Published in final edited form as:

Trends Parasitol. 2018 October ; 34(10): 843–860. doi:10.1016/j.pt.2018.07.011.

Opportunities for Host-targeted Therapies for Malaria

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Abstract

Despite the recent successes of artemisinin-based antimalarial drugs, many still die from severe malaria, and eradication efforts are hindered by limited drugs to target transmissible gametocyte parasites and liver-resident dormant *Plasmodium vivax* hypnozoites. Host-targeted therapy is a new direction for infectious disease drug development and aims to interfere with host molecules, pathways or networks that are required for infection or contribute to disease. Recent advances in our understanding of host pathways involved in parasite development and pathogenic mechanisms in severe malaria could facilitate the development of host-targeted interventions against *Plasmodium* infection and malaria disease. This review discusses new opportunities for host-targeted therapeutics for malaria and the potential to harness drug polypharmacology to simultaneously target multiple host pathways using a single drug intervention.

Keywords

Host-parasite interactions; malaria; cerebral malaria; liver; polypharmacology; drug discovery

Host-targeted therapy provides a new avenue for intervention against malaria

Malaria presents a significant global health burden with an estimated 216 million cases of disease and 445,000 malaria-related deaths in 2016 [1]. The mortality and morbidity associated with the disease in humans are caused almost entirely by two species of malaria parasites: *Plasmodium falciparum* and *Plasmodium vivax*. *P. falciparum*, the more virulent of the two, causes uncomplicated malaria characterized by fever, or severe malaria, manifesting as cerebral malaria (CM), metabolic acidosis and severe malaria anemia in children, or CM and multiorgan complications in adults [2, 3]. *Plasmodium* species have a complex biology, as the parasite invades different cells throughout its lifecycle within the

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human host, including both liver and blood stages of infection. Major strides against malaria have been made in recent years [1], but significant roadblocks to eradication remain. The most effective intervention strategies have targeted the *Anopheles* mosquito vector, and although they led to dramatic declines in parasite prevalence in many regions, parasite numbers rebounded after the interventions were removed [4]. Malaria vaccine strategies have been hindered by the population diversity of parasites [5, 6] and performance in the field that does not match efficacy in early clinical studies [7], while malaria parasites have developed resistance or tolerance to nearly every anti-malarial drug deployed to the field [8].

It has been suggested that host-targeted interventions, which have been more extensively explored for viral and bacterial diseases [9], are a reasonable strategy against malaria [10]. However, host-directed interventions against severe malaria disease have been largely unsuccessful [11] and no host-directed interventions against the liver stage have reached the clinic. Yet, new molecular insights into parasite-host interactions provide the opportunity to revisit the potential of host-directed interventions. A growing collection of inhibitors developed for use against non-infectious disease, with known targets and limited side effects, which target fast-acting cellular processes such as phosphorylation, provide a wealth of knowledge to inform drug repurposing. Importantly, many kinase inhibitors work by **polypharmacology** (see glossary) and interfere with multiple targets or disease pathways. This may be advantageous because malaria pathogenesis is complex and several host pathways are implicated in parasite development in hepatocytes. Here, we review points of host-dependence for the parasite and propose strategies for designing host-targeted interventions that aim to (1) eliminate parasites at points in the life cycle where conventional parasite-directed interventions are insufficient and (2) modulate host inflammatory responses and repair damage to the **blood-brain barrier** (BBB) in CM.

Host-targeted therapy against liver stage parasites

Halting malaria at the liver stage (LS) (Figure 1) is an attractive prospect because it precludes disease symptoms and production of transmissible stages of the parasite. Additionally, one of the major hurdles to malaria eradication is the persistence of *P. vivax* **hypnozoites**, dormant liver stages that can remain within the host hepatocyte for years and lead to relapses (Figure 1). Hypnozoites can only be eliminated with a single licensed drug, primaquine, and the use of primaquine is hampered by side effects and severe toxicity in individuals with glucose-6-phosphate dehydrogenase (G6PD)-deficiency [12]. Much of our knowledge to date on LS infection is based on rodent-infectious *Plasmodium* species, and there is a dearth of knowledge about *P. vivax*, due to the technical challenges of culturing *P. vivax* parasites in the lab. The extent to which our knowledge of LS biology holds true for *P. vivax*, and hypnozoites in particular, remains unknown, although genomic and proteomic studies suggest *P. falciparum* and *P. vivax* have at least partially overlapping metabolic capacities during liver stage schizogony [13].

The selectivity and host-dependence of LS parasites, makes host-targeted therapeutics a viable approach for control of infection. Host-targeted intervention at the LS could function in several ways: (1) prevent sporozoites from invading hepatocytes, (2) alter the host cell so that sporozoites are unable to select hospitable cells, causing them to infect cells that are

unable to support growth, (3) allow for infection but prevent maturation or exit. Treatments that arrest parasite development or clear infection after invasion may also promote protective immunity [14], as has been seen with live attenuated *Plasmodium* strains [15].

Host cell dependencies in LS parasite growth and differentiation

Not all hepatocytes are equally susceptible to, or suitable for, *Plasmodium* infection. Differential susceptibility has been observed among different human hepatocyte donors [16, 17], between mouse strains [18], and between hepatocytes within a single individual [19]. Several cell intrinsic properties that influence invasion have been described, including ploidy and expression levels of CD81, Scavenger Receptor BI, and Ephrin type-A receptor 2 [19–23]. Furthermore, the parasite's ability to select hospitable hepatocytes for invasion is crucial to maintenance of infection as selection of the wrong cell can lead to parasite clearance [22], although few host-targeted monotherapies completely prevent infection. The selectivity of the parasite and the regenerative properties of the liver allow for the possibility of prophylactically targeting hospitable hepatocytes or altering their biology in such a way as to prevent the parasite from identifying them.

