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Setting Our Sights on Infectious Diseases

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ABSTRACT: In May 2019, the Wellcome Centre for Anti-Infectives Research (*W*CAIR) at the University of Dundee, UK, held an international conference with the aim of discussing some key questions around discovering new medicines for infectious diseases and a particular focus on diseases affecting Low and Middle Income Countries. There is an urgent need for new drugs to treat most infectious diseases. We were keen to see if there were lessons that we could learn across different disease areas and between the preclinical and clinical phases with the aim of exploring how we can improve and speed up the drug discovery, translational, and clinical development processes. We started with an introductory session on the current situation and then worked backward from clinical development to combination therapy, pharmacokinetic/pharmacodynamic (PK/PD) studies, drug discovery pathways, and new starting points and targets. This Viewpoint aims to capture some of the learnings.

The general introductory session gave the background to the diseases and the problems involved,[1] which was started by Nicholas White. There is a very serious inequity in health care across the world. Child mortality across the world is inversely correlated to health spending per capita. Infectious diseases are still a major cause of mortality and morbidity and, globally, are the second leading cause of death; respiratory and diarrheal diseases as the major contributors, followed by tuberculosis and HIV. Treatments for many of these diseases are far from satisfactory, with many drugs currently in use dating from early in the 20th century, being essentially poisons; for example, arsenic used to treat sleeping sickness and antimonials, for leishmaniasis. Successes have been seen in some areas, a primary example being HIV-AIDS, which used to be a "death sentence". There are now effective treatments that have been made available to ∼60% of those infected with HIV, greatly extending life expectancy. However, many infected individuals are unaware of their status, meaning that many infected people continue to spread the disease. In other cases, the situation is static (tuberculosis) or worsening (malaria); although distribution of insecticide treated bed nets and the use of artemisinin-based antimalarials are thought to have saved 6.8 million lives, case numbers globally are currently increasing again. For some diseases, such as hepatitis B and hepatitis C, which affect huge numbers of people, there are drugs available, but they are outside the budget of most patients and health care providers. Bacterial infections remain a huge problem, which is exacerbated by the rise of antimicrobial resistance (AMR), which has a massive negative effect on global health and the economy. AMR needs to be approached on multiple levels. What used to be considered "last resort" antibiotics are widely used in pigs and chickens, for example, so better stewardship is required. The economic model for antibacterial drug discovery is broken and needs to be changed to encourage the pharmaceutical industry to invest in the development of new treatments. Newly emerging diseases, such as SARS, "bird flu" (H5N1 with mortality rates of approximately 50%), MERS, and Ebola, and these types of outbreaks, potentially with increased transmissibility, remain major threats.

There are relatively few "players" in the discovery of new drugs for neglected diseases, as discussed by Paul Herrling. These include some PDPs (Product Development Partnerships) and NGOs (Non-Governmental Organizations) such as Medicines for Malaria Venture (MMV), Drugs for Neglected Diseases *initiative* (DND*i*), and the TB Alliance. There are several University-Academic groups and several pharmaceutical companies that have institutes dedicated to global health and have drug discovery programs, and other pharmaceutical companies are giving significant resources. The Wellcome Trust, the Bill and Melinda Gates Foundation, and the NIH also make substantial contributions to Research and Development. However, the combined resources from the public, charitable, and private sectors, amounting to around $1 billion annually (for NTDs, malaria, and TB), pale in comparison with funds allocated to the development of drugs for the developed world conditions (annual BioPharma R&D spending is around $160 billion) and do not satisfy the clinical need.

With increased investment in drug discovery for neglected diseases has come a better understanding of the respective drug discovery paths. For dengue for example, it has become clear that target-based approaches are very challenging, while phenotypic screening has been more successful. In TB (and many other diseases), the need for pathophysiologically relevant *in vitro* assays to ensure correlation between *in vitro* and *in vivo* (animal models) models and patients has been shown to be critical. For instance, routine addition of glycerol to *in vitro* anti-TB screens to increase growth rates yielded several false positive hits, due to the nonphysiological interplay between glycerol metabolism and the inhibitors.[2] In the case of malaria, phenotypic screening against parasites in human red blood cells appears to be physiologically relevant as several compounds discovered in this assay are now in clinical development; however, assays looking at different life stages (blood stage forms, liver schizonts, gametocytes, hypnozoites) are required to understand fully the clinical potential of new inhibitors. Subsequent identification of the molecular targets for some of these malaria compounds has further increased the opportunities to find compounds with suitable properties for clinical development.

In addition to the drug discovery and development process, the search for new medicines for neglected diseases should also take into account the environmental, cultural, and social environment of those affected by these diseases and their access to healthcare. Finally, the main goal of this research is new medicines not publications. Publications are important but should not be allowed to drive the agenda.

By way of example of the challenges in neglected disease clinical trials, Shyam Sundar gave an overview of ongoing work on clinical trials for visceral leishmaniasis. This disease, fatal if left untreated, predominantly affects people living in India, East

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Africa, and South America but is also endemic in several European countries. A number of drugs are available, including antimonials, amphotericin B, miltefosine, and paromomycin. However, antimony is highly toxic, as is amphotericin B, unless in the liposomal formulation, which is very expensive, and miltefosine requires a 28-day treatment course and is teratogenic. Trials of combination treatments using existing drugs are underway, but there are significant differences in the efficacy of these therapies from one region to another. Particular challenges in eliminating visceral leishmaniasis include post kala-azar dermal leishmaniasis, which can occur after the initial infection has been treated, constituting a transmission reservoir; these patients often reject the relatively toxic treatment options. In addition, HIV−visceral leishmaniasis coinfection is mutually reinforcing, difficult to treat, and common in some areas. The long-term aim is to develop new drugs and new combinations for visceral leishmaniasis.

At the conference, we also wanted to highlight the importance of public engagement and increasing awareness of neglected tropical diseases. Toward that end, artist Jen Southern described a public engagement and contemporary art project, Para-Site-Seeing, created in collaboration with Rod Dillon (entomologist/microbiologist studying sandfly transmission of *Leishmania* in Brazil[3]) realized as part of both Scotland's NEoN Digital Arts Festival [www.northeastofnorth.com] and the LifeSpace Science Art Research gallery program [lifespace.dundee.ac.uk]. The multispecies relationships of *Leishmania* can be understood on both microscopic and global scales, and the project cites *Leishmania* research within geographical, cultural, and social histories. It communicates to audiences, in an engaging and accessible way, the journey of *Leishmania*, all told from the perspective of the parasite through a series of fictional travel blogs shared on social media (using Twitter, Instagram, and YouTube). Visitors to both the gallery and the online para-site-seeing.org Web site can follow the story of the parasite in photographs, poetry, video, and installations, in the gut of the sandfly, across the world, across the lab, and in its relationship with the drugs being developed to eliminate it.

CLINICAL DEVELOPMENT AND CONNECTIVITY TO THE PRECLINICAL SITUATION

Clinical development of candidate drugs takes a long time, costs a lot of money, and has a high attrition rate. Advances that can speed up the clinical trials process and provide increased understanding, such as why drugs work or fail, will be invaluable for infectious diseases, particularly where the patient populations are in areas with limited facilities for conducting clinical trials. The regulatory agencies (Food and Drug Administration [FDA] and European Medicines Agency [EMA]) are viewed as highly amenable and progressive in their thinking but consultation should begin very early on in the process. However, innovation is sorely needed to improve the economic model and to indicate direct clinical benefits.

Clifton Barry described work on tuberculosis (TB). TB continues to be a major problem worldwide with: at least 1.5 million deaths per year; a huge proportion of the world population latently infected; rapidly rising levels of drug resistant TB; long and complex treatment regimens. Current treatments typically require 6 months of therapy. The disease is further complicated by the presence of intracellular and extracellular bacteria, some in aerobic conditions and some in anaerobic environments. There are also bacteria found in caseous granulomas, where many drugs cannot penetrate. Further, there are also slow growing forms ("persisters") that are less susceptible to drugs. The complexity of human disease means that it is challenging to find an animal model that is predictive of human disease. Indeed, several expensive clinical trials have failed in the past.

