell into pregnancy category B, described as when “Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.” The three compounds in this category were daptomycin, meloquine HCl, and palonosetron HCl. Daptomycin is an anti-microbial, meloquine HCl is an anti-parasitic that disrupts autophagy, and palonosetron HCl is a 5-hydroxytryptamine-3 receptor antagonist with drug interactions with SSRIs. All other compounds identified for anti-Zika activity were in the C and D pregnancy categories, where adverse effects have been observed in either humans or animals (45).

Pascoalino et al., developed, validated and utilized an assay against the Zika virus (32). They screened the NIH Clinical Collection and found 5 structurally diverse, selective anti-Zika compounds. These compounds were lovastatin, a hypolipidemic agent; 5-fluorouracil, an anti-cancer agent, 6-azauridine, a broad spectrum antimetabolite; palonosetron, a 5-HT antagonist; and kitasamycin, a macrolide, broad spectrum antibiotic (32).

Xu et al., had observed increased caspase-3 activity in ZIka infected cells. They used this information to develop an assay and screen multiple libraries with a combined ~6,000 compounds (181). Initially, just over 100 compounds were identified as hits. Of these, emriscan, PHA-690509 and niclosamide showed significant activity without cytotoxicity. These were studied in combination and additive behavior was observed (181).

Retallack et al., performed a High-Throughput screen of 2,177 FDA-approved drugs, with an emphasis on drugs known to be safe in pregnancy (182). The screening was performed by monitoring inhibition of virus-dependent cell death in Vero or U87 cells. The authors showed that the antibiotic, azithromycin, reduces viral proliferation in glial cells, with low toxicity (182).

**Ebola virus**

The outbreak and spread of the Ebola virus (EBOV) in West Africa in 2014 highlighted the clear need for new antiviral drugs for this and other similar emerging viruses (183). A few research groups had previously undertaken high throughput screens and identified several already approved drugs with demonstrated in vitro growth inhibitory activities against the EBOV (48, 184). A study by Kouznetsova et al. used an assay that identifies compounds that block Ebola VLP (Virus-Like Particles) entry into host cells. From the 600 FDA-approved drugs that were screened 23 compounds were identified and the most potent group were microtubule inhibitors (44). The same group then identified 30 more hits from a larger library of over 2,000 compounds, and the authors went on to propose screening more from a 400,000 NCATS library (44).

In 2013, using an assay developed previously (185), Johansen et al., screened Food and Drug Administration (FDA)– and ex-US- approved drugs and molecular probes against an engineered strain and identified several compounds with activity against Ebola Zaire virus (51). Clomiphene, toremifene, tamoxifen, and raloxifene were particularly effective estrogen receptor antagonists along with diethylstilbestrol to a lesser extent. Quinestrol, equilenin, and hydroxyprogesterone caproate were ER agonists and also provided antiviral activity. Extensive work was performed with multiple cell lines to investigate selectivity (51). Two years later, this same group screened a library of 2600 US and non-US approved drugs through an in vitro high throughput screen of Ebola. Eighty compounds were considered initial hits, 30 were selected for further screening and eventually 4 top compounds were confirmed in mouse efficacy studies (50).

Edwards et al., optimized an Ebola minigenome assay and tested a library of over 2000 compounds with the transfected cells and then moved on to test 5 compounds under biosafety level 4 conditions with infectious virus (35). Mycophenolic acid and gedunin showed quite promising results with significantly reduced virus replication and low cytotoxicity (35).

In an exhaustive study performed by Anantpadma et al., 319,855 small molecules were screened at high concentration against Ebola and Marburg Virus Like Particles transduced into Hela cells (31). There were several steps to the screen which reduced final hits to 17 compounds that are active against both viruses (31).

Cheng et al., transfected human kidney cells with plasmids from multiple virus types including Marburg, EBOV, and HIV creating pseudovirions for their high throughput assay (186). They investigated the Prestwick Chemical Library and discovered 20 compounds that were effective against both Marburg and EBOV. A striking number of hits were GPCR (G-Protein Coupled Receptor) antagonists which provided additional insight to potential mechanism of action (186). Specifically, time of addition studies indicated that the inhibitors block viral entry after initial attachment and before cell fusion either by direct antagonism, indirectly via GPCR initiated cell signaling, or a combination. The compounds identified in this study were diverse in structure and target.
Histamine receptors, 5-HT (serotonin) receptors, muscarinic acetylcholine receptors, adrenergic receptors were found to block viral entry. Clozapine and trimipramine maleate salt fell into all of the above listed categories and 4 compounds were listed as having “other targets.” Benztrapine displayed broad spectrum antifilovirus activity against multiple isolates of EBOV and MARV (186).