LS parasites and host cell metabolism

Once they enter the liver, LS parasites undergo dramatic growth and replication from initial hepatocyte invasion to the release of merozoites (Figure 1). While the parasite can synthesize many of the cellular components for this growth, they also depend heavily on the host cell for nutrients. For many of these nutrients, the requirements of the parasite may exceed the requirements of uninfected cells, creating opportunities for selective targeting of infected cells. For example, infected hepatocytes have increased levels of the water and glycerol channel aquaporin-3 [24], and glucose transporter GLUT1 located at the plasma membrane and increase their glucose uptake beginning 30 hour post-infection. Glucose concentrations *in vitro* can be reduced to within a range that reduces LS infection but does not alter host cell viability [25]. Biotin and several classes of host lipids have been identified as contributing to successful LS development [26–29]. Knockdown of the *Plasmodium* fatty acid synthesis II pathway and phospholipid synthesis enzymes allowed for hepatocyte invasion and initial development but prevented the formation of merozoites, suggesting both host and parasite sources of lipids are needed for merozoite production [30–33]. Altering the fat content of hepatocytes through therapeutic or dietary [34] interventions could reduce LS infection.

Of particular interest from the perspective of host-targeted drug development, are infection-induced modifications of the host cell that resemble those seen in metabolic diseases. Targeting the molecular players in metabolic diseases would facilitate the use of ongoing drug development efforts. For example, endoplasmic reticulum (ER) stress restores cellular homeostasis by increasing protein folding and is activated in the context of type II diabetes, fatty liver disease, and viral hepatitis [35, 36]. ER stress is also induced in hepatocytes in response to LS infection and promotes infection maintenance at 24 hours post-infection, when parasite DNA replication has begun [37, 38]. Inhibition of AMP-activated protein kinase (AMPK), hypoxia, and increased levels of Hypoxia-inducible factor 1-alpha (HIF-1 α), conditions consistent with induction of ER stress, the Warburg effect, and

increased cell proliferation, all promote LS infection [39–42]. Hypoxia is a common feature of type II diabetes and liver diseases, and HIF-1 α expression is elevated in liver diseases, including nonalcoholic fatty liver disease and alcoholic liver disease [43, 44]. An AMPK agonist used to treat type II diabetes reduced *P. berghei* LS burden *in vivo* [41, 45]. Gaining a more comprehensive understanding of the altered metabolic state of infected hepatocytes may allow for the adaptation of existing drugs used to treat metabolic disorders to LS infection. Treatment post-infection is feasible in the case of *P. vivax* hypnozoites but only prophylaxis is feasible for other species as liver stage is brief and asymptomatic. Utilizing a prophylactic host-targeted drug could be envisioned for traveler's medicine, especially in areas where *P. vivax* is highly endemic to prevent hypnozoite formation, as well as, in areas with high intensity *P. falciparum* seasonal transmission since drugs developed for metabolic disease are typically intended for longer-term use.

LS parasites and host cell death

The potential for host-targeted therapeutics for LS infection is evident when examining the regulation of host cell survival post-infection. *Plasmodium*-infected hepatocytes are largely resistant to autophagy [46] and to external inducers of apoptosis [47, 48]. However, infected cells may be highly dependent on a single protein or pathway to avoid programmed cell death, a phenomenon that has been characterized in cancer and coined “**oncogene addiction**” [49]. This allows the selective targeting of aberrant cells, either cancerous or infected, while minimally affecting uninfected cells. For example, *Plasmodium*-infected cells are highly susceptible to mitochondria-driven apoptosis [48]. Treatment with chemical inhibitors of the B-cell lymphoma 2 (Bcl-2) family, which block mitochondrial pro-apoptotic signaling, significantly increased apoptosis of infected hepatocytes and are effective at concentrations below those used to treat cancer cells [48, 50]. Levels of the tumor suppressor protein p53 were found to be lower in infected hepatocytes and preventing p53 degradation with chemical inhibitors reduced *Plasmodium* LS infection [48, 50, 51]. Because Bcl-2 proteins and p53 were found to influence infected cell death through distinct mechanisms, the combined treatments dramatically reduce LS burden, demonstrating the potential for combination host-targeted therapeutics, which may impede the development of drug resistance [50].

The use of polypharmacology to target infected hepatocytes

Whole transcriptome and kinome screens suggest that *Plasmodium* infection dramatically alters signaling networks within hepatocytes [52–54]. Recent evidence also suggests that alterations in signaling in infected cells may change how cells respond to extrinsic stimuli (Glennon et al, under review) and provide targets for host-based intervention that are unique to infected cells. Adapting kinase regression (Box 1) to *Plasmodium* LS infection identified 47 host kinases whose activity is predicted to be important for maintenance of infection, as well as chemical inhibitors that target those kinases. These included FDA approved drugs which showed strong potency when tested *in vitro* [53], potentially due to “off-target” activity or activity against several kinases. Utilizing this polypharmacology allows for the targeting of multiple host signaling pathways and may reduce LS infection more robustly than a host- or parasite-targeted drug with a single target (Box 1). Moreover, the possibility

of drug repurposing can accelerate the development of new host-directed therapies for malaria and other infectious diseases (Figure 2).

