

OPINION

Hit and lead criteria in drug discovery for infectious diseases of the developing world

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Abstract | Reducing the burden of infectious diseases that affect people in the developing world requires sustained collaborative drug discovery efforts. The quality of the chemical starting points for such projects is a key factor in improving the likelihood of clinical success, and so it is important to set clear go/no-go criteria for the progression of hit and lead compounds. With this in mind, the Japanese Global Health Innovative Technology (GHIT) Fund convened with experts from the Medicines for Malaria Venture, the Drugs for Neglected Diseases *initiative* and the TB Alliance, together with representatives from the Bill & Melinda Gates Foundation, to set disease-specific criteria for hits and leads for malaria, tuberculosis, visceral leishmaniasis and Chagas disease. Here, we present the agreed criteria and discuss the underlying rationale.

There is an urgent need for new and more-effective drugs to treat the various diseases that take the heaviest toll on the developing world¹. This can only be achieved in a cost-effective manner by implementing robust and efficient processes to develop and deliver drugs that are safe, effective, affordable and available to those who need them most.

The quality of chemical starting points (known as 'hits') for drug discovery projects is a key factor for improving the likelihood of success of clinical candidates; starting a discovery project with poor-quality hits ultimately results in increased attrition of these compounds^{2,3}. The decisions to progress a hit into 'lead' identification (the HTL phase) and then on into lead optimization are crucial, as the downstream optimization phase may take years and require considerable financial investment. Setting the bar high by applying comprehensive, well-considered criteria for entry into lead optimization will improve overall success rates and focus resources on chemical series that stand a reasonable

chance of delivering a quality preclinical candidate⁴. The need to focus resources effectively is particularly important in drug research and development (R&D) for infectious diseases that affect people in the developing world, given the limited market incentives for R&D investment and the key role that philanthropic funding and public-private partnerships have in this field.

The Japanese Global Health Innovative Technology (GHIT) Fund (BOX 1) is a pioneering public-private partnership. It provides resources for research organizations in Japan to partner with international research groups and product development partnerships (PDPs) to screen and identify new hits and develop them into novel lead series for infectious diseases that affect people in the developing world. The initial areas of focus for the GHIT Fund are malaria (in partnership with the Medicines for Malaria Venture (MMV)), tuberculosis (TB; in partnership with the TB Alliance), Chagas disease and visceral leishmaniasis (in partnership with the Drugs for Neglected Diseases *initiative* (DNDi)).

Specific criteria have been proposed for defining hits and leads in the development of drugs for diseases such as malaria⁴⁻⁶; however, it is essential that such criteria are regularly reviewed and updated as part of evolving target product profiles (TPPs) to reflect accumulated experience from drug discovery projects and emerging scientific research, clinical experience, policy guidance and patient need. In order to reach a consensus on the HTL criteria to be used to guide its collaborative activities, the GHIT Fund recently convened an initial gathering of its key international partners involved in drug discovery — the MMV, the TB Alliance and the DNDi — together with representatives from the Bill & Melinda Gates Foundation. The objective of this meeting was to take the first steps towards creating a shared, flexible strategy to expedite the discovery of the next generation of drugs for these diseases by GHIT Fund collaborations. Here, after briefly discussing some of the general characteristics of the relevant screening assays and established target product profiles, we present the proposed generic and disease-specific criteria for hits and leads. We hope that these guidelines may also be valuable to drug discovery efforts outside these partnerships.

Hit discovery and assay development

For infectious diseases, hit candidates usually come from screens that involve intact pathogens, akin to phenotypic screens (or, more generally, high-content screens) rather than target-based screens. Which of these approaches is more productive is still being debated^{7,8}. Despite being fuelled by advances in genomics, target-based high-throughput screening, along with computer-assisted modelling, has not been as productive as phenotypic screening in the antibacterial area⁹. However, for subsequent optimization efforts there is no doubt that knowing the molecular target of a hit series is a major advantage. For instance, such knowledge allowed the clinical antimalarial candidate DSM265 to be specifically optimized to inhibit *Plasmodium falciparum* dihydroorotate dehydrogenase rather than its human orthologue¹⁰. Additionally, knowing the molecular target enables target-specific liabilities to be identified earlier in the R&D process. Furthermore, from a

Box 1 | The Global Health Innovative Technology Fund: unlocking Japanese innovation

