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Host-directed therapy, an untapped opportunity for antimalarial intervention

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SUMMARY

Host-directed therapy (HDT) is gaining traction as a strategy to combat infectious diseases caused by viruses and intracellular bacteria, but its implementation in the context of parasitic diseases has received less attention. Here, we provide a brief overview of this field and advocate HDT as a promising strategy for antimalarial intervention based on untapped targets. HDT provides a basis from which repurposed drugs could be rapidly deployed and is likely to strongly limit the emergence of resistance. This strategy can be applied to any intracellular pathogen and is particularly well placed in situations in which rapid identification of treatments is needed, such as emerging infections and pandemics, as starkly illustrated by the current COVID-19 crisis.

THE CURRENT CRISIS IN ANTI-INFECTIVE DRUGS

In the mid-20th century, newly deployed penicillin and sulfonamides saved countless lives from bacterial infections during World War II. In the decades that followed, new classes of antibiotics regularly came on the market, until the 1990s.1 Over this period, drugs targeting non-bacterial pathogens were also deployed with tremendous effect; arguably the one with the most impact was chloroquine, discovered in 1934 and used for mass treatment of malaria in the following decades, and whose outstanding efficacy against the multiple species of malaria parasites, tolerability, and low cost made it an antimalarial wonder.

Unfortunately, this golden age is over. The first cases of bacteria with decreased susceptibility to penicillin were reported in 1940,2 and the emergence and spread of chloroquine resistance in malaria parasites started in the 1950s, ultimately rendering the drug largely useless. The same trend affected essentially all molecules deployed as antimalarials. Resistance to one particularly promising drug, atovaquone, was observed even before mass deployment,3 and was subsequently shown to have emerged through the selection of mutations in the gene encoding its molecular target (in this case the cytochrome b of the parasite) that reduced susceptibility to the drug.4 Resistance caused by the selection of modified/altered targets under drug pressure has affected many other treatments, in the context of other infectious diseases beyond malaria.5 The rapidity with which drug-resistant pathogens can be selected through this mechanism is a grave concern in the context of global public health. The limited return on investment for research and development in infectious disease chemotherapy, in comparison to other types of indications such as cancer or chronic diseases, has exacerbated the problem by decreasing the overall investment from the pharmaceutical industry for the development of de novo anti-infectives. Today more than ever, additional strategies to combat infectious agents are urgently needed.

HOST-DIRECTED THERAPY

It is well established that intracellular pathogens of any taxon (viruses, bacteria, eukaryotic parasites) rely on host cell functions for their survival and proliferation. As a consequence of this reliance, inhibitors of host cell enzymes that are essential for pathogen viability have anti-infective properties. Furthermore, since the target is host encoded, the emergence of resistance based on the selection of mutations in the gene of a target whose affinity for the drug is decreased, and ensuing preferential proliferation of parasites with a resistant genotype under drug pressure, cannot occur. This offers opportunities for host-directed therapy (HDT), an innovative strategy that consists of targeting host functions that mediate diseases caused by infectious agents, and is being explored in the context of many infectious diseases. For example, the host methytransferase SETD3 was recently proposed as a target for the treatment of enterovirus and rhinovirus infections6; thus, the long-standing problem of a cure for the...
common cold may finally be solved through HDT. Alternatively, HDT can target host mechanisms implicated in pathogenesis, rather than enzymes directly required for pathogen survival as outlined above; studies with Leishmania and Mycobacterium tuberculosis exemplify this strategy. As a recent example, it was shown that ibrutinib, an inhibitor of the Bruton tyrosine kinase (BTK) that plays a critical role in B cell signaling, is more efficient than conventional anti-leishmanial drugs in an animal efficacy model; furthermore, the authors established that this effect is not mediated by microbicidal activity of the compounds, but by promoting protective immunity and targeting host pathways that mediate susceptibility. Perhaps surprisingly, HDT approaches have been underexplored for malaria, although its potential benefits have been discussed by our teams and others.10–14