Recently, the marmoset (*Callithrix jacchus*) has been used as a model, which is naturally infected with TB and more closely replicates human disease as well as treatment outcomes. Using linezolid, for example, TB lesions have been monitored by PET/CT (positron emission tomography/computed tomography) scanning in this model. This shows that the disease is very dynamic. From this, it is possible to see that treatment does not, and may not need to, give rise to complete resolution of lesions, but it pushes the equilibrium to a level where the immune system can re-establish control. This model for understanding disease progression has now been extended to human clinical studies. The imaging studies give a much clearer understanding of disease progression than measuring the numbers of bacteria in sputum, for example. Imaging allows one to study the effects of new treatments on individual lesions. This offers the possibility of having a major impact on phase 2 clinical studies.

James McCarthy presented the many benefits of malaria Volunteer Infection Studies (VIS). In this human challenge model, healthy volunteers are injected with *Plasmodium*-infected erythrocytes, resulting in a well standardized course of parasitaemia with little or no illness. Volunteers are treated with experimental drugs on day 8 with the collection of both pharmacokinetic data and pharmacodynamic data (parasitemia levels derived using PCR). This model allows early pharmacokinetic/pharmacodynamic (PK/PD) characterization of new drugs and is ideally suited to investigate new drug combinations. As such, VIS are a game changer for malaria clinical development.

In the case of Chagas' disease, the current burden of infection is primarily the chronic, indeterminate stage. This occurs after the initial acute infection. The infected subject experiences little or no clinical symptoms, and the parasites are difficult to detect in the blood but persist in various tissues. The disease is endemic in the Americas but is also found in Spain and several countries in Europe, primarily due to population flows. Isabela Ribeiro described a number of clinical trials using benznidazole, nifurtimox, and the CYP51 inhibitors posaconazole and fosravuconazole. On the basis of trial data generated in the 1990s, benznidazole was recently (2017) approved by the FDA for use in children aged 2 to 12 years with a 60-day dosing regimen. A more recent trial with benznidazole (BENDITA: NCT03378661) suggests that a shorter regimen of benznidazole may retain efficacy, while significantly reducing adverse side effects that currently lead to frequent treatment discontinuation. Trials assessing the CYP51 inhibitors all failed to show sustained parasite clearance.[4,5] More encouragingly, a recent trial testing the sleeping sickness drug fexinidazole (DNDi-CH-FEXI-001: NCT02498782) showed high sustained parasite clearance rates but also unexpected toxicity. A follow-up trial with lower doses is now ongoing in Chagas' disease patients (FEXI12: NCT03587766). Assessment of parasite clearance in clinical trials typically involves multiple PCR samplings to detect parasite DNA in blood; however, parasite densities in blood are typically very low, and negative PCR results do not equate to cure. This presents particular challenges in the chronic stage, where reservoirs of parasites may persist in inaccessible tissues and cardiac involvement may be present prior to therapy. To increase the sensitivity of the PCR method, multiple samples are

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tested at multiple time points, but the lack of a simpler, more robust measure of cure remains a key issue to date. In the case of Chagas', on one hand, we need shorter trials, but on the other, long-term follow-up may be needed for the qualification of markers and the correlation to clinical impact.

William Hope described aspects of clinical development of antibacterials. Late-stage clinical trials for antibacterials are currently too costly, and biomarkers are often poorly predictive of clinical benefit. Antibacterial stewardship will be particularly important to reduce the widespread and profligate use of valuable drugs that could otherwise have an extended lifespan. New antibacterial drugs need to be novel and address an unmet clinical need. Carefully planned clinical trials to demonstrate efficacy and safety are a critical part of the drug discovery process. Randomized clinical trials are the typical way to proceed; however, patient recruitment is very difficult, particularly MDR/XDR patients, at least in high income countries. A noninferiority study relative to the standard of care is usually the starting point. As a consequence, relatively prevalent complicated urinary tract infections are usually used for the initial studies. Other types of infection can be more challenging, because many of these patients are very sick, already having been exposed to antibiotics and may have very different PK and PD relative to healthy volunteers.

COMBINATION THERAPY: CLINICAL AND PRECLINICAL SELECTION

For many infectious diseases, combination therapy is used or is being considered. This can be for a variety of reasons: to reduce the risk of resistance; to increase the efficacy through synergy or additive action; to tackle pathogens in multiple states and/or locations within the body; to broaden the spectrum of activity; to potentially reduce toxicity through reduced dosing. The selection of combinations can be very challenging, however, and potential drug−drug interactions must also be considered.

Didier Leroy described how combination therapies are now standard for malaria treatment, but many currently accessible combinations contain artemisinin, meaning that artemisinin failure would have a major detrimental impact on multiple therapies. As noted above, VIS may facilitate the relatively rapid testing of a number of new drug combinations against malaria. The humanized mouse model of malaria, involving SCID mice transfused with human red blood cells (*Plasmodium falciparum* does not infect mouse red blood cells), allows for testing of even greater numbers of combinations, which may be prioritized for testing based on *in silico* predictions. Between March 2017 and April 2019, 23 combinations have been studied for PK/PD relationships by the team of Dr. Inigo Angulo-Barturen (CEO of the Art of Discovery in Bilbao), who pioneered the industrialized HuSCID mouse model for malaria. This approach is successfully revealing examples of both antagonism and synergy. Notably, dosing is typically applied at levels that yield recrudescence within 60 days such that drug efficacy can be compared in a quantitative manner.

Checkerboard or isobologram analyses are typically used to assess drug−drug interactions *in vitro* and their synergistic or antagonistic impacts on pathogen viability. Bree Aldridge described that there are now new, more efficient sampling and scoring methods that allow assessment of higher-order combinations, specifically applied to the TB assay, such as DiaMOND (diagonal measurement of *n*-way drug interactions).[6] These assays are now being applied to TB cultures in stress conditions rather than in "rich media" in the hope that the outputs will be more readily translated to *in vivo* efficacy. As noted above, however, TB tissue distribution, persisters, and dosing regimen and time frame are all factors that must be considered during the progression of potential combinations.

John Pottage discussed combination treatment in HIV infection. There are currently 1.8 million new cases of HIV infection per year. Since therapy is life-long, toxicity can be cumulative, so a key goal is to reduce dosing and long-term exposure. For HIV, approximately 40 medicines have emerged during a total of 38 years of the epidemic, but a cure has been documented for only two individuals following bone marrow transplant; latent reservoirs remain a major challenge. Combinations of nucleoside analogues, protease inhibitors, and integrase inhibitors are highly effective, but the GEMINI trials (NCT00105079) indicated that two-drug combinations can be equally effective compared to three-drug combinations. The question here is whether two-drug combinations will provide sufficient mitigation against the emergence of resistance over a long period of time. Notably, clinicians favor a daily oral formulation, whereas many patients favor a monthly parenteral administration. This has led to the introduction of several long acting parenteral antiretroviral agents into clinical development.

Olena Moshynets reported on the combination of a macrolide and colistin for treatment of carbapenem-resistant infections due to *Klebsiella pneumoniae*. The rationale for this study was that azithromycin is good at preventing biofilm formation in *K. pneumoniae* and increases the effectiveness of colistin. Biofilm formation by bacterial opportunists during an infection process reduces antibiotic effectiveness.[7] Colistin is a toxic antibiotic, with relatively low efficacy, which requires very high levels of dosing. It is often dosed with other antibiotics. Some early clinical data were presented. Specifically, a combined antibacterial therapy including azithromycin, colistin, and tienam was applied in two cases of severe sepsis associated with bad prognosis caused by carbapenem-resistant *K. pneumonia*. The infection was completely eradicated in both cases. The experimental treatment was performed by Head of the Department of Surgical Treatment of Infective Endocarditis of Amosov National Institute of Cardiovascular Surgery, A. Krykunov (Moshynets and Krykunov, manuscript in preparation).