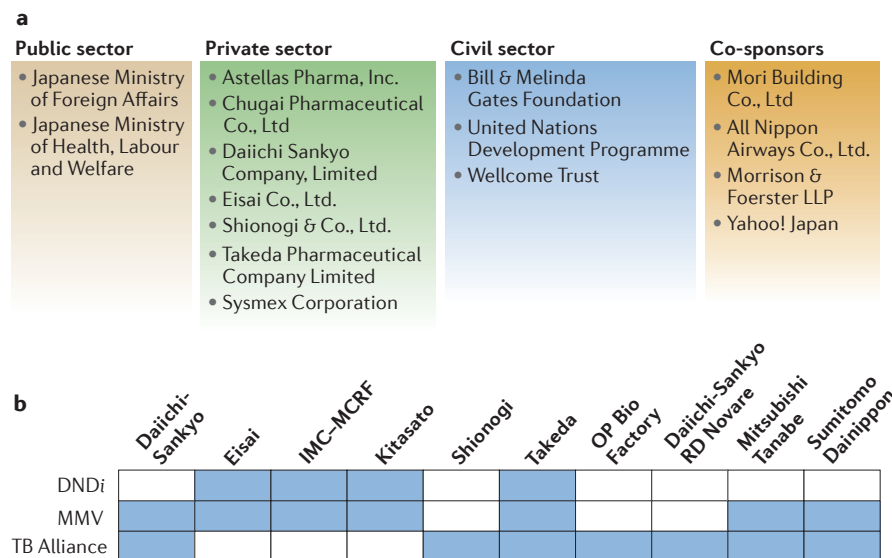
The Global Health Innovative Technology (GHIT) Fund of Japan was founded in April 2013 with a vision to alleviate the burden of infectious disease that prevents billions of people from seeking the level of prosperity and longevity commonly expected in industrialized nations. Uniquely, the GHIT Fund aims to do this by facilitating international partnerships that enable Japanese technology, innovations and insights to have a more direct role in making this vision a reality^{67,68}. Along with six Japanese pharmaceutical companies and one diagnostic company, the GHIT Fund is supported by two Japanese government ministries and numerous unexpected co-sponsors, including Mori Building Co., Ltd., All Nippon Airways Co., Ltd., Morrison & Foerster LLP, and Yahoo! Japan (see the figure, part a). The Japanese pharmaceutical industry is represented by the 69 members of the Japanese Pharmaceutical Manufacturers Association.

A key aim of the GHIT Fund is to link existing product development partnerships (PDPs) — responsible for discovering and delivering new medicines for malaria, tuberculosis (TB) and neglected tropical diseases (NTDs) — with Japanese partners that have expertise in drug research and development (R&D). This is achieved by establishing a portfolio of screening projects and a hit-to-lead (HTL) platform that use defined criteria to ensure that the most attractive chemical series are selected for further optimization and development. Research collaborations must comprise at least two distinct organizations, one of which is Japanese, in order to be eligible. For the screening and HTL platforms the compounds must originate from a Japanese entity. In addition, the HTL platform aims to maximize the skills and resources of existing global PDPs that operate in the field of NTDs and other infectious diseases that affect people in the developing world, such that each new partner must enter into a research collaboration with one of three specific PDPs (the Medicines for Malaria Venture (MMV), the Drugs for Neglected Diseases initiative (DNDi) or the TB Alliance).

Beyond the benefits of sharing expertise, collaborations of this type will allow diverse chemical libraries to be probed using various screening approaches. Importantly, Japanese compound assets of synthetic and natural origin could be a rich source of novel and chemically diverse compounds that have not previously been screened for infectious diseases of the developing world. Past examples of key natural-product-based drugs that originated from Japanese discovery efforts include ivermectin, an antiparasitic drug from the actinomycete *Streptomyces avermectinius*^{69–71}; the multiple sclerosis therapy fingolimod, which is a metabolite of the insect fungus *Isaria sinclairii*⁷²; and the founder of the statin drug class, mevastatin, which was discovered in *Penicillium* spp. extrolites by Akira Endo of Sankyo in the 1970s^{73,74}.

In just over 2 years since its inception, the GHIT Fund has facilitated more than 30 partnerships (for screening collaborations see the figure, part b) and invested more than US\$40 million in them. All proposals received are evaluated by external reviewers and a selection committee, both of which are completely independent from the GHIT Fund.

In addition to the existing three platforms (the screening, HTL and product development platforms), the GHIT Fund also initiated the 'Target Research Platform in Partnership with Grand Challenges' (TRP) for early-stage development of radically new and improved drugs, vaccines and diagnostics to prevent and treat infectious diseases that are prevalent in developing countries. With additional Japanese partnerships expected and additional compound screening and HTL proposals being submitted^{67,68}, the GHIT Fund encourages research and shows a commitment to the cause that is unprecedented in this area of research within Asia.



IMC, Institute of Microbial Chemistry; MCRF, Microbial Chemistry Research Foundation.

portfolio point of view, it ensures that a mix of mechanistic approaches are followed, which is especially relevant in diseases in which drug resistance develops and spreads quickly (for example, TB and malaria).

Fortunately, advances in genomics and related technologies usually enable the molecular target of a drug (or at least its mode of action) to be elucidated even when the compound was discovered in a phenotypic screen; such screens may therefore be viewed as discovery engines for druggable targets, along with the drug molecules themselves. The two crucial success components for screening are the availability of chemical libraries with 'interesting' chemistry and taking great care to develop assays that faithfully reproduce the microenvironment of a pathogen with a disease-relevant readout, while also maintaining throughput and robustness. Such advanced assays also eliminate compounds that do not penetrate cellular membranes or are otherwise unavailable to the target.

The organisms that cause malaria, TB and NTDs are biologically quite diverse, including viruses, bacteria and eukaryotes. Each of these comes with its own challenges and opportunities, resulting in differences in hit and lead criteria and assay conditions. Dogma holds that bacteria replicate quickly (~20-minute generation time) and eukaryotic (mammalian) cells double in number in ~24 hours, but these guidelines do not apply to the pathogens that cause the four diseases discussed in this article. *Mycobacterium tuberculosis* is an exception among bacteria in that it has up to 16-hour doubling times, whereas the doubling times of the eukaryotic *Leishmania* spp. parasites, which cause visceral leishmaniasis, are ~6–9 hours.