Wang et al. performed a screen of 1280 FDA-approved drugs using EBOV (the Zaire strain of Ebola virus) GP/HIV core pseudovirus that contains firefly luciferase reporter gene in order to identify new targets for the treatment of Ebola (46). Of the 1280 compounds, 137 compounds had greater than 50% inhibition of pEBOV at 10 μM. Of the 137 compounds, 15 compounds that had less than 15% inhibition of vesicular stomatitis virus (pVSV) were identified as selective primary hits. To validate the 15 primary hits, the cytotoxicity of each compound was assessed in parallel without the pseudovirus, this was necessary to rule out compounds that inhibited FLuc expression or HIV replication. Two compounds were identified as hits including teicoplanin which is a glycopeptide antibiotic used for the treatment of gram-positive bacterial infections (EC<sub>50</sub> 2.38 μM) and toremiphene (EC<sub>50</sub> 0.38 μM). Teicoplanin was selected for further characterization, and demonstrated complete inhibition of EBOV without apparent cytotoxicity, making it an ideal candidate for the treatment of Ebola (46).

Bayesian machine learning models were developed using data sets from previous drug screening against the Ebola virus (48). This data was used to generate Bayesian models that were used to score the MicroSource Spectrum library to predict compounds that would display anti-Ebola activity. Quinacrine, pyronaridine, and tilorone were identified and their activities were successfully confirmed in vitro as having good potency (187). Most recently, tilorone was shown to have 100% efficacy in a mouse model of Ebola infection (188).

Mirza et al. considered two target proteins of the EBOV-Z Virus and virtually screened 43,013 compounds after filtering compounds from a much larger compilation of libraries including phytochemical compounds and natural products (189). The proteins for which crystal structures were available were energy minimized using CHARMM and proteins for strains of Ebola without resolved crystal structures were homology modeled and energy minimized for docking. 749 hits were considered based on their predicted ADMET properties and 13 were selected for further study. The authors performed retrospective analysis, with known in vitro knowledge from the literature and aggregation prediction to account for what would likely be false positives in an in vitro assay. To date there has not been any in vitro validation (189).

Zhao et al., used a homology model for VP24, an enzyme critical to Ebola-human interaction and used molecular dynamics to find a conformation (49). Once this was found, they screened all FDA approved drugs and 259 nucleotide/ nucleoside drugs and investigated docked poses. Multiple docking programs were compared and 15 compounds were finally selected as potentially having activity that should be tested in vitro and in vivo against Ebola (49).

Chopra et al. conducted a high throughput screen of compounds with known side effects including FDA approved drugs through almost 50,000 protein structures (190). The interpretation of the resulting drug-protein interactions led to 46 compounds that had been previously established as having activity against Ebola by previous in vitro work. 8 novel suggestions were provided by this CANDO approach and are suggested for experimental follow up although this was not performed (190).

**OTHER VIRUSES**

Madrid et al. screened 1012 FDA-approved drugs at multiple dosages against multiple biological threat agents including Ebola, the Marburg virus, the Lassa virus, and Bacillus anthracis, Francisella tularensis, and Coxiella burnetii (causative agents for Anthrax, Tularemia, and Q fever respectively) (48). Initial inhibition was compared to positive controls (bafilomycin A1 for viruses, and ciprofloxin for bacteria) and compounds that performed comparably (within two standard deviations) were selected for further intracellular screens. The hits were further narrowed by selecting compounds with broad activities against more than one bacteria or virus (48). The Ebola dataset from this study was subsequently used in a machine learning model described earlier (187).

Mudhasani et al., investigated a library of protease inhibitors against the Rift Valley Fever Virus (RVFV) (191). 839 compounds from the ChemDiv library were screened and 34 compounds resulted in over 50% inhibition with four distinct structure types. Time of addition studies were also performed to gain insight into the mechanism of action, i.e. which compounds are involved with virus entry, viral RNA transcription inhibition, or protein synthesis disruption (191).

Cruz et al., performed similar work in which they screened a library of 4,000 kinase inhibitors against the Chikungunya Virus (192). They identified 76 compounds which provided greater than 50% inhibition. Of those, only 6 of those were dose dependent and were characterized by image-based screening (192).

Karlas et al. utilized a host genome-wide loss-of-function screen to validate 156 proviral and 41 antiviral host factors (193). Sixteen of these factors were relevant to multiple viruses and represented the central biological nodes for antiviral therapeutic intervention. To validate the sixteen factors, CRISPR/Cas9 technology was used to generate cells that were deficient in these genes and the functional response was observed. Gene enrichment analysis was used to analyze the biological processes and molecular functions that are required...
for viral infection. Karlas et al. next assessed the antiviral activity of chemical compounds that target the identified proviral factors involved in chikungunya replication (193). They utilized specialized drug databases to identify 52 compounds that interact with gene products of 14 distinct chikungunya proviral genes, and 20 compounds were shown to interact with six distinct proviral factors or pathways. RNAi (RNA interference) screens showed that five out of six proviral factors were druggable. Special knockout mice were generated to study these results in vivo, and it was demonstrated that tivozanib significantly reduced chikungunya viral load in vital organs. Pimozide, a calmodulin inhibitor and TOFA, a fatty acid synthesis inhibitor also showed promising results in vivo (193).