Maria Jose Lafuente reported on combination studies using *Plasmodium falciparum*. The tools available include *in vitro* isobolograms, *in vitro* PRR (parasite reduction ratio) assay, and the *in vivo* humanized mouse model. The time to recrudescence is used as the main PD end point, looking for an increase in time to recrudescence longer than that of each of the individual components.

PK/PD STUDIES AND ANIMAL MODELS

Animal models of infection can be highly valuable in optimizing PK/PD before progressing into the clinic. However, it is critical that the animal models developed should be representative of human disease.

There were two talks on cryptosporidiosis from Wes van Voorhis and Ujjini Manjunatha. The GEMS study[8] highlighted cryptosporidiosis as the second most common cause of diarrhea in children and of death in toddlers, and the MAL-ED study[9] highlighted the link to stunting and malnutrition. The only FDA-approved drug for cryptosporidiosis is nitazoxanide, but this drug provides only a 34% improvement over a placebo and is unsuitable for HIV-infected individuals or for children under 1 year old. The current Target Product Profile indicates the need

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for a therapy that is suitable for babies of less than 6 months, safe for presumptive disease (given the difficulties with current point-of-care diagnosis), inexpensive, and available in a liquid and stable formulation. A treatment for cattle is also desirable.

Mice (*Cryptosporidium parvum*), calf (*C. parvum*), and piglet (*C. hominis*) models of cryptosporidiosis are used preclinically. Two compound series were described: a series of bumped kinase inhibitors (BKIs) being developed by the University of Washington[10−12] and PI(4)K inhibitors being developed by Novartis.[13] For both of these series, efficacy in mouse models of infection is not dependent on plasma levels of compounds. In the case of the BKIs, gastrointestinal (GI) epithelial cell concentrations in the terminal ileum and cecum, where cryptosporidium predominately replicates, can be used to predict *in vivo* efficacy. BKI GI ileocecal epithelial concentrations can be modeled by GastroPlus, and these modeled levels have been confirmed in mice during a time course after administering BKIs and harvesting organs for tissue levels postmortem.[14] Interestingly, efflux by Pgps (P-glycoprotein efflux pumps) appears to reduce efficacy.[15] BKIs that are effective in mice have been shown to dramatically reduce diarrhea and parasite excretion in both calf[16,17] and piglet models[18] of established *Cryptosporidium* infection.

The *Cryptosporidium* PI(4)K inhibitors were identified from a phenotypic screen of a focused new chemical library, and *Cp*PI(4)K has been validated as a promising molecular target in both immunocompromised mouse and neonatal calf models.[13] The lead candidate displays low clearance and medium to low oral bioavailability across a range of species. Mass spectrometry imaging (MS-Imaging) is a label-free technique used to visualize the spatial distribution of compounds by their molecular masses. MS imaging in mice showed that the compound is localized in the gastrointestinal tract. Intravenous dosing of the compound is not efficacious, unlike the case of oral delivery, despite similar systemic exposure, indicating that systemic exposure alone is not sufficient for efficacy.

Soft drugs (SD) are commonly used to limit systemic exposure, thereby enhancing the therapeutic margin. An oral soft-drug approach, where systemic exposure was limited through fast metabolism after absorption, demonstrated that GI exposure is necessary and sufficient for efficacy in the immunocompromised mouse model (Manjunatha et al. unpublished results). As an alternative to the soft-drug approach, compounds with the right balance of properties (permeability, solubility) can be developed with limited systemic exposure, while retaining sufficient permeability to access parasites.

In patients, watery diarrheal symptoms and shorter GI transit time may reduce efficacy; thus, efficacy in the more clinically relevant calf model and in the controlled human infection model is important. For the soft-drug approach, it will be important to consider the therapeutic window in the target patient populations: diarrheal patients under 2 years; children with severe acute malnutrition (SAM); immunocompromised children due to HIV infection or malnutrition with reduced metabolic capacity, who are likely to have reduced metabolic activity.

PK/PD for cryptosporidiosis (at least in the mouse, calf, and piglet models of disease) is driven by gastrointestinal tract distribution rather than systemic distribution, given that the GI tract may be the sole relevant niche for the parasite for diarrhea and GI tract dysfunction, such as environmental enteric dysfunction and subsequent malnutrition and stunting. Though the primary site of *Cryptosporidium* infection is epithelial cells in the small intestine, infections outside the GI tract have been reported in humans,[19] mainly in chronic immunocompromised HIV-positive cryptosporidiosis patients. However, such reports in children are sparse, and it remains to be seen how relevant a recently reported *Cryptosporidium* respiratory infection is to intestinal cryptosporidiosis, diarrhea, and a possible persistence in the GI.[19]

For malaria, where many compounds are in the drug discovery pipeline, valid PK/PD models are essential to prioritize compounds for development. Nathalie Gobeau described how the models are established first on the basis of efficacy and PK data from the humanized SCID mouse model, and then together with the predicted human PK, predicted human doses are calculated. Compounds with the lowest predicted dose are prioritized for testing in the above-mentioned human challenge trials, which allows further tuning of the PK/PD model. It is critically important in malaria therapy to predict appropriate combination partners, and both PK and PD interaction models are used to rank potential combinations. The top combinations are then tested once again in the VIS model (see above).

In liver stage malaria (pre-erythrocytic development), Thomas Spangenberg reported that the activity on the *Plasmodium berghei* infected liver cell spheroids of a clinical compound could be correlated with the corresponding *in vivo* efficacy mouse model by comparing IC_{99} (*in vitro*) and average systemic concentration (*in vivo*) values.[20]

The cryptosporidiosis case clearly shows that systemic blood exposure is not always the relevant PK parameter and that drug exposure for the infectious organism will depend on the location of the organism in the human host. Thomas Spangenberg discussed PK/PD models in schistosomiasis. *Schistosoma mansoni* worms live in the mesenteric veins, and systemic plasma exposure of praziquantel in the *S. mansoni* mouse model is not predictive of efficacy. However, estimated portal vein drug concentrations do predict the efficacy of praziquantel, indicating that exposure prior to the first pass metabolism in the liver is critical. This was supported through the use of a cytochrome P450 metabolism inducer, which resulted in much lower systemic exposure but no reduction in efficacy of praziquantel.[21]

In some pathogens, compound access can be restricted, compounds effluxed or metabolized, affecting compound exposure within the pathogen. Understanding this is of key importance to understand PK studies. Caitlin Taylor outlined an approach using *Mycobacterium tuberculosis* spheroplasts that lack a mycomembrane and peptidoglycan. The concept is to identify potentially promising inhibitory compounds that are not normally taken up. In turn, this knowledge will inform current efforts to understand drug transport and uptake in mycobacteria with the aim of facilitating the development of more general strategies for anti-TB drug delivery. Since small changes in the structure of a compound can have a significant impact on activity, her work exploits click chemistry using azide-derivatized compounds developed by a collaborator (Dr. Mark Blaskovich, University of Queensland) to establish intracellular localization within the (myco)bacterial cell.

DRUG DISCOVERY PATHWAYS

In this session, Manu De Rycker discussed drug discovery pathways for kinetoplastid infections, Jennifer Keiser, helminth infections, Valerie Mizrahi, tuberculosis, and Lynn Silver, bacterial infections. There were common challenges among these disease areas.