The *in vitro* culture of all major strains of *P. falciparum* — the species responsible for the majority of deaths from malaria — has been an important breakthrough¹¹, but its doubling time is ~48 hours¹². Owing to these differences, biomass generation and assay set-up vary in difficulty between organisms.

The potency cut-offs for compounds to progress to the next stage of development varies by disease (see below) and is decided by a number of factors. Among these factors are empirical hit rates (the higher the hit rate, the lower the cutoff concentration). *Plasmodium* spp. hit rates are generally good, which is possibly related to the unusual (and vital) apicoplast organelles that these organisms harbour. Moreover, these organisms must actively overcome the toxicity of haem degradation and remodel their host cell (red blood cell) membrane transport capabilities, and these processes are druggable. By contrast,

it is perhaps not surprising that targeting and killing the intracellular amastigote form of *Leishmania* spp. parasites is more challenging, as amastigotes reside inside acidic phagolysosomes within macrophages, which present additional membranes and pH gradients that drugs must cross before reaching their target. *Trypanosoma cruzi*, the parasite that causes Chagas disease, presents its own challenges owing to its ability to infect many cell types, producing a highly dynamic infection. Furthermore, drugs that only target the replicating stages of the parasite may leave non-replicating forms, such as trypomastigotes, capable of maintaining infections long after the end of the treatment¹³.

Established target product profiles

It is important that hit and lead series are assessed as early as possible for conformity with the relevant disease TPPs and, if appropriate, target candidate profiles (TCPs). However, in most cases, further detailed biological or pharmacokinetic studies will be required to fully judge how a series can be strategically positioned. These TPPs and TCPs are typically developed by the PDPs in discussion with the research and medical communities.

TPPs and TCPs for malaria. In the case of malaria, two TPPs and five TCPs have been established, which reflect different patient populations and medicinal uses^{6,14}. In brief, malaria TPP1 is focused on treatment, ideally from a single dose, such that all symptomatic and asymptomatic parasites (gametocytes) in a host are cleared and transmission is blocked in addition to the patient being cured. TPP2 is focused on prophylaxis, because when attempting to eliminate a disease it is recognized that people in once-endemic regions, now with potentially reduced immunity, may require protection in the event of transmission outbursts. The TPPs focus on the profile of a medicine that is composed of two or more active ingredients (a criterion that is presently mandatory for artemisinins¹⁵). By contrast, the TCPs focus on attributes that individual molecules need to possess (acknowledging that one molecule may possess more than one attribute). The key features of the five TCPs are: fast parasite clearance (TCP1); a combination partner, ideally long duration, which provides post-treatment prophylaxis (TCP2); prevention of *Plasmodium vivax* and *Plasmodium ovale* relapse (TCP3a); prevention of transmission (TCP3b); and chemoprotection (TCP4)⁶. TCP1, TCP2, TCP3a and TCP3b all support TPP1, whereas TCP4 supports TPP2 (REF. 6).

Box 2 | Generic hit selection criteria for infectious diseases

The panel of drug experts convened by the Japanese Global Health Innovative Technology (GHIT) Fund identified the following generic hit selection criteria:

- A hit should have a potency that is consistent with the potential to deliver a lead compound (see BOXES 4–6 for details of criteria defined specifically for each disease)
- The chemical structure of a hit should be confirmed by identification (for natural products), re-synthesis or re-purification
- Primary screening data should be validated on a selection of hit compounds (>90% pure)
- A hit should have an acceptable *in vitro* response (typically, a sigmoidal concentration–growth inhibition curve reaching a maximal 100% efficacy, with a Hill coefficient ideally between 0.5 and 1.8)
- Preliminary knowledge of the structure–activity relationship (SAR; often available from analogues in the original screening library) of a hit is desirable
- A hit should have a tractable chemotype: it should have no highly reactive or unstable moieties in the pharmacophore and be amenable to structural variation by chemical (or biochemical) synthesis. Hits should pass basic drug-like filters, such as pan-assay interference filters (PAINS)⁷⁵, to eliminate promiscuous hits that lack target specificity^{76,77}. Conformity to the ‘rule of five’ (REF. 58) is preferred
- There should be a greater than 10-fold selectivity window for cytotoxicity using a mammalian cell line (for example, HepG2 or Vero cells)
- A hit requires adequate selectivity in a biochemical counter-assay (for example, a homologous mammalian target) where relevant. However, most infectious disease hit-to-lead projects are not target-based screens but phenotypic
- No serious intellectual property conflicts should exist (that is, a ‘freedom to operate’ is needed). However, with the value of US Food and Drug Administration (FDA) priority review vouchers now entering the pharmacoeconomic equation, there are further possibilities to develop drugs for infectious diseases even in absence of intellectual property protection
- No major synthesis or formulation issues should be anticipated (compounds should ideally be synthesized in ≤5 steps with an acceptable yield and acceptable solubility). For reasons of affordability, this criterion is more stringent than for drug discovery in general

TPPs for TB. TPPs for TB usually require new drugs to shorten the duration of treatment, demonstrate efficacy against drug-sensitive and drug-resistant *M. tuberculosis* strains and show potential for use in drug combinations in developing countries (for example, those with oral and once-daily dosing, and low cost of goods). These goals are based on the fact that the majority of TB drugs and treatments have not changed in the past half-century, even though two new drugs (bedaquiline¹⁶ and delamanid¹⁷) were recently approved — the first new drugs for TB in the past 40 years. Additional drugs are needed to develop new treatment regimens.

