Trends in Parasitology

CellPress

Opinion

Developing kinase inhibitors for malaria: an opportunity or liability?

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Highly druggable and essential to almost all aspects of cellular life, the protein and phosphoinositide kinase gene families offer a wealth of potential targets for pharmacological modulation for both noncommunicable and infectious diseases. Despite the success of kinase inhibitors in oncology and other disease indications, targeting kinases comes with significant challenges. Key hurdles for kinase drug discovery include selectivity and acquired resistance. The phosphatidylinositol 4-kinase beta inhibitor MMV390048 showed good efficacy in Phase 2a clinical trials, demonstrating the potential of kinase inhibitors for malaria treatment. Here we argue that the potential benefits of *Plasmodium* kinase inhibitors outweigh the risks, and we highlight the opportunity for designed polypharmacology to reduce the risk of resistance.

Highlights

New antimalarial treatments that are safe, effective, and affordable are urgently needed to alleviate the malaria disease burden and combat drug resistance.

Drugs targeting *Plasmodium* kinases have the potential to deliver potent antimalarials with multistage antiplasmodium activity, but there are notable challenges, including off-target activity and acquired resistance.

A *Plasmodium* phosphatidylinositol 4--kinase beta inhibitor advanced to Phase 2a clinical trials for malaria, and there are ongoing malaria drug-discovery programs targeting other validated kinase targets.

There is an opportunity for the development of kinase inhibitors for malaria with designed polypharmacology to lower the risk of acquired drug resistance.

The malaria burden and the propensity for resistance

Malaria, a parasitic disease caused by protozoa of the genus *Plasmodium*, remains a serious public health concern that most severely affects children and pregnant women. There are five *Plasmodium* species that cause malaria in humans, with two predominant species: *Plasmodium falciparum* responsible for the most severe form of disease, and *Plasmodium vivax* causing a relapsing form of malaria. Whilst the combination of vector control and artemisinin combination therapies (ACTs) have historically been successful at reducing the global disease burden, this has stalled in recent years with 247 million cases and 619 000 associated deaths reported in 2021 [1]. Partial resistance with a delayed parasite clearance phenotype to ACTs is prevalent in Southeast Asia and has more recently been reported in malaria-endemic East African regions [2]. This highlights the need for new antimalarial agents with novel modes of action, and no cross-resistance, to be developed and deployed in case of complete failure of current first-line malaria therapies.

Requirements for next-generation antimalarials

To reduce the disease burden and aid in disease eradication, several key challenges for next-generation antimalarials need to be addressed. New drugs need to be cheap, with simple dosing regimens, well tolerated in vulnerable populations including in young children (<3 years) and pregnant women (particularly in the first trimester) and effective against **multidrug-resistant malaria** (see Glossary) [3]. In addition to drugs for treating malaria symptoms, **chemoprotectants** and transmission-blocking drugs are also desirable. To preserve the efficacy of new drugs, new medicines for malaria need to be developed as combination therapies, where individual compounds with different modes of action are coadministered, to slow down the inevitable emergence of drug resistance. This adds complexity to drug development as suitable partner drugs need to be identified and tested in combination during clinical development. Medicines for Malaria Venture (MMV), a not-for-profit public–private product development partnership with the goal of developing and facilitating the delivery of new antimalarial drugs, has established Target Product Profiles

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(TPPs) and Target Candidate Profiles (TCPs) for malaria to guide the development of new individual drugs that can be used in combination to meet the TPPs [3]. While the ideal treatment for malaria is a drug regimen that can be administered as a single-dose cure, this is a high bar for drug development requiring molecules with long half-lives that are well tolerated. This is particularly challenging given that the drug regimen needs to be safe in young children, pregnant women, and other vulnerable populations.