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For many of these diseases, the preclinical development pathway is poorly understood and in need of further development. It is important to define the assays that are representative of human disease and can be used to triage compounds and guide the chemistry optimization. This is particularly the case where there are no current clinical treatments or the only drugs are reactive or form reactive intermediates. A particular challenge is heterogeneity in infectious organisms. This is very obvious in helminth infections where different developmental stages as larvae and adult worms might occur in infected people. Building *in vitro* assays that can assess all these stages is challenging, in particular for the adult worms, which typically need to be isolated from animals, severely limiting the throughput of any assays. Usually, more easily accessible stages are used for primary high-throughput screening, with the risk that compound activity will not translate to all disease-relevant stages.[22] Other pathogens, such as *Mycobacterium tuberculosis* and most protozoan parasites, are also present in multiple different states.

Persister organisms pose another challenge.[23] Persisters are usually defined as forms that are less susceptible to drug action, often due to some form of quiescence. Persister bacteria are well described in TB and divided into class I persisters, a small population that occurs stochastically, and class II persisters, where the microenvironment triggers the persister state in a local population. Recreating class I *in vitro* may not be possible, but under certain conditions (such as hypoxia or other conditions that induce a state of nonreplication), class II persisters can be obtained and used in phenotypic screens.[24] Recently, persister parasites were also identified *in vitro* for *Trypanosoma cruzi*, and the ability to kill these appears to be the best *in vitro* predictor of *in vivo* efficacy,[25,26] emphasizing the need to dedicate sufficient time and resources to developing appropriate *in vitro* assays.

Another issue is that, for many infectious organisms, phenotypic hits often target a small set of common targets. For example, for TB, cell wall inhibitors and Qcrb inhibitors are frequently identified,[27] whereas the ergosterol biosynthetic pathway enzyme CYP51 is a very frequent target in *T. cruzi* phenotypic screens. To contend with this, secondary assays need to be put in place to quickly identify such hits as compounds with novel modes of action are more desirable. Alternatively, primary screens can be adjusted to avoid finding hits for these promiscuous targets, especially those with a history of failure in clinical trials, such as *T. cruzi* CYP51.

Another consideration is that *in vitro* assays can never fully reproduce the *in vivo* situation and that key differences may exist between animal models and humans. In this respect, the *in vivo* microenvironment plays a key role and can trigger alternative metabolic states, provide bypass metabolites, or change expression of key targets. As such, a target that is essential for growth *in vitro* may not be required by all disease-causing stages. This brings us to the long-standing question of target versus phenotypic-based drug discovery approaches. Phenotypic hits have some key advantages, in particular that polypharmacology may drive their activity, potentially resulting in higher efficacy or lower resistance potential. Target-based programs have the key advantage of allowing rational design of inhibitors but may yield compounds with a higher likelihood of resistance, although this risk is attenuated when using such compounds in combinations. There is value in both approaches, and the choice between them will depend on many factors, including resistance generation potential of the infectious organism, the availability of disease-relevant validated targets, and the availability of appropriate cell-based assays.

Helminth infections are poorly treated and lead to short-term issues such as diarrhea, fatigue, and anemia. The long-term consequences include malabsorption of food and growth and cognitive retardation. Over 250 million people are infected with schistosomiasis. The only drug registered to treat this is praziquantel, and there is concern over resistance and hence a need for a new medicine. The parasite has a complex life cycle, and it is not suitable for high throughput screening. A screening cascade has been established, starting with the schistosomula, which has the highest throughput. Actives are then screened against the juvenile and adult worms,[22] which are much lower throughput assays. However, there is good interlaboratory confirmation of activity. Several screens have been conducted using the malaria or pathogen box from MMV, for example.[28] In addition, there are several interesting starting points from various sources; e.g., lead compounds investigated several decades ago at Hoffmann La-Roche are currently being studied.[29] There are also a number of species of soil-transmitted helminths (STH) that cause human disease: principally *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Necator americanus* and *Ancylostoma duodenale*). It is estimated that 1.5 billion people are infected with one or more STH, and only limited treatment options lacking a broad spectrum of efficacy are available.[30] Similar screening cascades have been developed for these pathogens. As is the case with schistosomes, these are low throughput. Unfortunately, there is very limited drug discovery ongoing against helminths, despite the immense clinical need.

Antibacterial drug discovery is proving very challenging, and the growing problem of antimicrobial resistance (AMR) is well documented. Despite the rise in antimicrobial resistance, the commercial drivers are low and most large pharmaceutical companies have pulled out of antibacterial drug discovery. As in other areas of anti-infectives research, both target-based and phenotypic approaches have been used. Target-based approaches have a poor track record for antibacterial drug discovery, for several reasons. Compounds either are not taken up by bacteria or are effluxed before they can exert their effect. Second, use of a single target has a high resistance potential. The qualifications for a target are as follows:

- It must be essential for survival of the bacterium both *in vitro* and *in vivo*.
- It must be possible to achieve selectivity compared to the human homologue, if any.
- It must have a useful antibacterial spectrum of activity.
- The target must be druggable.
- There must be a very low frequency of resistance, and there must not be existing resistant mutants. Ideally, targets should be selected where multiple mutations are required for resistance.

Single target antibacterials will almost certainly need to be combined with other agents as a fixed dose or standard combination. This brings challenges, such as matching PK between the individual agents, and it can be difficult to demonstrate clinical benefit to the regulators. There are limited models for testing resistance development; hollow fiber infection models (HFIM) are useful but lack *in vivo* factors, while high inoculum animal models are very difficult to achieve. Another approach is to prepare compounds that target more than one enzyme; however, this is very challenging from a design

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perspective and is further complicated by issues such as selectivity compared to human homologues.

Another approach to finding chemical starting points is phenotypic screening. However, this tends to have a low hit rate, probably due to the challenges associated with uptake and efflux. It also appears that chemical libraries screened are not optimal for the chemical space required for compounds active against Gram-negative antibacterials.[31,32]

The entry of compounds into bacteria is potentially complex and not well understood. The cell envelope contains lipopolysaccharides, which are hydrated and repel hydrophobic molecules. Penetration across the cell membrane is more typical of diffusion across lipid bilayers, requiring a degree of lipophilicity, which is contradictory to the properties for passage through the porins. Therefore, diffusion is very limited. In some cases, active transport is found across the plasma membrane. A Trojan horse strategy has also been used, piggybacking onto a molecule for active or facilitated transport. Further efforts should focus on understanding antibacterial drug delivery routes. Compound efflux is another issue, which is poorly understood and affects many compounds that penetrate the bacterial envelope, impacting compound levels within bacteria. Furthermore, there is a potential for metabolism of compounds by bacteria, and this also needs to be investigated.

Other strategies are being investigated, for example, externally exposed bacterial targets. Host driven strategies could be another fruitful area for further development.

TB in particular is a complicated disease, with the bacterium found in multiple states and with different tissue distributions.[33] There is a need for new drugs that can tackle problems such as drug resistance and dramatically shorten the current treatment regimens, which is 6 months, even for drug sensitive TB. There are many similarities with drug discovery for Gram-negative bacterial infections. Target-based approaches are problematic due to challenges associated with getting compounds into bacteria, efflux, and in the case of TB, metabolism of the compounds. As with Gram-negative bacteria (and most infectious diseases), there are very few highly validated drug targets. In contrast to Gram-negative bacteria, phenotypic screening has identified a number of different compound series; however, target deconvolution is complicated.[34]

NEW START POINTS AND TARGETS

In the case of infectious diseases, there are very few robustly validated drug targets. For many of these diseases, it is also challenging to identify quality chemical starting points. This is exacerbated in some disease areas where millions of compounds have been screened phenotypically, and accessing chemical matter for screening that occupies a differentiated chemical space is becoming increasingly challenging.