TPPs for Chagas disease and leishmaniasis. The DNDi has defined a TPP for Chagas disease with ‘acceptable’ and ‘ideal’ criteria (see the [DNDi website](#)). The benchmark for clinical efficacy is benznidazole, and this should therefore be the comparator for all hit and lead candidates. Similarly, the DNDi has published (with consultancy) ‘optimal’ and ‘minimal’ TPPs for visceral and cutaneous leishmaniasis. For visceral leishmaniasis, a safe oral drug with >90% efficacy within 10 days is crucial. Relevant for early compound triage is that a drug must be active against all resistant strains.

Generic criteria for hits and leads

Hit series definition and criteria. A number of generic hit criteria, identified by a panel of drug experts convened by the GHIT Fund, apply to all four infectious diseases, and these are listed in BOX 2. In general, the objectives at this stage are to build confidence in the quality of a compound series and the associated data, understand the liabilities and, if possible, generate data that guide medicinal chemists during the HTL phase¹⁸. Two main types of criteria are covered: disease-specific criteria, focusing on potency, efficacy and pathogenicity; and compound-specific criteria, which focus principally on the chemical scope of the compound and a risk assessment of drug metabolism and pharmacokinetics (DMPK), as well as the physical properties that are predictive of an oral therapy.

Lead series definition and criteria. During the HTL phase, the project team focuses on the optimization of a chemical series so as to improve any compound properties that could be an obstacle to further progression. Depending on the profile, the HTL strategy and medicinal chemistry plan can be very different between series. The milestone for

Box 3 | Generic lead selection criteria for infectious diseases

The panel of drug experts convened by the Japanese Global Health Innovative Technology (GHIT) Fund identified the following generic lead selection criteria:

- A lead requires an acceptable *in vitro* potency for the relevant disease (see BOXES 4–6 for disease-specific details). In general, high potency (a low half-maximal inhibitory concentration (IC_{50})) is highly desirable but not at the expense of poor physicochemical properties or drug metabolism and pharmacokinetic (DMPK) characteristics
- A lead should have oral efficacy in the appropriate disease model. Oral efficacy removes one key risk early on, namely, uncertainty about *in vivo* validation of the mechanism of action of the series (which is often unknown at this stage)
- The synthetic chemistry should be amenable to series expansion, as many more compounds are likely to be needed for testing in as short a time frame as possible
- A lead needs a greater than 10-fold selectivity in killing pathogens as opposed to mammalian cells in tuberculosis (TB) and a greater than 100-fold selectivity for the pathogens that cause the other diseases (malaria, Chagas disease and visceral leishmaniasis). This reflects the increased challenge of finding potent, attractive compounds that target TB
- A lead should have acceptable physicochemical properties (typically, solubility in phosphate-buffered saline $>10\ \mu\text{M}$; a sufficient level of solubility is expected to avoid problematic formulations⁷⁸. Acceptable lipophilicity; LogP values are typically <5 and ideally <3)⁵⁸
- A lead requires manageable drug metabolism and pharmacokinetic profiles. This involves liver microsomal and hepatocyte stability across species; understanding of the plasma protein, microsomal and media binding; good membrane permeability; and no unmanageable cytochrome P450 inhibition
- A lead needs to demonstrate good oral bioavailability in rodents (demonstrated $F>25\%$); ultimately, high oral bioavailability is highly desirable as it will reduce the potential for inter-patient variability, pill size, dose and cost of the medicine. It is therefore crucial that this parameter is tractable at the lead stage
- A lead needs an acceptable early safety assessment based on target (orthologue) and compound liabilities, *in vivo* observations, *in vitro* studies (for example, genotoxicity and the mini-Ames test), cytotoxicity, cardiac safety (as assessed using the hERG channel (QT prolongation)) and *in silico* approaches. A secondary pharmacology selectivity profile, consistent with achieving selectivity with the candidate compound, is also required. This would include human orthologues and paralogues of the targeted enzyme or receptor, if known; the number of these that are to be tested depends on the gene family size and their known or suspected safety risks (when targeted)
- All liabilities of the series should be understood (as a result of extensive profiling) and a rationale and medicinal chemistry plan generated for how they might be overcome in the subsequent optimization phase
- A lead should contain no known toxicophores or undesirable reactive groups and no detrimental chemical feature or characteristic associated with the pharmacophore indicative of, for example, adduct formation. This avoids the scenario in which a region of the molecule responsible for activity has a liability that cannot be overcome
- A lead should display no acute toxicity in *in vivo* efficacy studies. Although no formal *in vivo* safety studies are performed at this stage, careful observation of efficacy studies, particularly at high, repeated doses, can be informative
- Preferably, there should be no apparent intellectual property obstacles to the progression of any series (freedom to operate)

this phase is the delivery of a lead series. The generic criteria for such a lead are shown in BOX 3 (REF. 19).

Disease-specific hit and lead criteria

Guided by the specific requirements for each disease as well as existing target product and candidate profiles, the committee coordinated by the GHIT Fund devised disease-specific criteria for hits and leads, which are discussed below and summarized in BOXES 4–6.