Hypnozoites

Upon invading hepatocytes, *P. vivax* sporozoites form either dividing schizonts or non-dividing hypnozoites (Figure 1). Hypnozoites increase in size slightly over time and are thought to be metabolically active [55]. Numerous host pathways have been implicated in the growth and differentiation of LS parasites and it remains to be explored which of these and/or other host pathways are critical for the maintenance of hypnozoites. Recent studies on the hypnozoite transcriptome of *P. vivax* and *P. cynomolgi*, a related non-human primate malaria parasite, may also shed light on host processes and metabolites that are critical for parasite survival and inform the development of host-targeted therapeutics [56, 57].

In addition to targeting the host cell to eliminate dormant hypnozoite forms, host-targeted therapeutics could also be used in conjunction with existing antimalarials to activate hypnozoites and kill the resulting blood-stage parasites. The timing of *P. vivax* relapse ranges from weeks to months, with rapid rates of relapse occurring predominantly in tropical regions and prolonged relapses in temperate areas, corresponding with the continual or seasonal presence of mosquito vectors in each area [58]. Nearly nothing is known about host factors that influence relapse, although subsequent infection with *Plasmodium*, or other parasitic or bacterial pathogens, has been hypothesized to trigger hypnozoite activation [59, 60]. Comparisons with bradyzoites, the quiescent stage of *Toxoplasma gondii*, also suggest environmental stress, such as oxidative stress or changes in temperature, may trigger hypnozoite activation [61], although how accurate these comparisons are remains to be determined.

Hypnozoite infection is clinically silent and currently no biomarkers of infection have been identified in humans, making targeted treatment challenging. Recently, however, exosomes containing parasite proteins were detected in the plasma of human liver-chimeric mice infected with *P. vivax* hypnozoites [62]. The development of new *in vitro* and *in vivo* models of liver stage parasite development [57, 63, 64] [Table 1] has unlocked new possibilities to study this cryptic stage of parasite development and to evaluate interventions. These new experimental platforms will facilitate the investigation of host cell requirements for hypnozoite maintenance and may reveal new prophylactic therapeutic targets to eliminate or selectively activate hypnozoite reservoirs in the liver.

Host-targeted therapy against transmissible parasite stages

Mosquito to human transmission

During the blood meal, *Anopheles* mosquitoes deposit dozens to hundreds of *Plasmodium* sporozoites into the dermis (Figure 1). A great deal of our current knowledge of the parasite in the skin comes from intravital microscopy studies of rodent malaria parasites in mice [65, 66]. Malaria sporozoites interact with a variety of cell types before crossing the blood-vessel wall [65] and can traverse through cells in order to exit the dermis and enter the blood vessel. While the process of cell traversal is thought to occur in a cell-type-independent

manner [67], it remains unknown if it requires a ubiquitous host cell factor that could be targeted for intervention. Additional investigation into this biological property of sporozoites may result in molecular entities that could be targeted. Since no more than a few sporozoites transmitted in each bite successfully navigate the skin and liver sinusoid, even a slight enhancement of the host defense within the dermis or within the liver sinusoid might have the capacity to dramatically reduce liver infection, and subsequent disease and transmission. The host factor CD68 has been suggested to facilitate traversal through liver resident Kupffer cells, which might also serve as a target for host-based intervention [68].

The host response that is elicited prior to hepatocyte infection may also play a role in shaping the immune response to pre-erythrocytic malaria in the field. A single malaria vaccine candidate that elicits antibodies to the *P. falciparum* circumsporozoite protein (CSP) [69], has been licensed: RTS,S/AS01. Previous studies have demonstrated that antibodies against CSP immobilize the parasite in the skin [66]. Clinical studies have suggested that immunogenicity to RTS,S/AS01 differs between naïve individuals and those that have had previous exposure to *Plasmodium* infection [7, 70–72]. One hypothesis for this major discrepancy is that pre-existing immunity to malaria reduces vaccine efficacy in endemic areas. This suggests that antibody-dependent immune mechanisms within the skin contribute to natural malaria immunity, but they may be insufficient to prevent infection, or are even detrimental to the induction of long-lived, effective immune responses. It is possible that host-targeted interventions could be developed to increase the immunogenicity of whole parasite and/or subunit vaccines and improve vaccine efficacy.

Human to mosquito transmission

During the intraerythrocytic cycle, some parasites become gametocytes, the sexual stage which is transmissible to mosquitoes. Most antimalarial drugs target asexual parasites, but do not prevent gametocyte development and parasite transmission to mosquitoes. Gametocytogenesis occurs in infected erythrocytes (IEs) (Figure 1). Gametocyte levels in peripheral blood correlate with transmission efficiency, although submicroscopic levels still contribute to transmission [73]. *P. vivax* gametocyte-IEs appear within the blood earlier than *P. falciparum*, often before the onset of disease symptoms, making transmission possible before the infection has been detected and limiting the effectiveness of antimalarial drugs in preventing transmission. By comparison, early stage *P. falciparum* gametocyte-IEs predominantly sequester within the bone marrow and undergo an 8–10 day development period before reemerging into the blood circulation as mosquito-infective, stage V forms [74–76] (Figure 1). Host-targeted therapeutics that reduce gametocyte development, prevent the return to peripheral circulation, or skew the ratio of male to female gametocytes could limit transmission, however, very little is currently known about how host factors regulate these processes [75–79]. Host-directed therapies could be given with anti-parasitic drugs in mass drug administration approaches to eliminate host reservoirs of transmissible parasites, or given to travelers on a temporary basis (Figure 1). Mammalian host factors can also signal within the mosquito midgut upon ingestion to alter parasite transmission [80]. The conservation of signaling, such as the insulin signaling and mitogen-activated protein kinase (MAPK) cascades [81, 82], creates the possibility for dual-acting therapeutics that reduce disease and transmission by acting on both human and mosquito hosts. For example,

abscisic acid, reduced parasitemia in a mouse model of malaria and reduced parasite survival in the mosquito midgut by enhancing the immune responses of the mammalian and mosquito hosts independently of any direct effect on *Plasmodium* [83–85].