Susan Wyllie talked about the work of the Mode of Action (MoA) group in Dundee, who employ multiple orthogonal techniques to determine the molecular targets of compounds that are phenotypically active against kinetoplastid parasites. Targets identified in the course of these studies can be considered as high-value drug targets, since they are associated with a compound known to kill the parasite. Knowledge of the molecular target of phenotypically active compounds can facilitate the drug discovery process:

- It opens up the possibility of target (structure)-based projects against better validated targets, derisking medicinal chemistry optimization of compounds.
- It allows identification of any human homologues that can be used to optimize selectivity.
- It can be used to circumvent pharmacokinetic or toxicological issues using techniques such as scaffold hopping.
- If multiple series work through the same molecular target, it can assist in portfolio management by providing information that allows the series to be prioritized.
- It can be used to deprioritize compound series that work through less attractive mechanisms, allowing resource reassignment.
- It can assist in the rational selection of compounds for combination therapy.

The MoA group uses high-throughput genetics, cellular biology approaches, and proteomics approaches to determine the molecular targets of compounds. Their experience demonstrates that no one technique or methodology is sufficient to determine the mechanism of action of every compound or series; thus the use of multiple, orthogonal, and unbiased approaches is crucial.

Jacquin Niles has been developing new genetic tools for target identification in malaria. Chemical (recombinant) genetics approaches are proving very powerful for drug target deconvolution in *Plasmodium falciparum*, despite challenges associated with low transfection efficiency and with high genomic AT bias. An engineered translational switch system has been used to toggle gene expression up or down. The system can be used to screen for essentiality and also to assist in target deconvolution for phenotypic screening hits. tRNA synthetase inhibitors provide a proof of concept for a synthetic hypersensitivity approach, and further compounds have been confirmed as on-target with scaling and multiplexing currently underway.

Natural products remain one of the best sources of anti-infective drug leads. They contain an enormous chemical diversity and often exhibit new mechanisms of action.[35,36] A key challenge is identifying novel natural product scaffolds that are likely to have a therapeutic effect and obtain those in adequate amounts for further development. Jennifer Herrmann discussed how Myxobacteria are a rich source of novel natural products, which have diverse biological activities.[37] Modern bioinformatic and genomic approaches contribute to finding and modifying these new natural products. The pipeline at Helmholtz Institute for Pharmaceutical Research Saarland includes screening, compound isolation and structure elucidation, mode of action, resistance and biosynthesis studies, scale-up, and targeted modification of interesting candidates. Promising natural compound classes from Myxobacteria include antibacterial Cystobactamids exhibiting broad-spectrum activity against all major multidrug resistant pathogens,[38,39] antifilarial Corallopyronins depleting *Wolbachia* endosymbionts of nematodes through targeting RNA polymerase,[40,41] and antimalarial Chlorotonils exhibiting activity against all erythrocytic stages of *Plasmodium falciparum* including stages responsible for transmission.[42] The application of multidisciplinary methodologies supports and accelerates the preclinical development of natural products in various ways. To name a few examples, resistance genes and related mechanisms can be identified using biosynthetic gene cluster analysis, assisting mode of action studies.[43,44] Semisynthesis is a good route to diversify and optimize the potency and pharmaceutical properties of molecules. Heterologous expression systems can significantly

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increase product titers to improve natural product supply for further investigations.[45]

A group at Novartis Institute for Tropical Diseases has been working on a program for human African trypanosomiasis, as outlined by Srinivasa Rao. Their starting point was a phenotypic screen of >2 million compounds, which gave rise to about 1000 hits that targeted multiple trypanosomatids. These were further prioritized through a series of assays. Tractable scaffolds were identified through clustering and identifying clusters with a large variation in potency (∼3 log units). Further triaging used disease relevant assays. These included: cidality assays, looking at the concentration of compound and time to kill; a wash-off assay to predict reversibility; early assessment in animal models of infection; the use of physicochemical properties to optimize brain penetration. They also found that early identification of the mode of action was important to ensure compounds remain "on-target".

Fred Buckner from the University of Washington in Seattle contrasted target-based and phenotypic approaches. Analysis from Swinney and Anthony[46] indicates that the majority of first in class new medicines are discovered through a phenotypic approach, while the follow-on compounds are more often discovered through target-based programs. Starting with the Target Product Profile is key; in the case of human African trypanosomiasis (HAT), this includes blood brain penetration. They triaged compounds from a screen of >700 000 compounds against the bloodstream-form of *T. brucei*. Triaging included the rule-of-five, as well as avoiding singletons. Exemplars from the scaffolds identified were investigated for CNS penetration, leaving nine series. Following hit expansion, three series were further progressed. Compound series were terminated due to flat SAR, pharmacokinetic issues, and static rather than cidal mechanisms of action. This was contrasted with a program targeting methionyl tRNA synthetase that had the distinct advantage of being structurally enabled, facilitating preparation of highly potent and selective compounds. A major challenge is the CNS penetration.

A presentation from Richard Amewu at the University of Ghana highlighted the issues of coinfections that are often found in patients, a particular issue in malaria/TB. He discussed approaches for the design of compounds that target both pathogens.

An analysis of current drugs, by Paul Leeson, revealed some important information about what constitutes a good drug molecule.[47] Over the last 70 years, the average molecular weight of oral drugs has been increasing. This is in contrast to the clogP, which has remained reasonably constant (∼2.5), although it has risen slightly over the past few years, possibly due to the development of inhibitors of protein−protein interactions. It is also instructive to see how the properties of molecules change as the dose in increased.[48] It is generally reported that metabolism and toxicity problems are likely to increase as the MW increases above 400 Da and the clogP above 4. Therefore, it is important to see how safety margins are maintained as the dose increases. With smaller doses (<300 mg), clogP is about 3.0; average MW is about 360 Da, and about 50% of compounds are basic, while 35% are neutral and 10% are acidic. However, for high dose compounds (>1000 mg), the average clogP is ∼1; the MW is about 250 Da, and 45% of compounds are neutral, while 31% are acidic and 16% are basic.

Optimum physicochemical properties vary from target to target, due to differences in the size of the binding site, the interactions available, and also access to the target in phenotypic and pathogen screens. There is also a "practice" effect, since there are large differences between companies, when comparing the properties of compounds that are acting on the same target. This may be due to the culture within the company and how hits are selected and optimized.[49]

For oral drugs, the ligand efficiency (LE) and lipophilic ligand efficiency (LLE) values show wide variability. However, it is important to note that the LE and LLE values tend to be greater for drugs than the mean value of published inhibitors for the target.[50,51] This is true even for drugs and candidate drugs discovered using phenotypic screens such as antimalarials and for those in the "beyond rule-of-five" space. In general, where there is a low dose of compounds, the improvement of LE and LLE over the target mean is even higher.

John Overington discussed a number of issues around drug discovery that have been analyzed using a chemoinformatic approach. It is important to understand the molecular targets of drugs and compounds in the pipeline. Looking at antibacterial drug discovery, there are still relatively few different mechanisms targeted.[52] While some drugs target enzymes that are present in the bacteria but absent in the human host, actually, it is possible to obtain very good levels of selectivity between bacterial enzymes and their human orthologues, and we need to be careful not to discard potentially good targets just because there is a human orthologue. While overall the physicochemical properties of antibacterial compounds are different from those of human drugs, there is also an influence of the target; so, drugs that target the ribosome often have very different physicochemical properties from those that target a typical cytoplasmic enzyme.[53]

There are a number of different approaches that can be used for developing drug combinations. From the target perspective, there can be two different active sites on the same target, two different enzymes in the same pathway, or two enzymes in different pathways. From the compound perspective, there can be single drugs targeting single targets; single drugs targeting multiple targets; multiple drugs targeting the same target; multiple drugs targeting multiple targets.

There are a number of ways that "combinations" can be used to tackle resistance of a particular target. For example, in switched sequential therapy, there is a change to a drug that is active against the mutant protein. In "blended therapy", drugs that are competitive for the same active site but are active against different mutants are dosed simultaneously.