Malaria. There are several good treatments for malaria, but the challenge of emerging drug resistance is ever present, particularly

in South-East Asia where cases of increased parasite-clearance times are on the rise in patients treated with combination therapies that include artemisinin derivatives (for example, artesunate). This has revealed a new type of *Plasmodium* spp. resistance — essentially one in which the ring stage of the intra-erythrocytic cycle can tolerate drug intervention^{20–23}. As the mainstay malaria treatments rely on a component that is artemisinin-based, a global public health catastrophe could emerge unless new drugs that overcome this risk are delivered.

In addition, current antimalarials generally target the asexual blood stage of the disease.

Breaking the life cycle and killing parasites during other asymptomatic phases — such as the sexual-stage gametocytes and liver-stage hypnozoites and schizonts — will be crucial for providing treatments to block relapse in *P. vivax* infections, preventing transmission and protecting vulnerable populations. Indeed, only with such drugs can the global goal of eradicating malaria be realized. Extensive research on these two disease-causing pathogens (*P. falciparum* and *P. vivax*) over the past decade has helped to establish some very specific entry criteria for drug discovery programmes¹⁴.

The cellular potency criterion for a hit for malaria research (BOX 4) is based on the extensive screening efforts of the MMV and its partners over the past 7 years²⁴. Through partnerships in both industry and academia, more than 5 million compounds have been screened against the asexual blood stages of *P. falciparum* and the cutoff potency from these *in vitro* screens has mostly been around 1–2 μM . Nevertheless, more than 25,000 compound hits were available for follow-up^{25–27}. Screening against liver stages of *Plasmodium* spp. has also delivered many potent hits²⁸. Naturally, activity should be confirmed with a pure compound, and hits should have selectivity for the parasite over a mammalian cell line (for example, a greater than 10-fold difference between the IC_{50} and CC_{50} , the half-maximal inhibitory concentration against the parasites and the half-maximal cytotoxic concentration against the host mammalian cells, respectively) and display an acceptable concentration response, all of which provide confidence in a specific interaction and effect.

By the start of lead optimization there is a need for clarity on the TPPs and TCPs of the series, as these define the goal and tactics for the subsequent optimization phase. The potency required for progression depends on the TCP. For blood stages (TCP1 and TCP2), the crucial aspect is *in vivo* oral efficacy in the *P. falciparum* SCID (severe combined immunodeficiency) mouse model of infection²⁹, with the key feature for TCP1 compounds being rapid parasite clearance *in vivo*, at rates at least as good as those of chloroquine. For prophylaxis (TCP4), the crucial aspect is *in vivo* oral efficacy in a *Plasmodium berghei* sporozoite challenge model (or equivalent)³⁰. From experience, achieving a dose that eradicates 90% of the target pathogen (ED_{90}) $<50\ \text{mg per kg}$ provides confidence that the series has a parasitological foundation for the lead optimization phase; ultimately, a successful candidate will have an ED_{90} $<10\ \text{mg per kg}$ (often considerably lower). Typically,

this is achievable if a compound has an IC_{50} <100 nM against the blood or liver stages and appropriate DMPK properties. However, a compound may still be acceptable for follow up if one of these components does not meet the proposed criteria, provided the other parameter is outstanding.

Given the risk of resistance and a need for drugs with novel modes of action, potency *in vitro* across a panel of established drug-resistant parasite strains isolated from patients is also crucial. The genetics underlying the emergent resistance against artemisinins, as seen in the Mekong area in South-East Asia, are becoming increasingly understood^{31–33}, and this knowledge is being used to set up panels of parasites against which new drug candidates can be tested. For TCP1 at least, new drugs are most likely to be used in combinations (as presently mandated for all artemisinins). This means that a newcomer must be evaluated for potential drug–drug interactions with other TCP1 antimalarials.

For TCP3a (anti-relapse), it is necessary to have *in vitro* data supporting the activity of compounds on hypnozoites³⁴, whereas for TCP3b additional activity against mature and, ideally, early-stage gametocytes is required in addition to asexual blood-stage potency³⁵.

Tuberculosis. *M. tuberculosis* is so well adapted to its human host that almost one third of the world population is estimated to be infected. Although only 10% of infected individuals are believed to develop TB in their lifetime, this still results in ~1.5 million deaths annually³⁶ (see the [World Health Organization \(WHO\) website](#)). Therefore, there is an urgent need for new anti-TB drugs.

It still takes 6 months to cure drug-sensitive TB (DS-TB) and a minimum of 18 months to treat multidrug-resistant TB (MDR-TB). It is highly desirable to shorten treatment duration for both patients with DS-TB and those with MDR-TB to improve compliance and to limit the spread of drug-resistant TB (DR-TB). Standard care of patients with DS-TB includes a combination of isoniazid, rifampicin, pyrazinamide and ethambutol for the first 2 months and a combination of isoniazid and rifampicin for the remaining 4 months. Patients with MDR-TB, whose *M. tuberculosis* strains are resistant to isoniazid and rifampicin, are treated with second-line TB drugs that include aminoglycosides, quinolone antibiotics, cycloserine and capreomycin. An updated dataset was recently published for the most commonly used TB drugs with respect to *in vitro* potency, cidality, physicochemical and

Box 4 | Summary of main checkpoint criteria for antimalarial hits and leads

Guided by the specific requirements for the disease, and taking into account existing target product and candidate profiles, the committee coordinated by the Global Health Innovative Technology (GHIT) Fund devised the following disease-specific criteria for hits and leads for malaria.