**HUMAN TOXICITY POTENTIAL OF HDT AND DE-RISKING STRATEGIES**

The advantage of HDT in not targeting parasites directly, and thus limiting the risk for resistance, comes with the caveat of potential for toxicity, which clearly needs to be addressed. The main risk is that the inhibition of human targets could lead to toxicity, translating into potentially severe side effects in treated patients, which can affect a very small proportion of patients and thus may not be detected in safety clinical trials. It is therefore essential to embed a de-risking strategy when considering HDT. HDT hit and lead compounds should be profiled in vitro on panels of human receptors and kinases, which together represent a substantial proportion of key intracellular components essential for the viability and functionality in all human cells. This profiling could assist in the identification of the key host target(s) mediating the anti-parasitic activity. If not the case, then the off-target effect would be characterized as in any other classical drug discovery program. The potencies observed can then be compared to the antiparasitic potency of the compound in a relevant phenotypic assay, and the ratio can help to evaluate the selectivity factor and assess potential toxicity risk. It is important to note that a given inhibitory potency against a host cell target is not necessarily associated with toxicity. Should human enzymes or receptors associated with a given toxicity (e.g., cardiovascular, hepatic, reproductive) be inhibited in vitro by the HDT compound, medicinal chemistry would guide lead optimization to improve selectivity (host target versus off-target); in vivo toxicology studies should be conducted on animal models to further define the safety margin of the optimized compound. Determining the oral exposure profiles of the compound in animal toxicity and efficacy models will allow the safety margin to be evaluated. If available, human clinical, pharmacokinetic or efficacy data for the HDT compound would allow the calculation of an even more relevant therapeutic margin. In general, a safety margin of at least 10-fold is requested, but 20-fold is ideal to accommodate the diversity/variability of drug exposure profiles among different patients. Malaria being particularly severe in young children and pregnant women, juvenile and embryo fetal toxicity risk assessment will need to be conducted.15 Malaria treatments are based on 3-day dosing and ideally aim at full efficacy as a single-dose cure, which is the preferred treatment regimen in most clinical trials.16 It is therefore important to consider the difference in toxicity risks between the treatment of acute disease (which can be very brief) and the long-term treatment required for chronic disease (e.g., cancer, metabolic disorders), since the latter necessitate a repeated and sustained treatment that may lead to cumulative exposures and hence higher toxicity risk. Nevertheless, antimalarials must have long duration exposure even if the dosing regimen is infrequent. Regular infections in endemic regions also emphasize the safety required in any new antimalarial.

In summary, determining the effective exposed concentration needed to treat malaria while avoiding side effects in the host must govern the evaluation of risks. HDT can be accelerated by drug repositioning (see below); in such a framework, only the drugs displaying antimalarial potency equivalent to or below (in case of anticancer drugs) the concentration needed to treat patients of the primary indication disease can be considered.

**HOST CELL TARGETS DURING PLASMODIUM INFECTION OF HEPATOCYTES AND ERYTHROCYTES**

Malaria parasites have a complex life cycle (Figure 1). Human infection is initiated by the bite of an infected *Anopheles* mosquito, which delivers sporozoites at the bite site. These sporozoites gain access to the bloodstream and reach the liver, where they invade hepatocytes and develop into schizonts, producing tens of thousands of merozoites. After their release into the bloodstream, merozoites invade erythrocytes to produce more merozoites, causing malaria pathogenesis. Some of these merozoites develop into sexual forms (male or female gametocytes) that can be transmitted to a mosquito during a blood meal.

HDT can be implemented during the infection of either hepatocytes or erythrocytes (Figure 1), through targeting host cell functions that are directly required for parasite survival and proliferation at these stages. Some host targets may be relevant to both stages. Table 1 presents examples of ligands or inhibitors of host targets with known antimalarial activity.

