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Drug Discovery Strategies for Neglected Tropical Diseases: Repurposing Knowledge, Mechanisms and Therapeutics to Increase Discovery Efficiency

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1.1 Introduction

Neglected tropical diseases (NTDs) are a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries. These diseases include infections by bacteria, protozoans, helminths, and viruses. Analyses have estimated that NTDs affect more than one billion people and cost developing economies billions of dollars every year [1, 2]. Populations living in poverty, without adequate sanitation, and in close contact with infectious vectors and domestic animals and livestock are those worst affected. We refer readers to the websites of the WHO and CDC for more specifics on the individual diseases [3, 4].

Six of the infections caused by NTDs (dracunculiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths, and trachoma) can be controlled or even eliminated through mass administration of safe and effective medicines (mass drug administration, MDA), or other, effective interventions. Along with therapeutic interventions, efforts to control the vectors (e.g. mosquitoes, black flies) that transmit these diseases and to improve basic water, sanitation, and hygiene are highly effective strategies against these NTDs [4].

There are still many NTDs that cannot be controlled, due to their mechanism of transmission, or their presence in zoonotic reservoirs, among other reasons. Thus, there is a need for new affordable, effective therapeutics in addition to the plans to control the disease vectors and improve basic water, sanitation, and hygiene.

1.2 First-line Therapies for NTDs and Mechanisms of Action

Most medicines currently used to treat NTDs were discovered many decades ago, despite having limitations (Table 1.1). For example, suramin used for the treatment of human African trypanosomiasis (HAT) was discovered almost 100 years

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Table 1.1 First-line therapies for NTDs and how they were discovered.

First-line	Diseases	Mechanism	Year
Albendazole	Ascariasis, hookworm, echinococcosis, lymphatic filariasis	Tubulin inhibitor	1987
Ivermectin	Lymphatic filariasis, onchocerciasis	Ion channel	1981
Praziquantel	Schistosomiasis, foodborne trematodiasis, Taencasis/cysticerosis	Membrane disruption	1982
Benznidazole	Trichuriasis, chagas	Free radical toxicity	1966
Nifurtimox	Chagas, HAT	Oxidative stress	1970
Pentamidine	HAT	Cross-link DNA	1937
Suramin	HAT	Disrupt energy metabolism	1920
Melarsoprol	HAT	Trypanothione and pyruvate kinase inhibition	1949
Eflornithine	HAT	Ornithine decarboxylase inhibitor	1990
Amphotericin B	Leishmaniasis	Membrane disruption	1953
Miltefosine	Leishmaniasis	Membrane disruption	2002
Rifampicin	Buruli ulcer, leprosy	RNA polymerase	1971
Streptomycin	Buruli ulcer	Protein synthesis inhibition	1943
Dapsone	Leprosy	Dihydropteroate synthase inhibitor	1937
Clofazimine	Leprosy	DNA chelator	1969
Azithromycin	Trachoma, YAWS	Protein synthesis inhibition	1988
Triclabendazole	Foodborne trematodiasis, fascioliasis	Tubulin inhibitor	1989
Niclosamide	Taencasis/cysticerosis	Disrupt energy metabolism	1960s

ago and is still used, albeit a number of newer medicines are now available [5, 6]. Strikingly, most NTD medicines were discovered prior to the 1990s, when molecular biology, molecular genetics, and associated technologies became central to medicine and drug discovery.

The mechanisms of action of these medicines involve disruption of processes essential to an organism's survival. These actions include disruption of microtubules (albendazole, triclabendazole) [7], ion flux (ivermectin) [8], oxidative stress (benznidazole, nifurtimox) [6, 9], disruption of energy production (suramin, niclosamide) [10], inhibition of protein synthesis (streptomycin and azithromycin) [11], inhibition of RNA synthesis (rifampicin) [12], disruption

of membrane integrity (praziquantel [13, 14], amphotericin B [15], miltefosine [16, 17], clofazimine [18]), and inhibition of production of essential metabolites (eflornithine, dapsone) [5, 19].