The risk of generating resistance to new drug molecules is a key consideration for the prioritization of compounds and the eventual selection of candidates. MMV has outlined a resistance risk assessment for candidate selections taking into account *in vitro*, *in vivo*, and *ex vivo* resistance studies to categorize molecules as high, medium, or low risk [4]. It is noteworthy that this only serves as a guide and how accurately these predictions translate to resistance in the field remains unclear. These studies assess the **minimum inoculum for resistance (MIR)**, resistance genotype, shift in effective drug concentration (EC_{50}), and pre-existing resistance in the field. Resistance risk can be associated with the mode of action and can be used to prioritize/deprioritize drug targets. *In vitro* resistance selections are routinely used to identify the mechanism of resistance and can aid in deconvoluting the primary mode of action with single-point mutations and/or copy number variations commonly occurring in genes corresponding to the molecular target. Target identification can be more challenging for compounds that do not readily yield resistance *in vitro*, termed 'irresistibles', but these compounds are attractive for drug development due to an apparent lower risk of resistance [5]. The irresistible nature of such compounds can likely be attributed to a resistance-refractory mode of action or **polypharmacology** involving multiple *Plasmodium* and/or host cell molecular targets. Historically, malaria drug discovery has relied on **phenotypic-based drug discovery** approaches, but over the past decade significant progress has been made in target identification and validation, and increasing emphasis is being placed on **target-based drug discovery** for malaria largely driven by the Malaria Drug Accelerator (MalDA) amongst others [5]. A combination of phenotypic and target-based approaches can provide a powerful strategy for drug discovery. Once a target is identified and validated for an initial phenotypic hit, a target-based approach can be implemented either to guide the optimization of the original hit or to identify new scaffolds against the novel target.

Moreover, structural information on the molecular target provides valuable information for drug optimization for both potency and selectivity relative to related human off-targets. However, one potential drawback of this approach is that the drug is typically optimized to act through a single protein target, making it more prone to resistance as point mutations at the drug-binding site can lead to a disruption in target binding and reduced efficacy.

The antimalarial potential of small-molecule kinase inhibitors

As a result of the successful application of small-molecule kinase inhibitors in oncology, as well as inflammatory and autoimmune diseases, kinase-targeted drug discovery has gained traction in the past decade [6]. Both protein and lipid kinases are generally interesting molecular targets because they are highly druggable and play key regulatory roles in signal transduction and a wide range of essential cellular processes. Most kinase inhibitors compete with ATP for the highly conserved ATP-binding site (classified as type I or type II kinase inhibitors), but inhibitors with a range of other binding modes have been described, including allosteric and covalent inhibitors [7,8].

The *P. falciparum* kinome consists of ~100 kinases [9]. While many of these kinases can be classified into established eukaryotic protein kinase groups, the *Plasmodium* kinome shows substantial genetic divergence from that of other eukaryotic organisms, and importantly the human

Glossary

Beta hemozoin formation: during asexual blood-stage development, parasites within the red blood cells rely on the breakdown of hemoglobin as a source of amino acids leading to the release of toxic free heme [64]. Beta hemozoin formation is the detoxification process that the parasite uses to convert free heme to an inert crystalline form referred to as hemozoin. This process takes place in the parasite's digestive vacuole. Many of the most effective antimalarials that have been used clinically, including chloroquine, accumulate in the digestive vacuole and interfere with this process. However, acquired resistance leading to drug efflux has reduced the efficacy of many of these drugs.

Chemoprotectants: drugs used to prevent malaria (rather than treat malaria), by inhibiting the development of hepatic schizonts. Long-acting drugs with asexual blood-stage activity can also be used for chemoprotection.

Drug repositioning: the use of a drug that is active in one disease as a template/starting point for the synthesis of derivatives active in another disease.

Drug repurposing: the use of an already marketed drug for a different indication (without any modifications to the molecular structure of the active ingredient).

Minimum inoculum for resistance (MIR): the minimum number of *P. falciparum* asexual blood-stage parasites in culture required to yield resistance when exposed to a drug at a defined concentration. MIR is dependent on the drug concentration used, typically $3 \times EC_{50}$ or $3 \times EC_{90}$, and is reported as $\log_{10}$ of the minimum parasite inoculum (e.g., MIR = 9 implies that a minimum of 10^9 parasites were required to select for resistance at a given drug concentration) [4]. The MIR is used to predict resistance risk (MIR ≤7 high risk; MIR between 7 and 9 moderate risk; MIR >9 low risk at drug concentration $3 \times EC_{90}$).