Validated hit

- Cellular potency criteria: hits should have an effector concentration for half-maximum response (EC_{50}) <1 μ M for sensitive and multiple resistant strains of *Plasmodium* spp.
- Cytotoxicity criteria: hits require a greater than 10-fold selectivity between the half-maximal cytotoxic concentration (CC_{50}) for the mammalian cell line and the EC_{50} for *Plasmodium* spp.

(See also BOX 2 for the evaluation criteria regarding the acceptability of chemical structure, novelty and confirmation)

Early lead

- Cellular potency criteria: a lead requires EC_{50} <100 nM for sensitive and multidrug-resistant strains of *Plasmodium* spp.
- Cytotoxicity criteria: a lead should have a greater than 100-fold selectivity between mammalian cell line CC_{50} and *Plasmodium* EC_{50} . Frontrunners should be tested across the malaria life cycle and key mechanistic assays so as to ensure an understanding of the phenotype and target candidate profile (TCP) potential of each series and (preferably) novel mechanisms of action
- *In vivo* efficacy criteria: when administered orally in the blood stages of infection (TCP1 and TCP2), a lead should achieve parasite clearance at a dose that eradicates 90% of the target pathogen (ED_{90}) <50 mg per kg (typically four doses over 4 days) in the *Plasmodium falciparum*-infected SCID (severe combined immunodeficiency) mouse model. TCP1 should demonstrate a rapid rate of parasite clearance. For TCP3a, the anti-relapse TCP, there are no *in vivo* criteria for leads (in the absence of good models), but a lead should demonstrate anti-hypnozoite activity *in vitro*. For TCP3b, the transmission-blocking TCP, a lead should demonstrate potency in a gametocyte assay (for example, as measured by gamete formation⁷⁹) in a similar range to that of the *in vitro* asexual blood stage potency. For TCP4, the chemoprotection TCP, a lead should have efficacy in a prophylaxis model of malaria, with ED_{90} <50 mg per kg

pharmacokinetic properties generated under standardized conditions⁹. For patients treated for MDR-TB, the cure rate is only ~48% and this needs to be improved drastically, whereas the cure rate of patients treated for DS-TB is ~85%⁹.

There has been a steady increase in TB drug resistance; the WHO estimated that 3.5% of new cases and 21% of previously treated cases are MDR-TB³⁶. Among patients with MDR-TB, an estimated 9% have extensively drug-resistant TB (XDR-TB), meaning that the pathogens are resistant to all second-line TB drugs³⁶ in addition to rifampicin and isoniazid. A new drug must show efficacy against all resistant strains, which calls for agents with novel mechanisms.

The new candidates must also be orally available to ensure wide usage, especially in developing countries. For the same reason, the cost of goods needs to be low and pill size (which is determined by potency and pharmacokinetic properties) reasonable. Furthermore, as they will be used in combination with other TB drugs to stem the emergence of resistance, the new drugs must have a low risk for drug–drug interactions. As patients with TB are often co-infected with HIV, new agents also need to be compatible with most of the anti-retroviral

drugs. All of these requirements are especially stringent because TB drugs are administered for extended time spans.

Treatment shortening can only be achieved with an agent or a regimen that effectively eliminates non-replicating *M. tuberculosis*⁹. Non-replicating *M. tuberculosis* is metabolically less active and less susceptible to antibiotics than actively replicating bacteria⁹, and new hits must be evaluated for their capacity to kill both replicating and non-replicating *M. tuberculosis*, as measured in the microplate Alamar Blue assay (MABA³⁷) and low oxygen recovery assay (LORA³⁸), which are both suited for high-throughput screening. The desired hit and lead profiles are summarized in BOX 5.

The most-recent TB hits were discovered in phenotypic screens with cultured *M. tuberculosis*, typically using the MABA⁹. In addition, low-oxygen culture conditions (LORA³⁸) and other assays^{39,40} are used to identify hits that kill slow- or non-replicating bacteria⁴¹. However, these assays do not fully capture the little-understood mechanisms whereby TB bacteria move in and out of latency^{42–44}. In addressing this need, a new assay was recently developed for agents that kill *M. tuberculosis* bacteria that reside in macrophages⁴⁵, resulting in the discovery of Q208 (REF. 46).

Box 5 | Main checkpoint criteria for anti-tuberculosis hits and leads

Guided by the specific requirements for the disease, and taking into account existing target product and candidate profiles, the committee coordinated by the Global Health Innovative Technology (GHIT) Fund devised the following disease-specific criteria for hits and leads for tuberculosis (TB).