Most of these functions are not unique to the infectious agents. Selectivity over human homologs is required to achieve a useful safety profile. Differences in binding affinity between the microbe and human homologs provide the selectivity for some (albendazole, ivermectin), but not all, of the medicines. Perhaps, most interesting is that for some of the therapeutics, selectivity is thought to be achieved by the existence of compensatory mechanisms in humans. Greater free radical quenching in human cells versus parasite contribute the selectivity for benznidazole and nifurtimox [15]. Alternative uptake mechanisms for folic acid in hosts contribute to safety of dapsone [19]. Other exploitable differences include compound disposition (e.g. high-affinity uptake systems in trypanosomes by pentamidine) [6], and composition of membranes, which is a key selectivity feature for the function of amphotericin B [15].

1.3 Drug Discovery Efficiency

Drug discovery is an endeavor with very high attrition rates [20]. The high attrition rates are particularly detrimental for drug discovery for NTDs, owing to the disproportionately low research investment in this activity. As such, processes need to be employed to reduce the risk of attrition. Two important aspects relevant to medicinal chemistry are the strategies that provide therapeutic candidates and the critical components to identification and optimization of candidates with a greater chance of success. Drug discovery strategies are first addressed, followed by a discussion of the critical components of the drug discovery process and opportunities for repurposing.

1.3.1 Drug Discovery Process

The process of drug discovery and development is an iterative learn-and-confirm cycle addressing an unmet medical need (Figure 1.1) [21]. The process can be thought of as four stages that require different expertise and tools to define and test the therapeutic hypothesis.

1. Basic research creates new knowledge and **understanding of disease** that leads to tools created for discovery. This phase is most often accomplished in academia and government agencies. Some of the tools important to discovery that are created from basic research include models of disease, clinical relevant biomarkers, predictive phenotypic markers for use in screening assays, as well as potential mechanisms of intervention and drug targets.
2. The aim of the discovery/invention phase is to identify a potential therapeutic and its corresponding mechanism of action to be tested in patients. The strategies used for discovery, including assay formats and endpoints, are informed by the knowledge and tools created in basic research (discussed in Section 1.3.2). The invention phase has historically been the domain of the

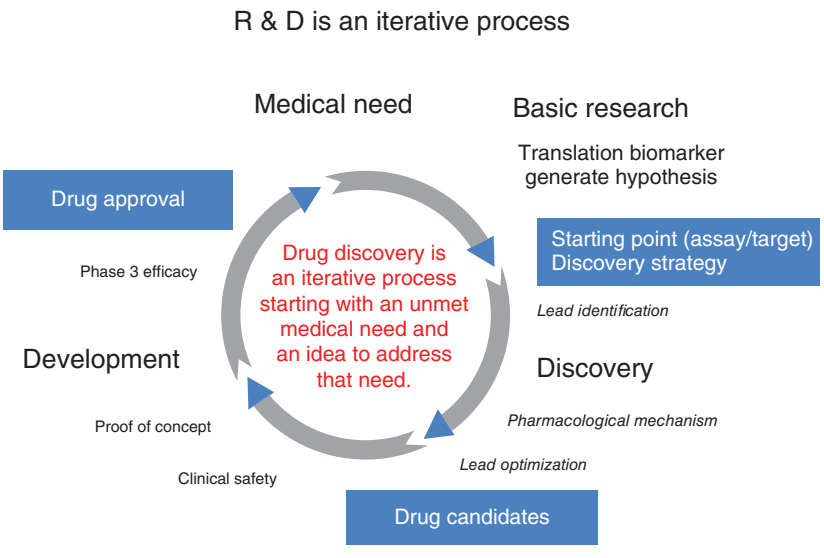

Figure 1.1 Drug discovery and development cycle. The process of drug discovery can be thought of as an iterative learn-and-confirm cycle with specific milestones. The process of discovery and development of a new medicine is initiated in response to an unmet medical need to treat a disease. Physiological, genetic, and chemical knowledge provides an understanding of the disease. This knowledge will lead to the identification of translation biomarkers and assays to enable discovery and invention of new medicines. These molecules will then be optimized for biopharmaceutic properties and safety to provide a drug candidate. At this point, the process of drug discovery is complete and the molecule should succeed or fail based on its own merit. Opportunities to improve efficiency in drug discovery will increase the probability that clinical candidates will make it to registration. The left-hand side of the circle (from 6 to 12 o'clock) is the development phase of drug discovery, which involves testing for safety and efficacy in humans leading to registration. Multiple iterations are generally required before a medicine with sufficient efficacy at a safe dose is discovered, tested in humans, and registered.