It must be kept in mind that it is not trivial to assign the efficacy of any HDT compound to the inhibition of the targeted host protein, as any compound could cause parasite killing through parasite- or host-encoded off-targets; off-target effects need to be characterized as in any other classical drug discovery program. This is particularly problematic for blood stages, as erythrocytes (unlike hepatocyte lines) are not directly amenable to genetic manipulations (e.g., replacement of wild-type alleles with alleles encoding drug-resistant targets) that can be used to tackle this problem. However, recent developments in the manipulation of erythroid lineage precursors to generate genetically engineered erythrocytes that are susceptible to infection with *Plasmodium* offer a (labor-intensive) window of opportunity for ascribing pharmacology to specific host-encoded targets. In parallel to this approach, pharmacological validation with a panel of reference inhibitors (when available) is possible.

**Liver stages**

The liver stage of infection represents a critical target for HDT, as blocking infection during this stage could prevent blood infection, and hence malaria pathogenesis and subsequent
transmission. However, prophylaxis using liver infection inhibitors would require a level of safety even greater than curative approaches. The establishment of hypnozoites, a dormant form that can cause relapsing infections, is one of the possible outcomes of hepatocyte infection with Plasmodium vivax; recent models suggest that eliminating a small fraction of hypnozoites could have a dramatic impact on P. vivax prevalence. Only two drugs, both belonging to the same 8-aminoquinoline chemical class, are approved for the elimination of hypnozoites. These two molecules, primaquine and tafenoquine, presumably have the same mechanism of action (although this is not yet fully elucidated), and both cause significant complications in G6PD-deficient individuals. Very few additional antihypnozoite drug candidates are in the pipeline. Identifying factors in the host hepatocyte that are required for hypnozoite establishment, maintenance, and/or reactivation could contribute to fill this gap.

Multiple host receptors have been demonstrated to be required for Plasmodium entry into hepatocytes. The roles of several of these factors is context dependent, and the two most important species with respect to global disease burden, P. falciparum and P. vivax, use different receptors for the invasion of hepatocytes. Targeting multiple factors is therefore critical to block invasion by diverse Plasmodium isolates and species. Once the parasite is within the hepatocyte, other factors are critical for the maintenance and development of the liver stage infection. The host cell fatty acids synthesis machinery is required for parasite development in the liver, presumably through facilitating nutrient uptake and parasite membrane growth. Recent studies have also shown that a series of host-derived vesicles sequester to the parasite. Targeting proteins that are involved in trafficking, such as coatomer protein complex I subunits (COPB2, COPG1) or the adaptor protein GGA1, impairs parasite development in hepatocytes. Further, the tumor suppressor p53 negatively regulates Plasmodium liver stage development by altering lipid peroxidation in the hepatocyte; boosting levels of p53 results in dramatically fewer liver-stage parasites, offering additional opportunities for interference with the parasite at this stage of its life cycle. The membrane water and small-molecule channel aquaporin-3 (AQP3) has also been shown to support development in multiple stages of the parasite, including liver stages. Excitingly, targeting AQP3 was recently shown to successfully impair P. vivax liver stages, suggesting that small-molecule inhibitors against AQP3 may have antihypnozoite activity, and could provide long-sought-after tools for targeting relapses. Finally, several host protein kinases, a family of enzymes with...
| Compound | Target | Target type | Proposed mechanism | Ref. |
|----------|--------|-------------|-------------------|-----|
| K252a    | c-MET and other kinases | protein kinase | inhibits kinase activity of c-Met and other tyrosine kinases | Carrolo et al. 18 |
| Blockers of lipid transport (BLTs) | scavenger receptor (SR)-B1 | lipoprotein receptor | inhibits SR-B1-mediated selective uptake of lipids from high-density lipoproteins | Rodrigues et al. 19 |
| Nutlin-3 | MDM2 | E3 ubiquitin-protein ligase | prevents degradation of p53, promotes lipid peroxidation in infected hepatocytes | Kaushansky et al. 20 |
| Serdemetan | MDM2 | E3 ubiquitin-protein ligase | minimizes degradation of p53, eliminate Plasmodium-infected hepatocytes | Douglass et al. 21 |
| SB505124 | TGF-β receptor 1 | protein kinase | inhibits the enzymatic activity of kinases involved in multiple cellular processes | Arang et al. 22 |
| Auphen | AQP3 | membrane protein | selectively and irreversibly inhibits glycerol transport by AQP3; effective against both liver and blood stages and against multiple human malarial parasites | Posfai et al. 23 |
| Brefeldin A | COPI | coatamer protein complex | blocks host intracellular protein trafficking | Raphemot et al. 24 |
| Golgicide A | GBF1 | guanine nucleotide exchange factor | blocks host intracellular protein trafficking | Raphemot et al. 24 |
| ABT-737, obatoclax | Bcl-2 family proteins | B cell lymphoma 2 family proteins | overcomes apoptotic block placed by parasites, eliminates Plasmodium-infected hepatocytes | Douglass et al. 21, Kaushansky et al. 25 |
| PP1 | Src family kinases | protein kinase | inhibits negative regulation of endothelial permeability by Src-family kinases, mediates dissociation of vascular endothelial (VE)-cadherin and redistribution of ZO-1 | Kaushansky et al. 25, Gillrie et al. 26 |
| Fasudil | Rho-associated protein kinase | protein kinase | decreases nuclear factor (NF)-κB activation and endothelial cell apoptosis, restores endothelial barrier integrity via rho-kinase signaling pathway | Gillrie et al. 26, Toufiq et al. 27 |
| Imatinib | receptor tyrosine kinases | protein kinase | inhibits erythrocyte band 3 phosphorylation, preventing parasite egress | Sicard et al. 28, Kesely et al. 29 |