Host-targeted therapy against parasite invasion of erythrocytes

Parasites released from the liver enter the bloodstream and begin a cycle of asexual replication consisting of erythrocyte invasion, intraerythrocytic growth and replication, and egress from mature IEs. Asexually replicating parasites, which are responsible for malaria disease symptoms, are effectively targeted by current anti-malarial drugs. Thus, there is not a pressing need for host-targeted therapy against erythrocyte invasion, however, such therapy may potentially augment parasite elimination achieved by parasite-targeted drugs. Two erythrocyte receptors, basigin (BSG) and CD55 are essential for parasite invasion of erythrocytes [86, 87], making them attractive therapeutic targets. However, any blockade of these receptors would have to account for their expression on other cell types, and their role in physiological processes in the body [88–90]. An alternative strategy that has been proposed is the use of soluble receptor [88], although recombinant proteins are expensive and present more complex storage issues than small molecule drugs for resource-poor settings.

The development of various *in vitro* [91] and *in vivo* models [92] will enable testing of candidate invasion-blocking host-targeted therapies. A recently developed human liver-chimeric mouse model that maintains human erythrocytes (FRGN huHep/huRBC) was used to demonstrate that an antibody targeting PfRh5, the parasite ligand for BSG, blocks erythrocyte invasion [93]. These mice and improved versions could also be used to test candidate therapies that target intraerythrocytic parasites.

Host-targeted therapies for cerebral malaria (CM)

CM is a severe neurological complication of *P. falciparum* infection characterized by coma and infected erythrocyte (IE) sequestration in brain microcirculation (Figure 1). The pathophysiology of CM is complex. Whereas CM is considered to be a largely neurological complication in children [94, 95], adult CM frequently occurs in conjunction with kidney or lung complications that interact to increase fatality risk [96]. Significant gaps remain in our understanding of the pathogenesis of CM, however, three major processes have emerged as being important: 1) occlusion of blood vessels by sequestered parasites resulting in impaired tissue perfusion [97, 98], 2) increased permeability of the BBB secondary to IE sequestration in cerebral microvasculature [94, 99–102], and 3) the host pro-inflammatory and pro-thrombotic response to high parasite burdens [103–108]. The relationship between these pathological mechanisms is influenced by both parasite and host determinants, and manifests to different extents in children and adults. Therefore, interventions may need to be tailored with age-dependent differences in mind. In the ensuing sections, we will discuss interventions that target the major pathogenic processes in CM (Figure 3). For a review of general severe malaria adjunctive therapies, we refer the reader to [11].

De-sequestration strategies

The primary goal of **de-sequestration therapy** is to clear obstructed vessels of adherent IEs, which may relieve hypoxic stress caused by decreased tissue perfusion, and remove pro-inflammatory and pro-thrombotic parasite stimuli. Alternatively, desequstration therapy may be targeted to reverse **pathogenic binding interactions** that interfere with protective host pathways. IEs adhere to endothelial cells via binding of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) to endothelial receptors, including intercellular adhesion molecule 1 (ICAM-1), CD36, heparin sulfate, and endothelial protein C receptor (EPCR) [109, 110]. The IE-EPCR6 interaction is strongly associated with severe disease and specifically with brain swelling in CM [111–113], a major risk factor for death [102]. Furthermore, some PfEMP1 encode dual EPCR and ICAM-1 binding properties [114, 115]. Thus, de-sequestration strategies may need to account for combinatorial PfEMP1 binding interactions.

Studies using recombinant PfEMP1 domains have suggested that IEs block the binding of EPCR's natural ligand, protein C, thereby inhibiting its activation and impairing the endothelial cytoprotective and anti-coagulant properties of the EPCR-activated protein C (APC) pathway [111, 116–118]. Coupled with loss of EPCR on cerebral microvessels [107], parasite blockade may lead to excessive pro-inflammatory and pro-thrombotic signaling in brain microvasculature. Disrupting the PfEMP1-EPCR interaction may assist in restoring this **cytoprotective host pathway**. Monoclonal antibodies that target host receptors such as EPCR and ICAM-1 partially inhibit cytoadherence [114, 115], but their therapeutic utility remains unknown. Furthermore, EPCR-binding PfEMP1s have a similar binding footprint as the native ligand, APC [119]. Thus, therapeutic anti-EPCR antibodies or single domain antibody fragments (nanobodies) will have to be engineered so as not to inhibit EPCR-APC interactions. An alternative approach being explored is recombinant soluble EPCR variants that bind PfEMP1 with high affinity but do not bind APC [117]. As discussed above, receptor blockade or soluble receptor strategies based on recombinant proteins present cost and storage issues that are challenging for resource poor settings.