Finally by mining data from different types of assays (biochemical, cell-based, functional, animal models, DMPK, and human clinical data), we should be able to improve the decision making process for when to progress a compound to further assays and what are the appropriate assays.

SUMMARY

Many common themes ran through the presentations. In short, infectious diseases represent a huge unmet medical need, which has a much more significant effect in Low and Middle Income Countries. There is a need for new medicines, and the current investment is insufficient. Furthermore, many of the patients are malnourished and many have multiple diseases, for example, TB and HIV coinfection. Many are also children. This complicates the clinical development and clinical trials and places a high bar on safety and efficacy.

There is a need for smarter clinical trials to give an earlier read out of likely efficacy, ideally before being taken to some quite challenging environments currently used for clinical trials.

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Approaches include using imaging technologies and human challenge models. There is also a need for biomarkers to assist in clinical trials for diseases such as Chagas' disease. Patient recruitment is not to be underestimated, for example, in the case of bacterial infections. In many cases, the majority of the patients are infants and children, and this needs to be factored into the clinical development of compounds.

Combination therapy is already standard in many disease areas, and in other areas, it is likely to become more important. Combination therapy can have multiple facets: improvement of efficacy, for example, to achieve a single dose cure; broadening the spectrum of efficacy as for the soil transmitted helminths; reducing the risk of resistance; dealing with pathogens with widespread tissue distribution and population heterogeneity. The selection of combination partners is very challenging. Ideally, this requires matched PK and that there are no clinically significant adverse drug−drug interactions. It is also important to make sure that the modes of action of the individual compounds are compatible (for example, not significantly antagonistic and, where there is population heterogeneity, that modes of action cover all forms of the pathogen).

Understanding the PK/PD is critical and can help to understand what is required for a drug. Therefore, the establishment of animal models of infection that are relevant to human disease is very important. Knowledge of where the pathogens are located within the body determines the required distribution of the compound. Various imaging technologies, including mass spectroscopy imaging, are under development, which can address these issues.

For many of these diseases, drug discovery pathways have not been established. Therefore, the determination of disease relevant assays and screening cascades is critical. It is important to develop assays to understand issues such as the susceptibility of persisters or slow growing pathogens to compounds and compound uptake and efflux from pathogens. The speed of kill of the pathogens is also an important factor in many cases. The development of resistance is also a major problem. It is not always clear what is the correlation between the development of resistance in the laboratory setting and that in the clinic and also the fitness cost of individual resistance mechanisms.

Recently, a lot of work has gone into determining drug mode of action, particularly for antikinetoplastid compounds and drugs, and this is having a major impact on decision making and prioritization in the drug discovery process. Attention also needs to be placed on understanding the physicochemical properties of the compounds that are drugs from the outset.

Clearly, there are a lot of common challenges in drug discovery for the diseases covered in this conference, and a better understanding and know-how in certain diseases (e.g., combination treatment in TB and malaria, mode of action studies in kinetoplastids, and persisters in TB and malaria) are invaluable to help build development paths for other, less well understood diseases. Thus, this conference clearly demonstrated the value of looking across disease areas to find parallels, new approaches, and new opportunities.

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REFERENCES

(1) De Rycker, M., Baragana, B., Duce, S. L., and Gilbert, I. H. (2018) Challenges and recent progress in drug discovery for tropical diseases. *Nature 559*, 498−506.

(2) Manjunatha, U. H., and Smith, P. W. (2015) Perspective: Challenges and opportunities in TB drug discovery from phenotypic screening. *Bioorg. Med. Chem. 23*, 5087−5097.

(3) Sant'Anna, M. R., Diaz-Albiter, H., Aguiar-Martins, K., Al Salem, W. S., Cavalcante, R. R., Dillon, V. M., Bates, P. A., Genta, F. A., and Dillon, R. J. (2014) Colonisation resistance in the sand fly gut: Leishmania protects Lutzomyia longipalpis from bacterial infection. *Parasites Vectors 7*, 329.

(4) Molina, I., Gomez i Prat, J., Salvador, F., Trevino, B., Sulleiro, E., Serre, N., Pou, D., Roure, S., Cabezos, J., Valerio, L., Blanco-Grau, A., Sanchez-Montalva, A., Vidal, X., and Pahissa, A. (2014) Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. *N. Engl. J. Med. 370*, 1899−1908.

(5) Torrico, F., Gascon, J., Ortiz, L., Alonso-Vega, C., Pinazo, M. J., Schijman, A., Almeida, I. C., Alves, F., Strub-Wourgaft, N., Ribeiro, I., et al. (2018) Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: a proof-of-concept, randomised, placebo-controlled trial. *Lancet Infect. Dis. 18*, 419−430.

(6) Cokol, M., Kuru, N., Bicak, E., Larkins-Ford, J., and Aldridge, B. B. (2017) Efficient measurement and factorization of high-order drug interactions in Mycobacterium tuberculosis. *Sci. Adv. 3*, No. e1701881.