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proven druggability, are important for parasite development in hepatocytes. Excitingly, some of these targets overlap with kinases that are also implicated in the subsequent blood stages (see below).

### Blood stages

After maturation within the hepatocyte, the parasite exits the liver and invades erythrocytes. Circulating erythrocytes comprise mostly mature normocytes, a simplified cell largely devoid of organelles and fully adapted for the transport of oxygen by hemoglobin, as well as a small number of the more complex early reticulocytes that contain several hundred additional proteins and remnant organelles. These host-cell differences are reflected in the tropism of some human malaria parasites, with *P. vivax* preferentially infecting reticulocytes, while *P. falciparum* can infect erythrocytes of all ages. We now understand that these host erythrocytes play an active role during *Plasmodium* infection and are beginning to elucidate which host factors are of specific or general importance for different human malaria. First, it was shown in the early 2000s that host erythrocyte heterotrimeric G proteins, upstream components of many signaling pathways, are activated by infection. Subsequently, a phospho-signaling pathway implicating host cell PAK (p21-activated kinase) and MEK1 (MAP/ERK kinase) was shown to likewise be activated by infection in the host erythrocyte. Treatment with U0126, a highly selective allosteric inhibitor of human MEK1, impaired parasite proliferation in both the hepatocyte and the erythrocyte. The toxicity associated with MEK inhibitors precludes their direct repurposing for malaria; however, these compounds offer new medicinal chemistry starting points to be developed, with the goal of reducing toxicity to a point that treatment of malaria infections becomes acceptable. More recently, AQP3 was found to be required for *P. vivax* development in reticulocytes, with an AQP3 inhibitor offering pan-malaria activity against both *P. vivax* in reticulocytes and *P. falciparum* in normocytes, in addition to its potential as a liver stage inhibitor (see above).

We recently published an antibody microarray-based global analysis of infected erythrocyte phospho-signaling pathways, which revealed that the host erythrocyte undergoes dynamic activation of numerous signaling elements. This strongly suggests that host cell signaling has a more central role in parasite development than previously thought. Highly selective inhibitors (that will remain selective until, as seen quite often, off-target activities are observed) targeting activated host cell enzymes identified in this study display nanomolar activity against both *P. falciparum* and *Plasmodium knowlesi* in culture and against the rodent malaria parasite *P. berghei in vivo*, suggesting that they constitute attractive targets for HDT against various *Plasmodium* clades (Table 1). These kinases are the subject of extensive drug discovery in the context of cancer chemotherapy, and highly selective inhibitors of these (and other) kinases display