pharmaceutical and biotech industries, although academic institutions are now frequently inventing new medicines. The invention is typically identified by evaluation of potential drugs in biological assays that measure a response related to the clinical outcome. The modalities evaluated can be of organic chemical, biological, and genetic material prepared synthetically or isolated from natural substances (e.g. natural products). The modalities for NTDs are all chemical in nature. Part of the reason for this is that the cost and stabilities of biological and genetic therapeutics are prohibitive for NTDs.

The active modality and its corresponding mechanism of action provide the **therapeutic hypothesis** that will be tested in patients. For NTDs, the therapeutic hypothesis will be that the molecule will kill the infectious organism and reduce morbidity and/or mortality. The mechanism may not be known until long after the drug is approved, or it may be never known. For example, the mechanism of action of acetaminophen is still not known. The mechanisms of action of most drugs for NTDs were determined long after the drugs were invented.

3. In order to test the therapeutic hypothesis in the clinic, the active modality must be tolerable and have suitable drug-like properties including pharmacokinetics and pharmaceutical to provide sufficient drug concentrations to achieve the response. The **optimization** phase can be facilitated by knowledge of the mechanism of action, but this knowledge is not mandatory. The optimization phase is considered the "Valley of Death" due to the high attrition rate. It is resource-intensive and typically conducted in the pharmaceutical industry, although there are now academic and government centers conducting this work. The optimization phase produces a clinical candidate that can then be used to test the therapeutic hypothesis in the clinic.
4. The central feature of the therapeutic hypothesis is predicting a dose–response relationship between mechanism of action and efficacy (or toxicity) in humans [22]. Clinical studies are designed to test a specific molecule for its therapeutic usefulness.

Multiple iterations of learn-and-confirm hypothesis testing are usually required to identify first-in-class medicines. This long-term investment is not feasible for NTDs; drug discovery for these diseases must be more successful, with fewer iterations and fewer failures.

1.3.2 Drug Discovery Strategies

The knowledge available from basic research will inform the drug discovery strategy. Important aspects of the knowledge that impact the drug discovery strategies are knowledge of mechanisms of action and targets, availability of robust phenotypic assays, and structures of active compounds [23, 24].

Medicinal chemistry-dependent drug discovery strategies are commonly differentiated into empirical strategies now known as phenotypic drug discovery (PDD) and hypothesis-driven strategies now commonly described as target-based drug discovery (TDD) [25].

Phenotypic assays measure a phenotype in a physiological system. The term "phenotypic assay" includes all preclinical assay formats that use physiological systems, e.g. animals, cells, and biochemical pathways [24, 26]. Phenotypic assays make few assumptions as to the molecular details of how the system works, provide an empirical method to probe effects in physiological systems, and are mechanistic agnostic. Therapeutics are identified by the effect upon a phenotype and, subsequently, the therapeutics are used to identify the mechanism of action. The identification of active therapeutics is accomplished through empirical trial and error, verifiable by observation rather than by theory. The therapeutics are identified in which disease-relevant phenotypes provide a chain of translation between the observation and clinical response [27, 28]. The phenotype most relevant to NTD is reduction in proliferation and death of the organism.

Empirical, phenotypic assays have always played an important role in drug discovery for NTDs [29, 30]. In his Nobel lecture entitled "Selective inhibitors of dihydrofolate reductase," George H Hitchings Jr. stated, "Those early, untargeted studies led to the development of useful drugs for a wide variety of diseases and has justified our belief that this approach to drug discovery is more fruitful than narrow targeting" [29].