DOI: 10.1021/acsinfecdis.9b00371
ACS Infect. Dis. XXXX, XXX, XXX−XXX

(7) Moshynets, O. V., and Spiers, A. J. (2016) Viewing biofilms within the larger context of bacterial aggregations. In *Biofilms*, pp 3−22, InTech Press.
(8) Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faruque, A. S. G., Zaidi, A. K. M., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., Kanungo, S., Ochieng, J. B., Omore, R., Oundo, J. O., Hossain, A., Das, S. K., Ahmed, S., Qureshi, S., Quadri, F., Adegbola, R. A., Antonio, M., Hossain, M. J., Akinsola, A., Mandomando, I., Nhampossa, T., Acácio, S., Biswas, K., O'Reilly, C. E., Mintz, E. D., Berkeley, L. Y., Muhsen, K., Sommerfelt, H., Robins-Browne, R. M., and Levine, M. M. (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet 382*, 209−222.
(9) Acosta, A. M., Chavez, C. B., Flores, J. T., Olotegui, M. P., Pinedo, S. R., Trigoso, D. R., Vasquez, A. O., Ahmed, I., Alam, D., Ali, A., Bhutta, Z. A., Qureshi, S., Shakoor, S., Soofi, S., Turab, A., Yousafzai, A. K., Zaidi, A. K. M., Bodhidatta, L., Mason, C. J., Babji, S., Bose, A., John, S., Kang, G., Kurien, B., Muliyil, J., Raghava, M. V., Ramachandran, A., Rose, A., Pan, W., Ambikapathi, R., Carreon, D., Charu, V., Dabo, L., Doan, V., Graham, J., Hoest, C., Knobler, S., Lang, D., McCormick, B., McGrath, M., Miller, M., Mohale, A., Nayyar, G., Psaki, S., Rasmussen, Z., Richard, S., Seidman, J., Wang, V., Blank, R., Gottlieb, M., Tountas, K., Amour, C., Mduma, E., Ahmed, T., Ahmed, A. M. S., Dinesh, M., Tofail, F., Haque, R., Hossain, I., Islam, M., Mahfuz, M., Chandyo, R. K., Shrestha, P. S., Shrestha, R., Ulak, M., Black, R., Caulfield, L., Checkley, W., Chen, P., Kosek, M., Lee, G., Yori, P. P., Murray-Kolb, L., Schaefer, B., Pendergast, L., Abreu, C., Binda, A., Costa, H., Di Moura, A., Filho, J. Q., Leite, A., Lima, A., Lima, N., Lima, I., Maciel, B., Moraes, M., Mota, F., Oria, R., Quetz, J., Soares, A., Svensen, E., Tor, S., Patil, C., Bessong, P., Mahopo, C., Mapula, A., Nesamvuni, C., Nyathi, E., Samie, A., Barrett, L., Gratz, J., Guerrant, R., Houpt, E., Olmsted, L., Petri, W., Platts-Mills, J., Scharf, R., Shrestha, B., and Shrestha, S. K. (2014) The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. *Clin. Infect. Dis. 59* (Suppl 4), S193−S206.
(10) Van Voorhis, W. C., Doggett, J. S., Parsons, M., Hulverson, M. A., Choi, R., Arnold, S. L. M., Riggs, M. W., Hemphill, A., Howe, D. K., Mealey, R. H., Lau, A. O. T., Merritt, E. A., Maly, D. J., Fan, E., and Ojo, K. K. (2017) Extended-spectrum antiprotozoal bumped kinase inhibitors: A review. *Exp. Parasitol. 180*, 71−83.
(11) Hulverson, M. A., Choi, R., Arnold, S. L. M., Schaefer, D. A., Hemphill, A., McCloskey, M. C., Betzer, D. P., Muller, J., Vidadala, R. S. R., Whitman, G. R., Rivas, K. L., Barrett, L. K., Hackman, R. C., Love, M. S., McNamara, C. W., Shaughnessy, T. K., Kondratiuk, A., Kurnick, M., Banfor, P. N., Lynch, J. J., Freiberg, G. M., Kempf, D. J., Maly, D. J., Riggs, M. W., Ojo, K. K., and Van Voorhis, W. C. (2017) Advances in bumped kinase inhibitors for human and animal therapy for cryptosporidiosis. *Int. J. Parasitol. 47*, 753−763.
(12) Huang, W., Hulverson, M. A., Choi, R., Arnold, S. L. M., Zhang, Z., McCloskey, M. C., Whitman, G. R., Hackman, R. C., Rivas, K. L., Barrett, L. K., Ojo, K. K., Van Voorhis, W. C., and Fan, E. (2019) Development of 5-Aminopyrazole-4-carboxamide-based Bumped-Kinase Inhibitors for Cryptosporidiosis Therapy. *J. Med. Chem. 62*, 3135−3146.
(13) Manjunatha, U. H., Vinayak, S., Zambriski, J. A., Chao, A. T., Sy, T., Noble, C. G., Bonamy, G. M. C., Kondreddi, R. R., Zou, B., Gedeck, P., Brooks, C. F., Herbert, G. T., Sateriale, A., Tandel, J., Noh, S., Lakshminarayana, S. B., Lim, S. H., Goodman, L. B., Bodenreider, C., Feng, G., Zhang, L., Blasco, F., Wagner, J., Leong, F. J., Striepen, B., and Diagana, T. T. (2017) A Cryptosporidium PI(4)K inhibitor is a drug candidate for cryptosporidiosis. *Nature 546*, 376−380.
(14) Arnold, S. L. M., Choi, R., Hulverson, M. A., Schaefer, D. A., Vinayak, S., Vidadala, R. S. R., McCloskey, M. C., Whitman, G. R., Huang, W., Barrett, L. K., Ojo, K. K., Fan, E., Maly, D. J., Riggs, M. W., Striepen, B., and Van Voorhis, W. C. (2017) Necessity of Bumped Kinase Inhibitor Gastrointestinal Exposure in Treating Cryptosporidium Infection. *J. Infect. Dis. 216*, 55−63.
(15) Arnold, S. L. M., Choi, R., Hulverson, M. A., Whitman, G. R., McCloskey, M. C., Dorr, C. S., Vidadala, R. S. R., Khatod, M., Morada, M., Barrett, L. K., Maly, D. J., Yarlett, N., and Van Voorhis, W. C. (2019) P-glycoprotein mediated efflux reduces the in vivo efficacy of a therapeutic targeting the gastrointestinal parasite Cryptosporidium. *J. Infect. Dis. 220*, 1188.
(16) Schaefer, D. A., Betzer, D. P., Smith, K. D., Millman, Z. G., Michalski, H. C., Menchaca, S. E., Zambriski, J. A., Ojo, K. K., Hulverson, M. A., Arnold, S. L., Rivas, K. L., Vidadala, R. S., Huang, W., Barrett, L. K., Maly, D. J., Fan, E., Van Voorhis, W. C., and Riggs, M. W. (2016) Novel Bumped Kinase Inhibitors Are Safe and Effective Therapeutics in the Calf Clinical Model for Cryptosporidiosis. *J. Infect. Dis. 214*, 1856−1864.
(17) Hulverson, M. A., Vinayak, S., Choi, R., Schaefer, D. A., Castellanos-Gonzalez, A., Vidadala, R. S. R., Brooks, C. F., Herbert, G. T., Betzer, D. P., Whitman, G. R., Sparks, H. N., Arnold, S. L. M., Rivas, K. L., Barrett, L. K., White, A. C., Jr., Maly, D. J., Riggs, M. W., Striepen, B., Van Voorhis, W. C., and Ojo, K. K. (2017) Bumped-Kinase Inhibitors for Cryptosporidiosis Therapy. *J. Infect. Dis. 215*, 1275−1284.
(18) Lee, S., Ginese, M., Beamer, G., Danz, H. R., Girouard, D. J., Chapman-Bonofiglio, S. P., Lee, M., Hulverson, M. A., Choi, R., Whitman, G. R., Ojo, K. K., Arnold, S. L. M., Van Voorhis, W. C., and Tzipori, S. (2018) Therapeutic Efficacy of Bumped Kinase Inhibitor 1369 in a Pig Model of Acute Diarrhea Caused by Cryptosporidium hominis. *Antimicrob. Agents Chemother. 62*, e00147-18.
(19) Mor, S. M., Ascolillo, L. R., Nakato, R., Ndeezi, G., Tumwine, J. K., Okwera, A., Sponseller, J. K., Tzipori, S., and Griffiths, J. K. (2018) Expectoration of Cryptosporidium Parasites in Sputum of Human Immunodeficiency Virus-Positive and -Negative Adults. *Am. J. Trop. Med. Hyg. 98*, 1086−1090.
(20) Arez, F., Rebelo, S., Fontinha, D., Simão, D., Martins, T., Machado, M., Fischli, C., Oeuvray, C., Badolo, L., Carrondo, M., Rottmann, M., Spangenberg, T., Brito, C., Greco, B., Prudêncio, M., and Alves, P. M. (2019) Flexible 3D cell-based platforms for the discovery and profiling of novel drugs targeting *Plasmodium* hepatic infection. *ACS Infect. Dis.*, in press, DOI: 10.1021/acsinfecdis.9b00144
(21) Abla, N., Keiser, J., Vargas, M., Reimers, N., Haas, H., and Spangenberg, T. (2017) Evaluation of the pharmacokinetic-pharmacodynamic relationship of praziquantel in the Schistosoma mansoni mouse model. *PLoS Neglected Trop. Dis. 11*, No. e0005942.
(22) Lombardo, F. C., Pasche, V., Panic, G., Endriss, Y., and Keiser, J. (2019) Life cycle maintenance and drug-sensitivity assays for early drug discovery in Schistosoma mansoni. *Nat. Protoc. 14*, 461−481.
(23) Balaban, N. Q., Helaine, S., Lewis, K., Ackermann, M., Aldridge, B., Andersson, D. I., Brynildsen, M. P., Bumann, D., Camilli, A., Collins, J. J., Dehio, C., Fortune, S., Ghigo, J. M., Hardt, W. D., Harms, A., Heinemann, M., Hung, D. T., Jenal, U., Levin, B. R., Michiels, J., Storz, G., Tan, M. W., Tenson, T., Van Melderen, L., and Zinkernagel, A. (2019) Definitions and guidelines for research on antibiotic persistence. *Nat. Rev. Microbiol. 17*, 441−448.
(24) Gold, B., and Nathan, C. (2017) Targeting Phenotypically Tolerant Mycobacterium tuberculosis. *Microbiol. Spectrum 5*, TBTB2-0031-2016.
(25) Sanchez-Valdez, F. J., Padilla, A., Wang, W., Orr, D., and Tarleton, R. L. (2018) Spontaneous dormancy protects Trypanosoma cruzi during extended drug exposure. *eLife 7*, No. e34039.
(26) MacLean, L. M., Thomas, J., Lewis, M. D., Cotillo, I., Gray, D. W., and De Rycker, M. (2018) Development of Trypanosoma cruzi in vitro assays to identify compounds suitable for progression in Chagas' disease drug discovery. *PLoS Neglected Trop. Dis. 12*, No. e0006612.
(27) Goldman, R. C. (2013) Why are membrane targets discovered by phenotypic screens and genome sequencing in Mycobacterium tuberculosis? *Tuberculosis (Oxford, U. K.) 93*, 569−88.
(28) Pasche, V., Laleu, B., and Keiser, J. (2019) Early Antischistosomal Leads Identified from in Vitro and in Vivo Screening of the