*Table 1. Continued*

| Compound  | Target | Target type | Proposed mechanism | Ref.            |
|-----------|--------|-------------|--------------------|-----------------|
| PHA-665752 | c-MET  | protein kinase | inhibits host c-MET activation during intra-erythrocytic development | Adderley et al.  |
| Crizotinib | c-MET/Alk | protein kinase | inhibits host c-MET activation during intra-erythrocytic development | Adderley et al.  |
| SB-590885  | B-Raf  | protein kinase | inhibits host B-Raf activation, which halts parasite development during intra-erythrocytic ring stage development | Adderley et al.  |
| IPA-3      | PAK1   | protein kinase | inhibits host MEK1 activation during intra-erythrocytic development | Sicard et al.    |
| U-0126     | MEK1/2 | protein kinase | inhibits MEK1/2 activation during intra-erythrocytic development | Sicard et al.    |
| Propranolol | G proteins | heterotrimeric G protein (G<sub>a</sub>) | blocks merozoite invasion of erythrocytes and blood-stage growth | Murphy et al.    |
| Erastin    | SLC7A11 | cystine/glutamate transporter | blocks host SLC7a11-GPX4 pathway to induce lipid peroxidation in *Plasmodium*-infected hepatocytes | Kain et al.      |
Table 2. FDA-approved kinase inhibitors of potential kinase targets for antimalarial HDT

| Host kinase | FDA-approved kinase inhibitor, with year of approval and original indication | Reference linking host target to malaria life cycle |
|-------------|--------------------------------------------------------------------------------|-----------------------------------------------------|
| MEK1        | trametinib (2013; melanoma)  
             cobimetinib (2015; melanoma)  
             binimetinib (2018; melanoma)  
             selumetinib (2020; neurofibromatosis type I) | Sicard et al.28 |
| SYK         | fostamitib (2018; chronic immune thrombocytopenia) | Pantaleo et al., Kesely et al.26,27 |
| MET         | crizotinib (2011; non-small cell lung carcinoma)  
             capmatinib (2020; non-small cell lung carcinoma)  
             tepotinib (2021; non-small cell lung carcinoma) | Carrolo et al.,18 Arang et al.,22 Adderley et al.,30 Leirião et al.,75 Prudêncio et al.76 |
| BRAF        | sorafenib (2005; hepatocellular, thyroid, and advanced renal cell carcinomas)  
             vemurafenib (2011; metastatic melanoma)  
             regorafenib (2012; metastatic colorectal cancer)  
             dabrafenib (2013; cancers associated with a mutated version of the gene BRAF)  
             encorafenib (2018; cancers associated with a mutated version of the gene BRAF) | Kain et al.,32 Adderley et al.30 |
| RET         | pralsetinib (2020; thyroid and lung carcinomas) | Adderley et al.30 |
| JAK2        | ruxolitinib (2011; myelofibrosis)  
             tofacitinib (2012; rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis)  
             baricitinib (2018; rheumatoid arthritis)  
             fedratinib (2019)  
             upadacitinib (2019) | Adderley et al.30 |
| CSK         | dasatinib (2006; chronic myelogenous leukemia)  
             ibrutinib (2013; mantle cell lymphoma) | Arang et al.22 |
| FGFR4       | nintedanib (2014; idiopathic pulmonary fibrosis)  
             erdafitinib (2019; urothelial carcinoma) | Arang et al.22 |
| FLT1        | sunitinib (2006; advanced renal cell carcinoma and gastrointestinal stromal tumors)  
             sorafenib (2005; advanced renal cell carcinoma)  
             nintedanib (2014; idiopathic pulmonary fibrosis)  
             vandetanib (2011; thyroid cancer)  
             cabozantinib (2012; medullary thyroid cancer)  
             pazopanib (2009; advanced renal cell cancer and advanced soft tissue sarcoma)  
             axitinib (2012; advanced renal cell carcinoma)  
             tivozanib (2021; advanced renal cell carcinoma)  
             lenvatinib (2015; progressive, differentiated thyroid cancer)  
             pexidartinib (2019; tenosynovial giant cell tumor) | Arang et al.22 |