Another de-sequestration therapy in development targets PfEMP1s that harbor the DBL α 1 domain shown to bind heparin or heparin-sulfate [120]. These PfEMP1s are frequently involved in rosetting of IEs with uninfected erythrocytes, a phenotype that may lead to greater microvascular obstruction [121]. Sevuparin, a heparin analog that lacks the antithrombin III-moiety and consequently has reduced anti-coagulant activity, caused transient de-sequestration of *P. falciparum* in adult patients with uncomplicated malaria treated with atovaquone/proguanil [122]. It has the added benefit of inhibiting merozoite invasion, but it remains to be seen if sevuparin will be effective in severe malaria patients treated with artesunate.

A major challenge with de-sequestration strategies is that PfEMP1s constitute a large and diverse protein family with cooperative binding between PfEMP1 domains. Desequestration strategies must overcome allelic variation between PfEMP1 sequences, as well as target key binding interactions contributing to the overall cytoadhesive activity of a given PfEMP1 variant. To ensure success, it will be important to determine if key PfEMP1-receptor

interactions are common between different virulent PfEMP1 forms. While immune sera mediated de-sequestration in a *P. falciparum* squirrel monkey model of malaria [123], it was ineffective in pediatric CM patients [124]. More research is needed to determine what makes an effective de-sequestration therapy.

Reducing endothelial dysfunction

Several processes have been implicated in **endothelial dysfunction** in CM, including localized dysregulation of clotting [94, 95, 107], excessive release of pro-inflammatory cytokines [104, 105], and disruption of the BBB [100, 101, 125]. The pathways that underlie these processes provide opportunities to intervene therapeutically.

As mentioned above, IEs potentially impair the EPCR-APC pathway (reviewed in [126]). Exogenous APC could potentially help restore BBB integrity by dampening the effect of thrombin or *P. falciparum* histone-induced permeability [127, 128] or counteracting endothelial activation by inhibiting the release of effectors such as IL-8 [128] and angiopoietin-2 (Ang-2) from **Weibel-Palade bodies**. Indeed, APC improved microcirculation in cases of sepsis [129]. There are some case reports of APC efficacy in severe malaria, but it has yet to be tested in a clinical trial. Like APC, exogenous Ang-1 may be effective at reducing endothelial activation in CM [130]. The host cell signaling pathways downstream of APC and Ang-1 may also be targeted for therapeutic benefit.

Several parasite products initiate barrier-disruptive signaling in endothelial cell monolayers and present opportunities for host-targeted therapy. For example, histidine-rich protein 2 (HRP2), a parasite protein that is released upon rupture of IEs and is a marker of disease severity [131, 132], caused disruption of brain endothelial barriers through activation of the inflammasome [133]. Other endothelial-activating and permeability-inducing stimuli include *P. falciparum* histones, thought to be partially dependent on Src family kinases [128], soluble factors from lysis of IEs [134], merozoite-associated proteins, and *P. falciparum* glycosphosphatidylinositols and food vacuoles [135, 136]. Modulation of angiotensin signaling, either via blockade of the angiotensin II type 1 receptor (AT1) or stimulation of the angiotensin II type 2 receptor (AT2) blocked parasite-induced β -catenin-dependent barrier disruption *in vitro*, and is being considered as a barrier-strengthening strategy [134].

Several studies have reported apoptosis of endothelial cells on exposure to *P. falciparum* [134, 137, 138]. While apoptosis was a major permeability mechanism in the study implicating β -catenin signaling [134], the kinetics of cell death suggested it was not the pathway that led to HRP2-induced endothelial barrier disruption [133]. *In vivo* evidence of apoptosis in brain tissue may help to clarify whether it is an important mechanism of BBB disruption.

RBC products released upon lysis of bystander RBCs may contribute to endothelial dysfunction. In a recent trial with adult CM patients, acetaminophen was evaluated as a renoprotective adjunctive therapy to reduce oxidative damage caused by cell-free hemoglobin. This approach helped protect against kidney injury in adults with severe and moderately severe malaria [139]. As kidney complications increase mortality risk from CM, this intervention may also have benefit in the context of adult multi-organ complications.

Hemoglobin also scavenges nitric oxide, the major vasorelaxant in microvessels and principal contributor to endothelial quiescence. Arginases decrease the bioavailability of L-arginine, the precursor of nitric oxide. This likely explains why children and adults with severe malaria have decreased bioavailability of nitric oxide and lower concentrations of L-arginine [140, 141]. Unfortunately, clinical trials delivering nitric oxide to CM pediatric patients through inhalation (iNO) have not been successful [142, 143] though there is some indication that iNO may improve neurocognitive function in children [144]. Other pathways to increase NO signaling would be worthwhile to explore.

The myriad pathways implicated in endothelial dysfunction in severe/cerebral malaria make a strong case for the application of polypharmacology to host-targeted therapy. Kinase inhibitors could prove very useful (Box 1). Kinases regulate many endothelial cell junction proteins, contributing to maintenance and integrity of the endothelial barrier [145, 146]. Multiple studies that observed permeability in endothelial cell monolayers upon exposure to various parasite stimuli detected abnormalities in the cellular distribution of adherens junction, tight junction and focal adhesion proteins [133, 134, 136], suggesting dysregulation in phosphorylation. In support of this, pretreatment of dermal endothelial cells with PP1, an inhibitor of Src family kinases, restored the discontinuous staining of the tight junction protein, ZO-1 and blunted the ability of *P. falciparum* merozoite proteins to induce permeability [136]. Importantly, patterns of discontinuous and overall diminished staining of junctional proteins (ZO-1, occludin, and vinculin) are also observed in vessels from fatal CM cases [99]. Further evidence that kinase inhibitors may restore the integrity of the BBB is seen in the ability of fasudil, a Rho kinase inhibitor, to help repair a disrupted lung endothelial monolayer after adhesion of IEs [147]. Additional points at which kinase inhibitors could be beneficial, include modulation of the Ang1/2-Tie-2 axis [148] and actomyosin contractility [146].