Validated hit

- Cellular potency criteria: a hit should have a minimum inhibitory concentration (MIC) against the *Mycobacterium tuberculosis* laboratory strain H37Rv of <10 µM under replicating growth conditions, for example, under microplate Alamar Blue assay (MABA) conditions³⁷
- A hit requires a greater than a 10-fold difference between cytotoxicity against mammalian cells (Vero cells) and the MIC against *M. tuberculosis* H37Rv
- Hits should show evidence of a preliminary structure–activity relationship (SAR) among analogues (See also BOX 2 for the evaluation criteria regarding the acceptability of chemical structure, novelty and confirmation)

Early lead

- *In vitro* potency criteria: a lead should have a MIC against the *M. tuberculosis* strain H37Rv of <1 µM under MABA conditions and a MIC <10 µM under non-replicating anaerobic conditions (low-oxygen-recovery assay, LORA³⁸ conditions)
- A lead should exhibit *in vitro* activity against *M. tuberculosis* strains that are resistant to a single TB drug, such as isoniazid or rifampicin, indicating a new mechanism of action
- A demonstration of the *in vitro* bactericidal activity of a lead is required as indicated by time–kill curves showing that the number of colony-forming units (CFUs) decreases over time (for example, 14 days) compared with the number of CFUs at the beginning of the experiment
- The oral bioavailability of a lead must be demonstrated in rodents
- A demonstration of the oral efficacy of a lead in a mouse acute infection model is required⁸⁰
- A preliminary indication of the safety of a lead must be demonstrated (in hERG⁸¹ and cell health assays⁸²)

The second challenge in discovering drugs that target latent TB requires an understanding of TB pathogenesis. *M. tuberculosis* primarily infects and replicates in activated macrophages but can persist in a non-replicating state in foamy macrophages⁴⁷. This host–pathogen system can form granulomas in which infected macrophages are surrounded by layers of active macrophages and other immune cells. The central granuloma region is oxygen-deprived and necrotized but contains latent *M. tuberculosis*; in the lung this results in cavities. The inner necrotic area is poorly irrigated, complicating local drug exposure. Granulomas do not normally form in *in vivo* models; however, recently, animal models of granulomas were developed^{48,49}. In one of these models, it was shown that treatment with a vascular endothelial growth factor (VEGF)-specific antibody can restore vasculature and drug access in TB granulomas⁴⁹. Continued research into new assays and models is crucial, as well as pursuing completely different approaches that aim to stimulate host immune responses against TB⁵⁰.

Visceral leishmaniasis and Chagas disease.

For visceral leishmaniasis, existing drugs have variable efficacy and serious toxicities⁵¹; only one (miltefosine) is administered orally and the others are given by intravenous or intramuscular injections, which are impractical.

The goal for research must be to transform patient therapy from poorly adapted antimonial treatments (for example, sodium stibogluconate (SSG)) to simple, patient-adapted oral therapies that are affordable, safe and efficacious in both children and adults.

In the past 15 years, combinations of drugs with similar or improved efficacy to the older antimonials, but with improved safety and tolerability profiles, have been developed. These include combinations containing liposomal amphotericin B, paromomycin and/or miltefosine⁵². However, these drugs remain costly, are difficult to administer, have poor stability at the high temperatures that occur in endemic regions, require lengthy treatment and/or are poorly tolerated. In addition, there is a dichotomy in drug efficacy in regions of the world where visceral leishmaniasis is endemic. In South Asia, the medical needs for visceral leishmaniasis are presently moderately to well met. However, in East Africa and Latin America, the efficacy and tolerability of current visceral leishmaniasis therapies remain a challenging area for improvement.

Ideally, what is needed in the treatment of visceral leishmaniasis is a simple oral combination therapy that would prove to be advantageous and/or effective in maintaining or improving efficacy, improving tolerability and preventing or delaying the emergence of

resistance. Furthermore, a treatment adapted to field conditions, with a shorter treatment duration and that could be used globally, would be optimal. In addition, to address the development of resistance by the parasite to drug monotherapies — as documented for current therapies — drugs with new and distinct mechanisms of action should be developed as combination therapies early in development and not used as monotherapies.

In the case of Chagas disease, there are even fewer treatment options than there are for visceral leishmaniasis. Monotherapy with nifurtimox or benznidazole (both from the same nitroheterocycle class) remains the only recognized treatment, but these agents require long treatment courses, have variable efficacy and cause serious side effects, resulting in discontinuation of treatment in ~20–30% of patients. It is crucial that new classes of effective, well-tolerated, orally acting and short-course treatments are progressed into the clinic to provide improved options for patients. New classes of drugs for Chagas disease are also essential to enable the development of combination therapies to improve efficacy and toleration, reduce treatment duration and combat the risk of the development of resistance to monotherapies. Recently, a trial in chronic Chagas disease was described for posaconazole, an inhibitor of *T. cruzi* 14- α demethylase (CYP51) that has a different mechanism of action to the currently used nitroheterocyclics, as exemplified by benznidazole⁵³. In spite of good preclinical efficacy, the new compound did not meet expectations, resulting in more treatment failures than benznidazole. It is believed that the standard Chagas mouse model that was used to validate posaconazole only represents the early acute phase of infection; thus, it is important to use models and assays that also allow testing of the chronic late stage of the disease^{13,54}.

In order to promote the discovery of safe, efficacious and orally acting treatments for visceral leishmaniasis and Chagas disease that overcome the limitations of existing regimens, discovery needs to focus on new chemical series, leaving behind the flawed classes in current use. Until recently, the steady identification of new chemical series has been hindered by a limited screening capacity coupled with very low hit rates. However, progress has been made and larger compound collections are now being screened. More than a million compounds have been screened against *T. cruzi* and *Leishmania* spp.^{54–56}, but the rate of hit discovery still lags far behind that for malaria. It remains necessary to avoid discarding precious chemical series by setting the hit

selection criteria too high. Thus, hit criteria focus on the identification of new series and mechanisms of action with even modest activity against the intracellular forms of *Leishmania donovani* and *T. cruzi*, the causative agents of leishmaniasis and Chagas disease, respectively⁵⁷.