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potent antimalarial activity in parasite proliferation. The fact that many of these enzymes do not have orthologs in the kinome of the parasite reduces the likelihood that their inhibitors have parasite-encoded off-targets, although this needs to be demonstrated by reverse genetics in each case (see above). The potential of inhibitors that target host kinases with no ortholog in the parasite is illustrated by the potent antimalarial activity of imatinib (Gleevec), the first kinase inhibitor to have reached the market as a treatment for chronic myelogenous leukemia and acute lymphocytic leukemia. As mentioned above, some of the host kinases required for parasite proliferation in erythrocytes are also essential for the hepatic stage of the life cycle (and likely for the development of gametocytes as well, although this needs to be investigated), offering opportunities for multi-stage, pan-malaria intervention and potentially leading to HDT compounds with additional prophylactic or transmission-blocking properties.

HDT TARGETING MALARIA PATHOGENESIS

The infected erythrocyte evades clearance in the spleen through cytoadherence to the endothelium of the peripheral vasculature, which is a major driver of the pathogenesis of *P. falciparum* infections. Cytoadherence is mediated by products of the *var* gene family, which are expressed at the surface of the infected erythrocyte and interact with specific receptors on endothelial cells (reviewed in Bernardes and Smith, and Wassmer et al.). Cerebral malaria, the main cause of malaria-induced mortality, is dependent on the cytoadherence of infected erythrocytes in the brain vasculature and is strongly associated with inflammation-mediated brain swelling, possibly triggered by the breakdown of the blood-brain barrier. Molecules that interfere with parasite cytoadherence and/or barrier-strengthening compounds represent an opportunity to target the most severe symptoms of malaria (reviewed in Glennon et al.). Importantly, while current antimalarials that inhibit parasite-encoded functions have facilitated major strides in reducing malaria mortality and morbidity, they are not entirely effective at eliminating death due to cerebral malaria. Fast-acting host-targeting interventions that block rapid signaling processes involved in brain inflammation or blood-brain barrier disruption may act rapidly enough to save the lives of patients suffering from cerebral malaria episodes. This is supported by studies in mouse models of cerebral malaria: for example, treatment of animals with MEK inhibitors decreases organ inflammation and immune cell recruitment, thus limiting tissue damage; this cooperates with a direct effect of MEK inhibitors on parasite proliferation (see above) to prevent the death of treated animals. Likewise, treatment with neuregulin-1 (NRG-1, a neuronal growth factor) attenuates cerebral malaria pathogenesis in infected mice by regulating ErbB4/AKT/STAT3 signaling, leading the authors to suggest that augmenting NRG-1 may be an effective adjunctive therapy to reduce brain tissue injury during cerebral malaria.

THE OPPORTUNITY FOR DRUG REPURPOSING

Targeting the host to eliminate infection or reduce pathology associated with disease presents an opportunity for the repurposing of molecules originally developed to target non-communicable diseases. One advantage of targeting the host is that a wealth of information is available for many potential host targets. As an example, the aforementioned tumor suppressor protein p53, which has been shown to regulate both liver-stage malaria in the laboratory and clinical symptoms of malaria in the field, has prompted nearly 100,000 publications in PubMed, approximately the same number of publications as in the entirety of the malaria field. Furthermore, large collections of well-characterized small-molecule inhibitors of host targets are available.
which is not the case for parasite-encoded proteins. One example is the 12,000-compounds ReFRAME (Repurposing, Focused Rescue, and Accelerated Medchem) library, an open-access drug repositioning screening set combining 3 widely used commercial drug databases (Clarivate Integrity, GVK Excelra GoStar, and Citeline Pharmaprojects). This library has been screened against parasites such as Trypanosoma cruzi, the malaria-related Cryptosporidium, and pathogenic amoebae, as well as viruses such as Zika and Lassa, and it would be of great interest to implement a screen against malaria parasites.