The main challenge to host-targeted therapy that aims to reduce endothelial dysfunction is our incomplete understanding of the interplay of pathways that results in vascular dysfunction. Despite the progress made, many questions remain. Do all pathologic processes converge on a common pathway? Are some pathways more important than others? The answers to these questions will aid efforts to develop successful targeted therapies for CM. The use of higher strength magnetic resonance imaging (MRI) machines to image the brain of CM patients has confirmed and provided new insights into processes that underlie brain swelling in children and adults [100, 101]. **Vasogenic edema** and posterior reversible encephalopathy syndrome (PRES) were common findings in two studies [100, 101], whereas **cytotoxic edema** was identified in one study [100], but was not present in the other [101]. State of the art neuroimaging paired with improved *in vitro* models of the BBB (Table 1) may clarify mechanisms. Ultimately, adjunctive therapy that corrects multiple pathologic processes may be needed to reverse or decrease brain swelling, an important endpoint for host-targeted therapy given the strong correlation between severe brain swelling and death.

Immunomodulation

The goal of immunomodulatory host-targeted therapy is to dampen the excessive pro-inflammatory signaling that occurs during severe malaria, and thereby reduce endothelial

activation and platelet and monocyte recruitment, which may continue even after parasite killing. Fatal pediatric CM cases expressed significantly elevated levels of tumor necrosis factor (TNF) [104, 105], IL-1 α [105], CXCL10 and CXCL4 [108] implicating these cytokines and chemokines in CM pathogenesis.

Several immunomodulators tested in humans have been largely unsuccessful [11], underscoring the difficulty of finding the right balance between an anti-inflammatory and a pro-inflammatory state. Even for a single immunomodulatory drug, pentoxifylline, tested in both children and adults, there were contradictory results across five clinical trials ([11] and references therein). So far, rosiglitazone, a peroxisome-activated receptor- γ (PPAR- γ) agonist approved for the treatment of type-2 diabetes, is one of the most promising immunomodulator candidates. It has been shown to reduce the inflammation induced in brain disease/injury of various kinds [149]. In clinical trials with uncomplicated malaria patients, it was safe and well-tolerated [11] and enhanced parasite clearance [150]. Rosiglitazone is also reported to have neuroprotective and anti-oxidant properties [149, 151], and may exert these effects in malaria treatment [152]. A clinical trial is in progress to test rosiglitazone as an adjunct to artesunate treatment in severe malaria (NCT02694874).

Immunomodulation may also be achieved by strengthening counter-inflammatory pathways. Several small molecules are being tested that are reported to have anti-inflammatory properties among other benefits, for example, curcumin, atorvastatin and erythropoietin (reviewed in [11]). Knowledge of the anti-inflammatory pathways that they act upon may reveal opportunities for drug development. The nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, which is a transcriptional regulator of genes encoding antioxidant proteins, is activated by dimethylformamide and is used in the treatment of multiple sclerosis and psoriasis [153]. It will be valuable to test the utility of Nrf2 activators in severe malaria.

As above, a challenge of anti-inflammatory cytoprotective strategies is the variety of pro-inflammatory processes implicated in endothelial dysfunction in CM. Host-directed therapy that can simultaneously target multiple pathways may be necessary to restore endothelial function (Box 1).

Concluding Remarks

The *Plasmodium* parasite moves through different organs in the human host while undergoing changes that enable it to survive, thrive and transmit. As effective as current artemisinin-based antimalarials are, many people still die from severe malaria. Furthermore, there are limited drug options to target gametocytes and the only drug for clearing *P. vivax* hypnozoites is contraindicated in people with G6PD-deficiency. Host-targeted therapy could revolutionize the prevention and treatment of malaria.

A common theme that has emerged from our growing knowledge of malaria infection is the network of host pathways that the parasite either relies on or dysregulates that can potentially be targeted. There is increasing appreciation that many drugs work via polypharmacology and that this feature can be exploited to simultaneously target multiple pathways (Box 1). The growing collection of small molecule drugs used to treat diseases like

cancer, diabetes or multiple sclerosis, may be repurposed for malaria. As described above, some of these drugs already show efficacy in malaria studies. The major limitation to more of such studies is the slowness with which we are building our understanding of host-parasite interactions at specific stages of malaria infection. These efforts will be significantly aided by an *in vitro* culture system for *P. vivax*, a better *in vitro* model of the BBB, and the application of systems biology approaches to the study of malaria (see Outstanding Questions) [154]. The increasing range of host factors that is responsible for parasite development and malaria pathophysiology offer new opportunities for host-directed therapeutics in malaria and encourage more effort to bring these new therapeutic approaches to the clinic and the people that need them most.

Acknowledgements:

We acknowledge Adriana Lippy for illustrations. This work was supported by National Institutes of Health grants R01GM101183 (AK), T32 Post Doctoral Fellowship AI07509 (EKKG), and NIH RO1 HL130488 (JDS).