Although this inclusive approach to hit selection provides scope for subsequent HTL projects it does bring with it a high rate of early attrition and often leads to long optimization campaigns before quality leads are produced. The HTL entry criteria stated in BOX 6 are a combination of the current well-documented 'best practices' for targeted drug discovery projects⁵⁸ and a modest phenotypic *in vitro* activity hurdle⁵⁹. Once the encouraging *in vitro* anti-parasitic activity of a new series can be coupled with sufficient *in vivo* plasma exposure, ideally following oral dosing, the next hurdle is to demonstrate a robust reduction in the parasite burden at target organs in infected rodents, which is the defining characteristic of a lead that is ready for subsequent optimization. Unfortunately, we have little knowledge regarding how *in vitro* potency correlates with *in vivo* activity in visceral leishmaniasis and Chagas disease, with a lack of pharmacokinetic and pharmacodynamic (PK/PD) examples. For *T. cruzi* infection, oral dosing is required for *in vivo* proof-of-concept studies, which is in line with the requirement for orally acting therapies. Although oral dosing is also preferred for studies with rodents infected with *Leishmania* spp., intraperitoneal or intravenous administrations are also acceptable given that a short-course treatment administered by the parenteral route could fulfil the TPP for visceral leishmaniasis.

Discussion

Our consultation among drug discovery experts collaborating with the GHIT Fund revealed differences in the criteria considered most conducive to cost-efficient drug discovery, which are associated with the specific requirements for the diseases reviewed here. One of the dangers in establishing stringent, clear-cut criteria for entry into the HTL phase and then progression into lead optimization is that valuable chemicals are discarded at an early stage. It is obvious that a molecule such as artemisinin, were it to emerge today as a hit in a high-throughput screening campaign, would severely struggle to be taken forward, scoring extremely low in chemical tractability, ease of synthesis and chemical suitability (the compound has a highly reactive peroxide). Even if it were taken further (without analysis of the

Box 6 | Hit and lead criteria for Chagas disease and visceral leishmaniasis

Guided by the specific requirements for the disease, and taking into account existing target product and candidate profiles, the committee coordinated by the Global Health Innovative Technology (GHIT) Fund devised the following disease-specific criteria for hits and leads for Chagas disease and visceral leishmaniasis.

Hit and lead criteria for Chagas disease

- Key hit selection criteria: cellular potency — a hit should have a half-maximal inhibitory concentration (IC_{50}) <10 μ M against intracellular *Trypanosoma cruzi*
- Key lead selection criteria: in an acute mouse model of Chagas disease, a hit should demonstrate an 80% reduction of parasite burden in organs or tissues, or there should be no parasites detected at the end of treatment and an increase in lifespan with up to 10 doses at 50 mg per kg delivered orally

Hit and lead criteria for visceral leishmaniasis

- Key hit selection criteria: cellular potency — a lead should demonstrate an IC_{50} <10 μ M against intracellular *Leishmania donovani*
- Key lead selection criteria: in a mouse (or hamster) model (infected with *L. donovani* or *Leishmania infantum*), treatment with a lead should result in a >70% reduction in liver parasite burden after at most 5 doses at 50 mg per kg delivered orally once or twice per day

structure–activity relationship (SAR)), scientists would discover that the compound has a very short *in vivo* half-life (minutes), is metabolically unstable and its mode of action and target are poorly understood⁶⁰. Along with serious preclinical safety concerns^{61,62}, there is every reason to believe that the modern drug discovery process would have discarded the drug long before it had a chance to save the lives of millions. In fact, algorithms that select 'drug-like' molecules generally exclude artemisinin-like molecules from the chemical libraries used for HTS in the first place. Chemical tractability is important, but it must be kept in mind that there are many other molecules that have progressed straight from a phenotypic screen (that is, without further chemical modification) into patients, such as tamatinib (also known as R406)⁶³, paclitaxel⁶⁴, rapamycin⁶⁵ and cyclosporine⁶⁶.

Conversely, there are numerous examples in which chemical series were pursued far too long (in hindsight), soaking up valuable resources, time and careers that should have been invested elsewhere. As an additional complication in navigating between these two extremes, the TPPs may shift with changes in the clinical landscape for infectious diseases that affect people in the developing world; for example, such changes may include a shift in policy from curing patients ad hoc to eradication programmes that require mass drug administration, a decision to focus on the prevention of transmission rather than on cures, changing disease priorities and pharmacoeconomics, the emergence of co-infections (with concomitant drug–drug interaction risks) or the spread of resistance.

Nevertheless, in the midst of all these complexities, project teams can attempt to steer their compounds using the set of criteria and

considerations presented here in the light of the four sets of disease TPPs as beacons. Our analysis has split recommendations for entry into HTL and lead optimization phase into a generic part and a more specific part for each disease. As stated earlier, these guidelines were established in the context of GHIT Fund-coordinated collaborations, but we hope that they will find wider adoption and aid the acceleration and expansion of pipeline drug candidates for these serious diseases.

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Competing interests statement

The authors declare no competing interests.

FURTHER INFORMATION

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 Global Health Innovative Technology (GHIT) Fund: <http://www.ghitfund.org>
 Japanese Pharmaceutical Manufacturers Association: <http://www.jpma.or.jp>
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