Likewise, as alluded to above, host protein kinases carry more tremendous potential in repurposing approved drugs for infectious diseases: a large proportion of the human kinome is already targeted by drugs that have been used in humans, with >60 kinase inhibitors approved by the US Food and Drug Administration (FDA), mostly for the treatment of various cancers. In addition, there are >200 kinase inhibitors in clinical trials. Many of the host kinases shown to be essential for parasite survival in hepatocytes and/or erythrocytes are the targets of approved inhibitors, as exemplified above for the tyrosine kinase inhibitor imatinib, whose potential in antimalarial drug development could be rapidly tested in clinical trials. Table 2 provides examples of FDA-approved medicines that inhibit some of the host kinases (MEK1, SYK, MET, BRAF, RET, JAK2) implicated in malaria progression and pathogenesis. As these drugs have been approved and thus extensively studied in both preclinical animal models and humans, the requisite data are available to facilitate evaluation in malaria models and guide dosing in humans, should they prove effective in malaria preclinical studies. The availability of pharmacokinetic and safety data will allow for careful delineation of dosing regimens to establish an appropriate therapeutic window. It is important to note that kinase inhibitors are not approved solely for cancer, and there is increasing interest in non-oncology indications, reflecting the ability to design selective kinase inhibitors and the accumulating evidence that suitable safety margins for these molecules can be achieved in various contexts. In addition to the potential utility of approved medicines, it would also be of particular interest to identify compounds that passed early-phase clinical trials, and hence are supposedly tolerated enough for use in humans, but failed for efficacy against their original target indication. This would provide an incentive to the industry for repurposing dead-end compounds for clinical trials in an infectious disease context.

### IN SILICO-GUIDED DRUG REPURPOSING

In silico-guided drug development strategies can capture the important features of both the drug and the target space, and enable down-selection and accurate identification of target candidates for further validations. Computational methods for drug repurposing include signature matching, molecular docking, network-based strategies, and machine learning methods (Table 3).

**Signature matching** infers drug-disease associations based on the transcriptomic, proteomic, or metabolic characteristics of host cells in healthy and disease conditions. One application is to identify drug candidates that alter gene expression signatures. The logic is that if a drug can reverse the gene expression signatures for the disease phenotype, it may restore a healthy cellular state. Studies using human colon cancer cells showed that signature matching can effectively predict drugs to treat colorectal cancer. The integration of signature-based in silico tools with patient-specific disease signatures could facilitate the establishment of therapeutic pipelines for treating infectious diseases, including malaria.

**Molecular docking** predicts drug-target binding using three-dimensional (3D) structure-based modeling and computational simulation, and can be target centered (in which drugs are screened against the disease-associated protein) or ligand centered (in which compounds are docked into an
array of protein targets). This approach requires the 3D structural information on both compounds and protein targets, as well as the validation of the host target for the primary disease indication. While molecular docking is a prohibitive limitation for pathogen-encoded targets whose structure has not been solved, it offers the opportunity to facilitate host-directed drug discovery and has been applied to various infectious agents such as human immunodeficiency virus (HIV), Mycobacterium tuberculosis (MTB), malaria, and severe acute respiratory syndrome-coronaviruses-1 and -2 (SARS-CoV-1 and -2). Network-based approaches for drug repurposing and discovery have been facilitated by the expansion of genomic, proteomic, transcriptomic, and metabolomic data. Disease-specific protein-protein interactions (PPIs) or gene regulatory networks are constructed, allowing the comparison of the molecular underpinnings of a comparatively under-studied disease with the molecular basis of a well-studied disease with effective therapeutics. In addition to their therapeutic applications in cancers and coronary artery disease, network-based approaches have facilitated drug discovery and repurposing to treat infectious diseases such as pandemic and seasonal respiratory infections by redirecting the usage of known drugs to target host protein regulators of viral infection-related processes. As our knowledge of host-Plasmodium molecular interactions grows, network-based methods could facilitate the development of HDT for malaria.