In the last decades of the twentieth century, the emphasis of drug discovery changed to a more reductionist, target-based approach, and phenotypic assays were primarily used to confirm efficacy and evaluate safety. The drug target is a gene product that provides a mechanistic hypothesis to focus discovery research to identify a therapeutic that modulates the protein's activity [31]. A target can be validated with many technologies, including genetics [22]. Molecular technologies such as X-ray structure and computational chemistry are tools that help medicinal chemists in the rational design and optimization of molecules that bind to the target [32]. The central features of TDD are (i) identification and validation of a drug target, (ii) identification of a molecule that binds to that target, (iii) optimization of the selectivity over anti-targets, and (iv) optimization of the biopharmaceutic properties such that the drug concentrations in the body are sufficient to ensure that the drug is bound to the target throughout the dosing interval. This target-based paradigm has been envisioned to provide a more rational approach to drug discovery, analogous to a design and engineering approach [23, 32].

Most medicines for NTDs were discovered decades ago using empirical strategies (PDD) involving testing the ability of compounds to kill the infectious organisms (Table 1.1), essentially agnostic to the mechanism of action. Some of the key components of PDD success are the robustness of the assays and the composition of the screening libraries, both of which are addressed in more detail later [27].

Not all NTDs were discovered via phenotypic screening. Eflornithine was discovered on the basis of the hypothesis that an ornithine decarboxylase (ODC) inhibitor would be efficacious for HAT. Eflornithine is an irreversible inhibitor originally developed for cancer and repurposed for HAT. It was not efficacious for cancer due to the fast resynthesis of the ODC enzyme. Differential activity in the parasite was achieved due to much slower enzyme resynthesis in the trypanosome parasite [5].

1.3.3 PDD versus TDD for NTDs

As already noted, historically, PDD has provided most of the medicines for NTDs. A likely contributor to this success is the feasibility of assays measuring viability of parasites, worms, and bacteria, termed the chain of translatability [27]. The translatability of the microbe viability as a phenotypic measure of infectious disease pathology is very strong. This contrasts with the more uncertain translatability that modulation of a new target will provide selective cytotoxicity.

In general, the choice between a phenotypic (PDD) versus target-based strategy (TDD) for medicinal chemistry-dependent, first-in-class drug discovery is strongly influenced by the robustness, feasibility, and translatability that a phenotype will predict clinical efficacy (its chain of translatability) versus the predictability that a drug target and corresponding molecular mechanism will provide efficacy and selectivity. Molecular mechanisms of small molecules interacting with a target to provide sufficient efficacy and safety are more complex than simple binding. They involve conformational changes, kinetics, and are dependent on physiological context. This was the conclusion of an analysis of first-in-class medicines across all disease areas showing that the majority

of medicinal chemistry-driven medicines were discovered with phenotypic screening [25]. The molecular mechanisms are very difficult to predict and incorporate into reductionist assay formats [33, 34]. It was also noted that TDD was more successful for followers, presumably because the mechanism of action had already been validated [25].

An aspect of discovery strategies for NTDs that is rarely appreciated is that the selectivity of drugs was identified in many cases as a consequence of the empirical nature of the strategy. Differences in binding affinity between the microbe and human target determined the selectivity for some but not all of the medicines. As noted earlier, the selectivity is thought to be achieved by other mechanisms including compensatory mechanisms in humans (e.g. greater free radical quenching [6], alternative uptake mechanism for folic acid [19]), compound disposition (high-affinity uptake systems in parasites) [6], and composition of membranes (amphotericin B) [15].

1.4 Critical Components for Successful Drug Discovery

1.4.1 Finding a Starting Point

Identification of suitable chemical matter for optimization is paramount. In tropical medicine drug discovery, both phenotypic and target-based screens have been applied to a number of small-molecule chemical libraries, including FDA drug libraries [35] and natural products [36], as well as collections arising from industry [37] or product development partnerships such as the MMV, which has released the Malaria Box [38] and Pathogen Box [39], each of which contains 400 Lipinski-compliant chemistries with validated antiparasitic activities. In addition, repurposing of established drugs or preclinical chemotypes that inhibit homologous function in other eukaryotic systems can be a fruitful approach.