Dyall and coworkers suggested the use of Emetine dihydrochloride hydrate, an antibacterial agent, and several other compounds to combat MERS-CoV and SARS-CoV (194). The authors began with an exclusive library of approved drugs that had shown in vitro activity tested in relevant cell lines and virus strains consisting of 290 compounds and found 66 which had efficacy against one or both of the viruses tested (194).

**MOLECULAR PROPERTY ANALYSIS**

We are not aware of others performing analysis across compounds identified by repurposing screens. Therefore we have curated the molecules derived from these various drug repurposing efforts and calculated molecular properties for the compounds. Several molecular descriptors were generated for compounds using Discovery Studio version 4.1 (Biovia, San Diego, CA) and summary statistics performed using JMP (Cary, NC). Table III and Figure S1 shows that for over 1,225 compounds the mean properties are very similar to drug-like properties summarized previously (195). In particular, we find the average MW to be 394.39 +/- 190.17 g/mol (less than 500), the number of H-bond donors to be 1.69 +/- 2.71 (less than 5), H-bond acceptors to be 5.05 +/- 3.50 (less than 10) and the average octanol-water partition coefficient to be 3.12 +/- 1.84 (less than 5). Since the majority of these compounds were identified through screening of large libraries (bioactives, FDA approved drugs, etc.) this result is not entirely surprising.

**MACHINE LEARNING MODELS**

For several NTDs datasets we have developed Bayesian models using Assay Central (196–198) with datasets published by ourselves or others, for example Chagas disease, Ebola, Zika, etc (Figure 1). Several recent publications have described Assay Central in more detail as well as made comparisons with other algorithms (196–198). The NTD models can therefore be used to score input molecules and prioritize them for testing which is ongoing in our labs and those of collaborators. This work expands upon our previous efforts for Chagas (79), Ebola (199) and other diseases such as tuberculosis (196). We believe there are considerable opportunities for drug discovery that could be achieved by mining the growing public datasets for neglected diseases.

**CONCLUSIONS**

The clear trend towards using phenotypic screens in place of target-based screens is particularly persuasive for NTDs, as well as for other bacterial, viral and fungal pathogens. For these diseases, it is certainly considered more difficult to convert a strong targeted hit into a cell permeable, non-toxic drug than it is to identify the target of a non-toxic compound with phenotypic, whole-cell activity (200). This is certainly true in the case of intracellular parasites in which the compound has to traverse one or more of the host membranes to reach its final target. This study indicates that many different research groups globally have performed considerable amounts of work to identify compounds for repurposing for neglected diseases. These efforts still do not cover all the diseases defined by the WHO (Table I). Due to space restrictions we have not covered all the diseases eligible for a priority review voucher (Table II) for which there have been repurposing efforts (e.g. tuberculosis, malaria, etc). The 1,225 compounds identified from the various studies described (Supplemental data 1) appear to have generally “drug-like” properties as one would expect (Table III).

In some cases when disease transmission is rampant and the risk begins to outweigh costs, certain government agencies and academic institutions are more likely to be granted funding to pursue treatments for these
diseases, as happened with Ebola and Zika viruses. In some instances, academics are able to partner with companies or increasingly they have access to their own large libraries of compounds that they can screen, to produce hundreds or tens of compounds with high likelihood of repurposing. Some of these groups have the tools and access to perform in vivo work and suggest clinical studies as well. Other groups perform in vitro screening and suggest follow-up with in vivo studies. Those various researchers using computational tools such as, molecular dynamics, docking, and machine learning models can provide considerable insight to specific mechanisms, perform high throughput screening very quickly, and even leverage machine

Fig. 1 Examples of Bayesian models for (a) Chagas disease, (b) Dengue virus, (c) Ebola virus, (d) L. donovani, (e) L. infantum, (f) Toxoplasma gondii, developed with Assay Central.

Fig. 2 Examples of molecules that have multiple activities against NTDs. (a) Clofazimine, (b) pyronaridine.
learning to predict novel structures. However, it is common for these scientists to conclude with suggestions for validation in vitro and in vivo. Ideally this should be performed at the same time. From our own experience of collaborations on Chagas disease we have been able to go from in silico to in vitro to in vivo in a single study (79) and then for Ebola we went from in silico to in vitro in one study (187) and in vivo efficacy study in another (188). Most groups generally illustrate that they can go from in vitro to in vivo.

Repurposing drugs for neglected diseases likely requires the successful integration of multiple fields; it also helps to be aware of promising compounds and conversely potentially difficult compounds. Occasionally we see the same compound having disparate activities. For example clofazimine as an anti-trypanosomal agent (34) and active against Cryptosporidium parvum and active against Trypanosoma brucei (41), and pyronaridine active against Chagas disease (79) and Ebola (187) (Fig. 2). Fexinidazole, which recently completed clinical trials for HAT (201), is also being considered for treatment of Chagas (202,203). Such molecules that have multiple potential activities against neglected tropical diseases may be more common than we think and could point to common targets across diseases. Further screens like those described herein will help identify such compounds and bring more treatments into the pipelines for these diseases. We hope that this review will inspire others to search for new molecules for NTDs.

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