Multidrug-resistant malaria: malaria caused by *Plasmodium* parasites displaying reduced susceptibility to multiple classes of clinically used drugs.

Phenotypic-based drug discovery: drug discovery focused on optimizing compounds to alter the phenotype of a cell, tissue, or organism in a desired manner. This is typically done without knowledge of the mode of action but is

kinome. In addition to protein kinases, the *P. falciparum* genome encodes seven putative phosphoinositide kinases [10,11].

The focus on kinases in malaria drug discovery has grown with the identification and phenotypic validation of several vulnerable *Plasmodium* kinase targets. *Plasmodium* phosphatidylinositol 4-kinase beta (PI4Kβ) is the primary target of the 2-aminopyridine MMV390048 that reached Phase 2a clinical trials before development was stopped [12,13]. MMV390048 showed good safety in Phase 1 trials in healthy patients and impressive efficacy with fast-killing kinetics in a volunteer *P. falciparum* infection study [14,15]. In a Phase 2a trial, a single oral dose of 120 mg of MMV390048 cleared asexual blood-stage parasites in all eight *P. vivax* malaria patients in the treatment group and was well tolerated [13]. Despite the good efficacy in the clinic, Phase 2 trials were stopped based on data emerging from embryofetal studies in rodents, flagging potential risks of **teratogenicity** [16]. The cause of the inhibitor-induced species-specific developmental toxicity observed in rats (but not in rabbits) is still unknown, and whether this would occur in humans is unclear, but given the requirement that new antimalarials are safe in pregnant women, this posed sufficient risk for a decision by MMV to discontinue development. Despite MMV390048 showing remarkable selectivity for *Plasmodium* PI4Kβ relative to related human kinase off-targets in early *in vitro* studies [12], it has been speculated that inhibition of human kinases PI4Kβ, MAP4K4, and MINK1 may contribute to the observed toxicity – but this association has not yet been mechanistically proven [16]. It is important to note that the toxicity observed for MMV390048 is likely chemotype-specific and is not necessarily a general feature of *Plasmodium* PI4Kβ inhibitors as distinct chemotypes display different off-target kinase activity and *in vivo* toxicity profiles. Several promising backup *Plasmodium* PI4Kβ inhibitor programs in preclinical development proceed with understandable caution, with efforts to de-risk series early in terms of teratogenicity using the *in vitro* zebrafish assays [17], the human-induced pluripotent stem cell model, and off-target human kinase inhibition assays with particular focus on human PI4Kβ, MAP4K4, and MINK1. Mechanistic understanding of the inhibitor-induced teratogenicity observed in rats for MMV390048 will be important for the development of future *Plasmodium* PI4Kβ inhibitors.

Other *Plasmodium* kinase targets that have been phenotypically validated, and for which inhibitors have demonstrated *in vivo* proof-of-concept, include *Plasmodium* cGMP-dependent protein kinase (PKG) and cyclin-dependent-like protein kinase 3 (CLK3) [18–20]. Kinases offer the potential to deliver multistage antimalarials as many play an essential role at multiple stages of the *Plasmodium* life cycle [21,22]. This is illustrated by *Plasmodium* PI4Kβ, PKG, and CLK3 inhibitors which show liver-stage, asexual blood-stage, and transmission-blocking antiplasmodium activity in *in vitro* and/or *in vivo* models [12,18–20,23,24]. Whether this will translate to multistage activity in a clinical setting is more complicated and will depend on the tolerated dose relative to the effective concentration required to clear different life cycle forms of the parasite (i.e., gametocytes or liver schizonts) as well as the dosing regimen and half-life of the compound.