1.4.2 Assays Robustness and Hit Selection Criteria

As with drug discovery programs for any other indication, it is essential that screening assays are sufficiently robust and reproducible, and of reasonable throughput, to drive chemical optimization. Assays must have sufficient sensitivity to reproducibly identify modifications that affect a compound's activity, and it would be highly desirable to utilize orthogonal assays that measure the same biological endpoint as the primary assay but utilize a different readout. This can help avoid false-positive results that arise due to assay artefacts.

When selecting and defining a compound hit, different disease indications will have different requirements, overall. However, all programs share the same essential criteria: (i) sufficient potency against the target or pathogen, with some indication of a potential selectivity window. (ii) A hit compound is preferably a member of a series of structurally similar compounds that display differences in activity across 2–3 orders of magnitude. (iii) An assessment of compound ADME properties; while such properties are typically measured, computed

properties can also provide useful insights. (iv) A hit series would contain at least several compounds that meet at least some of the desired criteria measured or computed earlier. This will provide high confidence that the chemical series will be a tractable substrate for medicinal chemistry optimization.

It is essential that all hit compounds are assessed against other metrics of tractability. For example, the employment of a Pan-Assay Interfering compound (PAINs) assessment can identify potentially promiscuous chemotypes that, while appearing to be strong optimization starting points, are artefactual findings. Similarly, any active compounds identified in a screening campaign should be carefully assessed for features that are generally undesirable in a hit compound. These would include highly electrophilic moieties (alkyl halides, aldehydes), hydrolysable features (such as esters or acetals), or any other sort of chemically unstable moiety. Lastly, substructure searches using freely available databases such as PubChem or ChEMBL can often uncover potentially promiscuous or toxic moieties to help inform compound series selection.

It is highly desirable to pursue a chemotype that is readily pursued by organic synthesis (often described as "parallel-enabled"). In particular, the ability to easily and rapidly prepare analogs simultaneously is a major benefit to the speed of an optimization program, and it also allows exploration of a diverse chemical space. While many drugs do indeed trace their roots back to natural products [25], challenges in chemical synthesis of natural product analogs can frequently frustrate analog synthesis while searching for new compounds with appropriate properties.

1.4.3 Optimization Processes

Any successful chemical drug discovery program has, at its center, a well-informed medicinal chemistry effort. Noting that target product profiles for new drugs for many NTDs have been described [40–42], optimization programs must design and employ a series of assays that ensure direction toward the desired endpoint. Rather than an exclusive focus on antiparasitic potency and selectivity, it is critical to include considerations of absorption, distribution, metabolism, and excretion (ADME) properties, pharmacokinetics and pharmacodynamics, and selectivity against important anti-targets, such as hERG.

A project team should design an assay cascade that is fit-to-purpose, both in terms of measuring desired endpoints, as well as in maximizing efficient use of resource (which is frequently limited in NTD drug discovery). An example assay cascade is shown in Figure 1.2, which would lead to a compound that is <100 nM in potency, >100× selective over host cells, with adequate solubility and ADME properties and animal pharmacokinetic exposure, that can be tested in an *in vivo* efficacy experiment. Note that transition to each step of the cascade has defined property cutoffs, in terms of potency and properties. Depending on the goals on a given project, this diagram could be modified to include aspects such as screens in a panel of anti-targets (ion channels, G protein-coupled receptors (GPCRs), kinases, etc.), hERG, or other endpoints that are central to optimization.

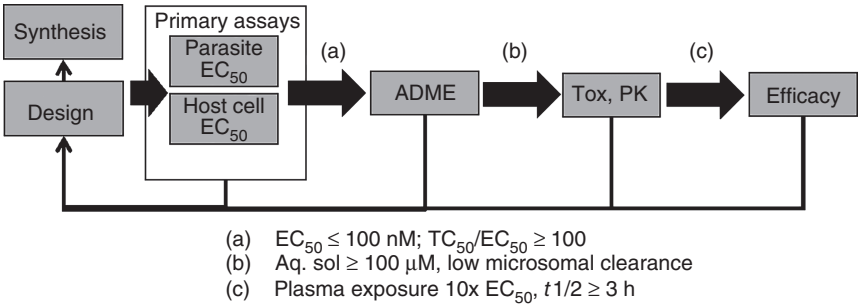

Figure 1.2 Example project optimization cascade.