Glossary

Blood-brain barrier

the network of endothelial cells and perivascular cells that maintain a highly selective barrier, preventing the movement of most substances and cells from blood into the underlying brain tissue

Cytoprotective host pathway

signaling pathways that may result in anti-inflammatory, anti-apoptotic and anti-coagulant activity, as well as preservation of endothelial barrier integrity

Cytotoxic edema

edema that occurs when cells swell due to dysfunction of sodium/potassium pumps, which causes an accumulation of sodium ions and water in cells

De-sequestration therapy

therapy that detaches already adherent IEs. It is distinct from anti-sequestration agents, which prevent the binding of IEs to endothelial cells

Endothelial dysfunction

any deviation from the normal activity of endothelial cells to regulate blood flow and vessel wall permeability

Hypnozoite

liver-resident malaria parasite that does not replicate its DNA upon initial infection and is the origin of relapsing infection

Oncogene addiction

The reliance of a cancer cell on a single gene for survival

Pathogenic binding interaction

IE-receptor binding interactions that interfere with normal endothelial cell physiology, and contribute to disease processes.

Polypharmacology

the property of small molecules to have multiple targets

Vasogenic edema

in the case of the brain, it is edema that results from disruption of the blood-brain barrier, which allows fluid and other substances from the blood to leak into extracellular space

Weibel Palade bodies

membrane-bound storage vesicles whose contents are released via exocytosis under conditions of endothelial cell activation

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Trends Box

- Host-targeted therapy is a strategy for eliminating or reducing symptoms of infectious disease, and holds promise for application to malaria
- New insights into the malaria parasite-host interaction may lead to novel interventions to target liver-dormant forms called hypnozoites or to counteract the complications associated with cerebral malaria.
- Systematic approaches that exploit the polypharmacology of drugs developed for other indications could facilitate repurposing drugs that impact one or more points in the malaria parasite life cycle and also address severe complications of the disease.

Outstanding Questions Box

- To what extent are host factors determinants of infection across *Plasmodium* life cycle stages?
- Can we exploit polypharmacology of small molecule inhibitors, or broadly important signaling cascades, to target multiple parasite life stages?
- What host factors regulate hypnozoite formation, maintenance, and reactivation?
- Can drug polypharmacology be exploited to restore BBB integrity?
- For de-sequestration therapies, what are the key receptor interactions that need to be abrogated to relieve blocked microvessels?
- Are there common pathways in the development of brain swelling in CM that can be targeted for treatment?
- Can host-targeted therapeutics promote immune-mediated protection from subsequent infection?
- Can host-targeted interventions be designed to eliminate multiple *Plasmodium* species or co-infections?

Box 1.**Kinase Regression**

Humans have 518 kinases, which are involved in a wide range of cellular processes. Kinase inhibitors are a major component in the pharmacological pipeline with 38 inhibitors approved to date, predominantly for the treatment of cancer (reviewed in [155]). Although kinase inhibitors are often described as inhibiting a single kinase or family of kinases, many of them exhibit **polypharmacology**, influencing the activity of a wide range of kinases. This has important implications both for the use of kinase inhibitors as research tools and as therapeutics.

Recently, an approach called kinase regression (KiR) (Figure I) was developed to use **polypharmacology** to deconvolve kinase inhibitor targets [156]. KiR integrates experimental data from a small-scale kinase inhibitor screen with published biochemical data on the inhibitory activity of 178 kinase inhibitors against 300 human kinases [157] using a machine learning algorithm. The algorithm predicts kinases that contribute to the regulation of a specific phenotype, as well as kinase inhibitors that exhibit the same phenotype. KiR has been applied to study cell migration and metastasis [156, 158] and was recently utilized to identify host kinases that regulate *Plasmodium* LS infection [53]. Forty-seven host kinases were predicted to influence the maintenance of LS infection, many of which had not previously been studied in the context of malaria, as well as several FDA-approved kinase inhibitors which showed high efficacy *in vitro* [53]. This type of approach may be particularly relevant when determining host points of susceptibility for field-isolated samples, where resources are limited. Furthermore, the ubiquity of kinase inhibitors in publicly available compound libraries will make it easier to test the efficacy of kinase inhibitors in the laboratory.

As therapeutics, chemical inhibitors that exhibit polypharmacology have the potential to be more potent by targeting multiple host processes. Additionally, targeting more than one host component may limit the development of resistance to a single therapeutic. Understanding and exploiting the polypharmacology of kinase inhibitors has become a major focus for the development of cancer therapeutics [148] and is currently a relatively untapped resource for the repurposing of drugs for use against malaria and other infectious diseases.

Figure 1. The complexity of the malaria parasite life cycle and opportunities for host-targeted therapies.

The *Plasmodium* parasite undergoes extensive cell biological changes throughout its life cycle within the human host, accompanied by expansions and contractions in parasite numbers starting from (A) the small number of parasites that are initially deposited in the dermis by the bite of an *Anopheles* mosquito. Within the dermis most parasites are eliminated, but a small number cross into a blood vessel and then are carried within the blood circulation to (B) the liver where they traverse the sinusoidal endothelium to invade hepatocytes. Both *P. falciparum* and *P. vivax* undergo LS schizogony, in which parasites asexually replicate within the hepatocyte to produce upwards of 20,000 merozoite parasite forms over 7–10 days. Some species of parasites (e.g. *P. vivax*) also form dormant parasite forms, called hypnozoites, which can reactivate later. Once parasites complete LS development, the merozoites reenter the bloodstream to infect erythrocytes and undergo repeated cycles of asexual development. (C) During the asexual blood stage of *P. falciparum*,

infected erythrocytes exit the blood circulation by cytoadhering to vascular endothelium, using members of a clonally variant gene family called PfEMP1, expressed on the surface of the erythrocyte. The site of endothelial cytoadhesion is associated with organ-specific disease complications, most prominently in cerebral malaria. **(D)** During intraerythrocytic replication, parasites can also differentiate into sexual forms, called gametocytes, which sequester in bone marrow to complete their maturation before reemerging as stage V forms that are transmissible to mosquitoes. Opportunities for host-targeted intervention can be envisioned at multiple points within the malaria life cycle, either as prophylaxis, treatment, or adjunctive therapies.