Given that over a third of *Plasmodium* kinases are thought to be genetically essential for asexual blood-stage development and/or the sexual stages of the parasite based on genetic knockout studies [21,22], there must be other attractive *Plasmodium* kinase targets for the development of drugs for malaria treatment and/or transmission blocking. Inhibitors for a range of other *Plasmodium* protein and phosphoinositide kinases have been reported, although in most cases further investigation is required to conclusively link kinase inhibition to phenotypic antiplasmodium activity, and further assessment of these kinases is required to evaluate their value as targets. Key considerations for selecting an appropriate *Plasmodium* kinase for target-based drug discovery, beyond genetic essentiality, are discussed in detail in our previous article

generally followed by target deconvolution studies. For malaria, phenotypic drug discovery relies on *in vitro* whole-cell antiplasmodium activity to guide compound optimization.
Polypharmacology: a single drug acting on more than one molecular target to produce its therapeutic effect.
Target-based drug discovery: drug discovery focused on developing agents that interfere with the function of a validated target in a way that has been shown to have the desired effect on the disease state. Also referred to as rational drug discovery, the target-based approach typically incorporates data from biochemical assays designed to measure the effect of drugs on a given target or pathway *in vitro* and structural information on the target to optimize compounds for binding.
Teratogenicity: abnormalities in a developing fetus caused by a toxin.

[10]. Recent review articles provide detailed overviews of progress targeting *Plasmodium* kinase for malaria drug discovery [25,26].

Selectivity: make-or-break

The major question at the forefront of all kinase-targeted drug discovery is whether sufficient selectivity can be achieved to avoid toxicity resulting from off-target human kinase inhibitory activity. Considering the high conservation of the ATP-binding site across the kinase superfamily, kinase inhibitors with impressive selectivity have been developed, and over 70 kinase inhibitors have been approved by the FDA for oncology and a range of other indications [6]. Despite this, some level of off-target activity is inevitable and needs to be carefully monitored. At the early stages of kinase-targeted drug discovery, it is not clear what level of selectivity is required and to what extent kinase inhibition will be tolerated for any given human kinase target or off-target. This will depend on drug exposure at the off-target sites, treatment period, and safety requirements for the given disease indication. Nevertheless, off-target human kinase activity is typically considered a liability and leads to the deprioritization of many otherwise promising compounds.

The *Plasmodium* and human kinomes are divergent, displaying key differences which can be exploited for selectivity [9]. Strategies include targeting *Plasmodium*-specific kinases for which human kinase orthologs do not exist, or exploiting key differences within the ATP-binding site between the *Plasmodium* kinase target and human kinase homologs, including the size of the gatekeeper residue using bumped kinase inhibitors (Figure 1) [27], a strategy commonly used for selectively targeting human tyrosine kinases.

Identifying initial starting points/scaffolds with narrow spectrum kinase activity is preferable, but it should be possible to design in selectivity using a structure-based approach with sufficient chemistry resources and data from routine *in vitro* compound profiling against the *Plasmodium* kinase target and the closest human orthologs/key kinase off-targets. The challenge here is that the major human kinase off-targets may not necessarily be the human orthologs, and while a compound is optimized to reduce potency for one human kinase off-target, activity against other human kinases that are not being monitored may unknowingly be introduced. *Plasmodium* PKG inhibitors exploit the presence of a small gatekeeper residue for selectivity relative to the human PKG orthologs PRKG1 and PRKG2 which have a large residue at the equivalent position,