DOI: 10.1021/acsinfecdis.9b00371
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Medicines for Malaria Venture Pathogen Box. *ACS Infect. Dis. 5*, 102−110.
(29) Keiser, J., Panic, G., Vargas, M., Wang, C., Dong, Y., Gautam, N., and Vennerstrom, J. L. (2015) Aryl hydantoin Ro 13−3978, a broad-spectrum antischistosomal. *J. Antimicrob. Chemother. 70*, 1788−1797.
(30) Moser, W., Schindler, C., and Keiser, J. (2017) Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis. *BMJ. 358*, j4307.
(31) Brown, D. G., May-Dracka, T. L., Gagnon, M. M., and Tommasi, R. (2014) Trends and exceptions of physical properties on antibacterial activity for Gram-positive and Gram-negative pathogens. *J. Med. Chem. 57*, 10144−10161.
(32) Tommasi, R., Brown, D. G., Walkup, G. K., Manchester, J. I., and Miller, A. A. (2015) ESKAPEing the labyrinth of antibacterial discovery. *Nat. Rev. Drug Discovery 14*, 529−542.
(33) Dartois, V. (2014) The path of anti-tuberculosis drugs: from blood to lesions to mycobacterial cells. *Nat. Rev. Microbiol. 12*, 159−167.
(34) Evans, J. C., and Mizrahi, V. (2018) Priming the tuberculosis drug pipeline: new antimycobacterial targets and agents. *Curr. Opin. Microbiol. 45*, 39−46.
(35) Newman, D. J., and Cragg, G. M. (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod. 75*, 311−335.
(36) Shen, B. (2015) A New Golden Age of Natural Products Drug Discovery. *Cell 163*, 1297−300.
(37) Herrmann, J., Fayad, A. A., and Muller, R. (2017) Natural products from myxobacteria: novel metabolites and bioactivities. *Nat. Prod. Rep. 34*, 135−160.
(38) Huttel, S., Testolin, G., Herrmann, J., Planke, T., Gille, F., Moreno, M., Stadler, M., Bronstrup, M., Kirschning, A., and Muller, R. (2017) Discovery and Total Synthesis of Natural Cystobactamid Derivatives with Superior Activity against Gram-Negative Pathogens. *Angew. Chem., Int. Ed. 56*, 12760−12764.
(39) Baumann, S., Herrmann, J., Raju, R., Steinmetz, H., Mohr, K. I., Huttel, S., Harmrolfs, K., Stadler, M., and Muller, R. (2014) Cystobactamids: myxobacterial topoisomerase inhibitors exhibiting potent antibacterial activity. *Angew. Chem., Int. Ed. 53*, 14605−14609.
(40) Schaberle, T. F., Schiefer, A., Schmitz, A., Konig, G. M., Hoerauf, A., and Pfarr, K. (2014) Corallopyronin A - a promising antibiotic for treatment of filariasis. *Int. J. Med. Microbiol. 304*, 72−78.
(41) Srivastava, A., Talaue, M., Liu, S., Degen, D., Ebright, R. Y., Sineva, E., Chakraborty, A., Druzhinin, S. Y., Chatterjee, S., Mukhopadhyay, J., Ebright, Y. W., Zozula, A., Shen, J., Sengupta, S., Niedfeldt, R. R., Xin, C., Kaneko, T., Irschik, H., Jansen, R., Donadio, S., Connell, N., and Ebright, R. H. (2011) New target for inhibition of bacterial RNA polymerase: 'switch region'. *Curr. Opin. Microbiol. 14*, 532−543.
(42) Held, J., Gebru, T., Kalesse, M., Jansen, R., Gerth, K., Muller, R., and Mordmuller, B. (2014) Antimalarial activity of the myxobacterial macrolide chlorotonil a. *Antimicrob. Agents Chemother. 58*, 6378−6384.
(43) Fu, C., Sikandar, A., Donner, J., Zaburannyi, N., Herrmann, J., Reck, M., Wagner-Dobler, I., Koehnke, J., and Muller, R. (2017) The natural product carolacton inhibits folate-dependent C1 metabolism by targeting FolD/MTHFD. *Nat. Commun. 8*, 1529.
(44) Kling, A., Lukat, P., Almeida, D. V., Bauer, A., Fontaine, E., Sordello, S., Zaburannyi, N., Herrmann, J., Wenzel, S. C., Konig, C., Ammerman, N. C., Barrio, M. B., Borchers, K., Bordon-Pallier, F., Bronstrup, M., Courtemanche, G., Gerlitz, M., Geslin, M., Hammann, P., Heinz, D. W., Hoffmann, H., Klieber, S., Kohlmann, M., Kurz, M., Lair, C., Matter, H., Nuermberger, E., Tyagi, S., Fraisse, L., Grosset, J. H., Lagrange, S., and Muller, R. (2015) Antibiotics. Targeting DnaN for tuberculosis therapy using novel griselimycins. *Science 348*, 1106−1112.
(45) Sucipto, H., Pogorevc, D., Luxenburger, E., Wenzel, S. C., and Muller, R. (2017) Heterologous production of myxobacterial alpha-pyrone antibiotics in *Myxococcus xanthus*. *Metab. Eng. 44*, 160−170.
(46) Swinney, D. C., and Anthony, J. (2011) How were new medicines discovered? *Nat. Rev. Drug Discovery 10*, 507−519.
(47) Leeson, P. D. (2016) Molecular inflation, attrition and the rule of five. *Adv. Drug Delivery Rev. 101*, 22−33.
(48) Leeson, P. D. (2018) Impact of Physicochemical Properties on Dose and Hepatotoxicity of Oral Drugs. *Chem. Res. Toxicol. 31*, 494−505.
(49) Leeson, P. D., and St-Gallay, S. A. (2011) The influence of the 'organizational factor' on compound quality in drug discovery. *Nat. Rev. Drug Discovery 10*, 749−765.
(50) Hopkins, A. L., Keseru, G. M., Leeson, P. D., Rees, D. C., and Reynolds, C. H. (2014) The role of ligand efficiency metrics in drug discovery. *Nat. Rev. Drug Discovery 13*, 105−21.
(51) Young, R. J., and Leeson, P. D. (2018) Mapping the Efficiency and Physicochemical Trajectories of Successful Optimizations. *J. Med. Chem. 61*, 6421−6467.
(52) Santos, R., Ursu, O., Gaulton, A., Bento, A. P., Donadi, R. S., Bologa, C. G., Karlsson, A., Al-Lazikani, B., Hersey, A., Oprea, T. I., and Overington, J. P. (2017) A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discovery 16*, 19−34.
(53) Mugumbate, G., and Overington, J. P. (2015) The relationship between target-class and the physicochemical properties of antibacterial drugs. *Bioorg. Med. Chem. 23*, 5218−5224.

DOI: 10.1021/acsinfecdis.9b00371
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