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Figure 2. Exploiting polypharmacology and drug repurposing for host-targeted therapies in malaria.

Polypharmacology and repurposing of drugs have the potential to reduce the time, cost, and failure rate of production of malaria therapeutics, as well as create new options for host-targeted therapies. Calculations for de novo cancer therapeutic development estimate 10–17 years for de novo drug development with a 10% success rate, while drug repurposing requires 3–12 years, with a 30% success rate and only 50–60% of the cost [159, 160]. Potential compounds for repurposing can include those that are FDA approved, still within the development pipeline, or discarded for reasons other than safety. Many drugs exhibit polypharmacology, targeting more than one protein or process within a cell, and the ability to target multiple pathways may be what makes them efficacious in the context of malaria treatment. Therefore, rejected drugs, or drug derivatives, that passed human safety trials but were not carried forward could be effective against *Plasmodium* infection through a mode of action other than that for which they were originally developed.

Figure 3. Proposed host-targeted interventions to reverse the major pathogenetic processes in cerebral malaria.

(A) IEs accumulate in cerebral microvasculature by PfEMP1 binding to specific receptors on endothelial cells. The depicted PfEMP1 variant is a dual EPCR-ICAM-1 binder. Desequestration strategies include host receptor biologics such as anti-EPCR antibodies and modified soluble EPCR variants [117], both of which have yet to be demonstrated as desequestration therapies. Sevuparin, a modified heparin analog, caused transient desequestration of IEs in uncomplicated malaria patients [122]. **(B)** Parasite factors including PfHRP2 and histones, released during IE rupture, as well as host factors including Ang-2 released from activated endothelial cells and the soluble plasma protein thrombin, are believed to contribute to endothelial barrier disruption. EPCR-binding IEs block binding of APC to EPCR and impair the cytoprotective properties of endothelial cells, contributing to barrier disruption and a pro-coagulant and pro-thrombotic state. Host-targeted therapeutics include barrier-strengthening exogenous factors such as Ang-1 and APC, and small molecule modulators of host-signaling pathways. **(C)** Elevated levels of pro-inflammatory cytokines and chemokines (TNF α , CXCL10) contribute to excessive pro-inflammatory signaling, which exacerbates endothelial dysfunction. Immunomodulators such as rosiglitazone [150], may neutralize cytokines, boost anti-inflammatory mechanisms, and reduce pro-thrombotic signaling. This, in turn, may reduce endothelial cell activation and dysfunction, as well as the continual recruitment of platelet and monocytes in brain microvasculature. Abbreviations: IEs, infected erythrocytes; ICAM-1, intercellular adhesion molecule 1; EPCR, endothelial protein C receptor; PfHRP2, *Plasmodium falciparum* histidine-rich protein 2; Ang-2, angiopoietin-2; APC, activated protein C; TNF α , tumor necrosis factor alpha.

Figure I.

Kinase inhibitors (A and B) have varying and overlapping specificities for host kinases (1, 2 and 3) and have different effects on LS infection: inhibitor B reduces LS infection while inhibitor A does not. Kinase 3 is therefore identified as important for maintenance of LS infection. Inhibitor C can then be predicted to reduce LS infection based on its known activity against kinase 3. In this schematic the importance of 3 host kinases in maintaining LS infection are probed using only 2 inhibitors. Scaled up, kinase regression uses roughly 30 kinase inhibitors to predict the role of 300 kinases and the effect of 178 inhibitors on LS infection.

Table 1.

Models for malaria host-pathogen interactions.

Model	References
<i>In vivo</i> LS models	
<i>Plasmodium cynomolgi</i> non-human primate malaria model with dormant liver stages resembling <i>P. vivax</i>	[161]
Humanized liver-chimeric FRG huHep mouse model for <i>P. falciparum</i> and <i>P. vivax</i>	[162] [55]
Mice with human ectopic artificial livers (HEALS) that support <i>P. falciparum</i> LS infection	[64]
Hepatocyte co-cultures	
Micropatterned primary human hepatocyte co-cultures supporting <i>P. falciparum</i> and <i>P. vivax</i>	[16] [57]
Hepatocyte mono-cultures	
384-well plate primary human hepatocyte culture system for <i>P. falciparum</i> and <i>P. vivax</i>	[17]
Hepatocyte-like cell line imHC for <i>P. vivax</i>	[63]
Induced pluripotent stem cell-derived hepatocyte-like cells for <i>P. falciparum</i> and <i>P. vivax</i>	[163]
3D human microvessel and lumenized models	
Human umbilical vein endothelial cells grown in a lumenized collagen matrix	[164]
Human umbilical vein, lung and dermal endothelial cells grown in a lumenized hydrogel matrix	[165]
Perfusable microfluidic device with endothelial cells, astrocytes and pericytes	[166]