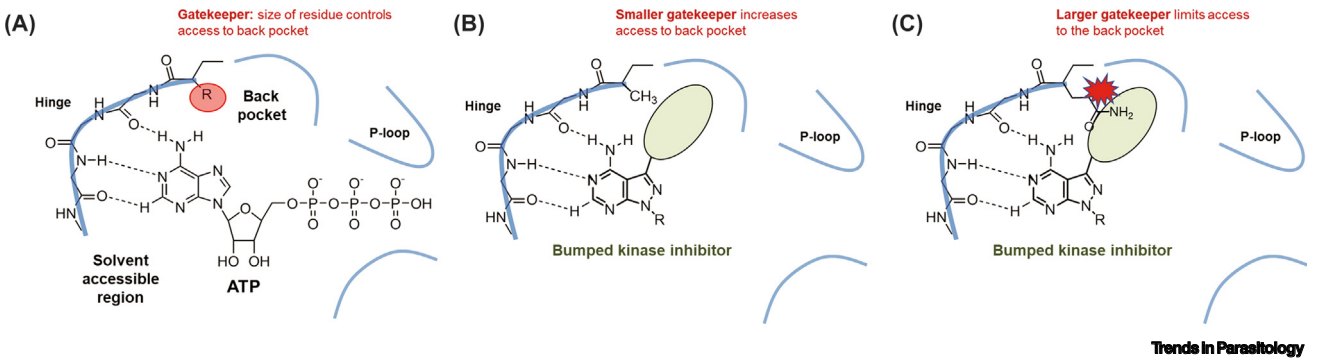

Figure 1. Exploiting the gatekeeper residue for selective kinase inhibition. (A) Schematic of ATP bound to the kinase catalytic site. The size of the so-called gatekeeper residue affects access to a hydrophobic pocket adjacent to the ATP-binding site called the back pocket. Most kinases have a gatekeeper residue with a bulky side chain that limits access to this pocket. (B) Schematic of a bumped kinase inhibitor bound to the catalytic site of a kinase with a small gatekeeper residue. In the case of kinases with a smaller gatekeeper residue (e.g., glycine, alanine, serine, or threonine), kinase inhibitors can be designed to extend into the back pocket leading to potent and selective inhibition. (C) Schematic showing how a larger gatekeeper residue leads to a steric clash, preventing binding of bumped kinase inhibitors to the ATP-binding site.

but this does not guarantee selectivity relative to more distantly related human kinases. Consequently, human kinases with small gatekeeper residues are the key off-targets that should be carefully monitored during the development of *Plasmodium* PKG inhibitors. For example, the *Plasmodium* PKG inhibitor MMV030084 is a potent inhibitor of the human serine-threonine kinase BRAF that also has a small (threonine) gatekeeper residue [19,28].

There is a wealth of structural information available for human kinases but structural information for key *Plasmodium* kinase targets is still very limited. Inhibitor-bound high-resolution structures of *Plasmodium* PKG are available [29], but high-resolution structures of PI4Kβ and CLK3 have not been reported. Subsequently, homology models need to be relied on, making structure-based drug design even more challenging [30].

Resistance risk for kinase inhibitors

In oncology, acquired resistance to human kinase inhibitors is a major problem and is inevitable given the inherent genetic instability of neoplastic cells. Mutations to the gatekeeper residue, particularly in tyrosine kinases, are frequently responsible for acquired resistance as a change at this position is typically tolerated in terms of ATP-binding and kinase function (and in some cases even increases ATP-binding affinity/catalytic activity) but is highly effective at disrupting the binding of bumped kinase inhibitors. For example, the tyrosine kinase epidermal growth factor receptor (EGFR) T790M gatekeeper mutation is a major resistance mediator responsible for almost half of EGFR inhibitor resistance resulting from acquired mutations [6,31].

Given the remarkable ability of *Plasmodium* parasites to acquire drug resistance, it is likely that a similar issue will arise for selective *Plasmodium* kinase inhibitors that are developed for malaria. This has been observed for *Plasmodium* PI4Kβ inhibitors in *in vitro* evolution experiments, with selections resulting in a range of different single-point mutations within the PI4Kβ kinase domain leading to shifts in the effective drug concentration (EC_{50}) [12,23,24,32,33]. The risk of resistance is a property of both the target and the chemotype, and may depend on both the binding mode and the selectivity of the compound. The *Pf*PI4Kβ inhibitor MMV390048 is predicted to have a high to moderate risk of resistance based on an MIR of 6 (at a drug concentration of 3 × EC_{50}) and the identification of several point mutations resulting in a three- to sevenfold EC_{50} shift [4,12]. Careful pairing of PI4Kβ kinase inhibitors with partner drugs will be required to slow down the emergence of resistance.