Machine learning aims to facilitate pattern exploration and extract the most informative relationships between drugs, targets, and disease phenotypes through learning from diverse, large-scale data. Machine learning has been used to identify novel disease-relevant targets based on drug polypharmacology and cell phenotype data collected from small-scale drug screens. For instance, applying an elastic net regression algorithm on a small-scale drug screen dataset led to the identification of host kinases that regulate Plasmodium liver stage infection and the prediction of kinase inhibitors that inhibit parasite growth in the hepatocyte.

In silico-guided drug repurposing holds great promise for identifying the next generation of host-targeted anti-malarial drugs.

CONCLUDING REMARKS

Many major pharmaceutical companies (Novartis, Sanofi, GSK, Takeda, Merck KGaA, Zydus Cadila) have collaborated, or are engaged in discovery and development, with the Medicines for Malaria Venture to provide the antimalarial community with a strong pipeline of drug candidates, focusing on whole-cell active compounds and compounds acting on parasite-encoded targets (for a recent overview of the MMV pipeline, see Hoof van Huijsduijnen et al. In particular, it is possible that some of the “irresistible” compounds identified in whole-cell screens may have host targets. It is tempting to propose an evolution of this collaborative work by inviting the pharmaceutical industry to consider some of their advanced compounds for repositioning in the HDT approach. Similar to the modus operandi when companies provide their compound libraries for phenotypic screening, the repositioning exercise could be performed on the small sets of clinically relevant candidate drugs available within each company. Drugs for protecting endemic populations of low- to middle-income countries against Plasmodium infection must be distributed with a price tag that will be much lower than the one for travelers coming from high-income countries. As such, a mechanism to ensure affordability in endemic regions for those most in need would need to be established for any such repurposed drug.

As detailed above, safety assessment will be a crucial element in applying HDT to the malaria context, and it is essential to take into account the specifics of malaria (versus cancer) when addressing this problem. Cancer drugs are allowed to have a relatively aggressive profile, especially in cases in which the specific disease has a high mortality rate, such as pancreatic, liver, or lung cancers, whose 5-year survival rate are as low as 10%, 20%, or 21%, respectively. The much lower mortality rate for malaria (in 2019, there was an estimated 409,000 deaths for 229 million cases, or a >0.2% mortality rate) makes any risk associated with antimalarial drugs deployed for mass treatment essentially unacceptable, even more so if one considers liver stage-targeting prophylactics. Severe malaria complications, such as cerebral malaria (which has a mortality rate of ~20% and can have very serious sequelae in survivors), may arguably justify the use of drugs with higher toxicity; however, here again, tolerability should be very high, as exemplified by the current treatment with artesunate; furthermore, these drugs would need to act very quickly, as death can occur very rapidly after the onset of severe malaria symptoms.

Finally, multiple pathogens may have comparable needs from the host, or initiate symptoms using similar mechanisms. As an example, most intracellular pathogens, including viruses, bacteria, and eukaryotic parasites, rely on the host cell cytoskeleton machinery to permit invasion. Likewise, many pathogens are dependent on the same set of kinases in their host cells. For example, receptor tyrosine kinases have been implicated not only in the infection of hepatocytes and erythrocytes by Plasmodium spp but also in the infection of their cognate cell types by the bacterium Listeria and by several viruses, including Ebola, hepatitis C, and influenza viruses (reviewed in Haqshenas and Doerg). As a further and highly topical example, the possibility of repurposing kinase inhibitors for the treatment of COVID-19 was recently reviewed. Thus, targeting the host provides an opportunity to develop interventions that could be useful to treat various infectious diseases, including co-infections. This is particularly the case for well-studied infections that are challenging to address pharmacologically understudied or neglected infections, or even novel outbreaks such as the crisis caused by the SARS-CoV-2 coronavirus, where little is known about the specific host-pathogen interactions at the time treatments need to be developed. This strategy would require testing on pathogens beyond malaria, and would fill in the dangerous gap in new anti-infectives that has developed in recent decades. A major advantage of repurposing approved drugs is the rapidity with which new treatments can be deployed, a feature whose crucial importance is dramatically demonstrated by the coronavirus disease 2019 (COVID-19) pandemic.
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