1.5 Repurposing Knowledge Mechanisms and Therapeutics

The process of *de novo* drug discovery can be too resource expensive for NTDs. Opportunities to address this deficiency come from repurposing molecules and mechanisms. Repurposing is not a new concept for NTDs. Many of the currently used medicines were repurposed. For example, the benzimidazoles were originally developed as plant fungicides and later as veterinary anthelmintics [43]. The first benzimidazole to be developed and licensed for human use was thiabendazole in 1962. Although thiabendazole was very effective, it was also moderately toxic, which led to enormous efforts by animal health companies to find better and safer compounds. This led to the benzimidazole carbamates, such as mebendazole, flubendazole, oxfendazole, albendazole, and oxibendazole. Subsequently, several veterinary anthelmintics were developed and marketed, including parbendazole, fenbendazole, oxfendazole, and cambendazole. The first benzimidazole carbamate to make it into humans was mebendazole, followed by flubendazole (both Janssen products).

More recent examples of repurposing mechanisms include eflornithine. As noted earlier, eflornithine was discovered on the basis of the hypothesis that an ODC inhibitor would be efficacious for HAT [5]. Eflornithine is an irreversible inhibitor originally developed for cancer and repurposed for HAT and is one of the few therapeutics discovered with TDD.

There is growing optimism in the NTD community that more drugs will become available through repurposing. In 2018, moxidectin was approved by the U.S. FDA for onchocerciasis. Moxidectin, a macrocyclic lactone, was repurposed from animals and clinical studies showed superiority to ivermectin [44]. Fexinidazole, originally developed in the 1980s, was rediscovered in 2005 by DNDi researchers looking for possible antiparasitic compounds. In late 2018, an EMA scientific committee announced its "positive opinion" for fexinidazole, opening the way for individual countries to approve its use in HAT, with the first patients to receive the drug by mid-2019.

Repurposing, known as exaptation, has been an effective source of discovery and invention across many industries. The most obvious and exploited approach for NTDs is to identify molecules that have been developed for another disease

or industry (agriculture, animal health) and then test those against the specific NTDs. This practice is ongoing and the major source of leads for the NTDs, as noted in the chapters on specific NTDs in this book. This is often pursued using a phenotypic approach. A significant bottleneck for this approach is acquiring the funding and infrastructure to test the new hypothesis in the clinic. Organizations such as DND*i*, BMGF, and Welcome Trust provide mechanisms to evaluate promising compounds. However, as noted earlier, the limited resources only allow for the most promising candidates to be evaluated. Sources of candidates for repurposing include selective cytotoxic agents from other infectious diseases including malaria, TB, antibiotics, and HIV. The anticancer pharmacopeia can also provide a source of compounds. Indeed, many compound libraries have been already been evaluated against NTDs, and it is important to follow the criteria described in Section 1.4 to ensure that the actives have sufficient properties to warrant further investment.

In many cases, these evaluations are mechanistically agnostic. A collection of compounds is screened for viability against the pathogenic microbe. The increasing availability of genomic and mechanistic knowledge and bioinformatic and computational biology tools provide opportunities to focus the screening around specific mechanisms and target classes. For example, two species may have homologous essential enzymes such as a MAP kinase or a protease. Therefore, a compound library identified in one species can be used to identify leads in another to provide a starting point for medicinal chemistry optimization.

1.6 Summary

In summary, the expansion of NTD drug discovery, and the progress made to date are encouraging. New programs in this area are bolstered by sophisticated assay technologies, deep understanding of the infectious agent's biology, modern, metric-driven medicinal chemistry campaigns, and excellent disease models in animals. However, because of the resource cost of drug discovery and lack of available resources for NTDs, repurposing compounds and mechanisms provides the best opportunities for new medicines.

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