This contrasts with what has been observed for inhibitors targeting the *Plasmodium* serine/threonine protein kinase PKG. Somewhat unexpectedly, experimental *Plasmodium* PKG inhibitors ML10 and MMV030084 did not lead to mutations in the *pfpkg* gene in *in vitro* evolution experiments [18,19]. Rather, tyrosine kinase-like protein 3 (TKL3) was identified as a low-level resistance mediator for MMV030084. Given that these inhibitors exploit the presence of a small gatekeeper residue in PKG, and that engineered parasite lines with a gatekeeper mutation (*Pf*PKG T618Q) are viable and resistant to bumped PKG inhibitors, one might expect that a gatekeeper mutation would be selected for. While it cannot be ruled out that polypharmacology is contributing the low resistance risk observed for these inhibitors, conditional knockdown of *Pf*PKG, but not other putative targets such as *Pf*CDPK1 and *Pf*TKL3, led to the sensitization of the parasites to MMV030084 – suggesting that *Pf*PKG is the primary efficacious target. Similarly, for ML10, an engineered gatekeeper mutation (PKG T618Q) led to a loss in antiplasmodium activity (i.e., resistance), suggesting that the low propensity for resistance is not a result of multiple targets. The apparent resistant refractory nature of PKG makes it a very appealing target, but this needs to be investigated further to see if this holds true for other PKG inhibitors and is likely to translate into a clinical setting.

Nonetheless, resistance remains a key concern for kinase inhibitors and other inhibitors emerging from target-based programs, and novel strategies to address this are required.

Kinase inhibitors for designed polypharmacology

Traditionally target-based drug discovery focuses on improving potency for a single target and reducing promiscuity which is associated with potential toxicity. However, it is becoming increasingly apparent that complex multifactorial diseases such as cancer, neurological disorders, and infection may require modulation of multiple pathways to ensure drug efficacy (and potentially safety due to lower dosing requirements and the requirement for only partial inhibition of individual targets, reducing target-related adverse reactions) in the long term [34]. Polypharmacology offers a range of potential advantages, including (i) additive or synergistic effects resulting from the modulation of multiple pathways by a single compound resulting in a lower effective dose, (ii) delayed drug resistance, and (iii) reduced pill burden and costs associated with complex combination therapies. Aptly referred to as 'death by a thousand cuts' [35] or 'the magic shotgun approach' [36], partial inhibition of multiple targets may result in superior efficacy than complete inhibition of a single target. Many drugs, particularly kinase inhibitors, serendipitously act through polypharmacology with both the target and 'off-targets' contributing to favorable clinical outcomes. For example, sunitinib, a multikinase inhibitor approved for use in various cancer indications, was found to unintentionally inhibit the 'off-target' Axl receptor protein-tyrosine kinase (in addition to tyrosine receptor kinases KIT, FLT3, PDGFR, and VEGFR2) [37]. Inhibition of Axl receptor protein-tyrosine kinase is now believed to contribute to the clinical efficacy of sunitinib, and this kinase is now intentionally targeted by inhibitors as a primary or secondary target [38]. Notably, at least a third of approved kinase inhibitors are (intentionally or unintentionally) polypharmacological in nature, with multiple targets contributing to drug efficacy [6]. In fact, it could be argued that no ATP-competitive kinase inhibitors have exquisite selectivity for a single target. An early designed multikinase inhibitor lapatinib was the first approved dual tyrosine kinase inhibitor optimized to simultaneously inhibit EGFR and human epidermal growth factor receptor 2 (HER2), while reducing unwanted off-target effects observed for previous-generation EGFR inhibitors [39]. Designed polypharmacology, while significantly more challenging than optimizing a drug for a single target, is gaining traction to address resistance and poor efficacy for a range of indications, including infectious diseases [40]. The potential benefits of designed polypharmacology for malaria are exemplified by a study on WM382, a dual inhibitor of the *Plasmodium* aspartic proteases plasmepsin IX and X [41]. In contrast to inhibitors specific for plasmepsin X, WM382 displayed potent multistage antiplasmodium activity and attempts to select for drug-resistant parasites *in vitro* were unsuccessful, suggesting a high barrier to resistance attributed to the compound's dual action.

It is also worth noting that the mode of action of some of the most successful antimalarial drugs developed using a phenotypic approach has remained elusive despite extensive target identification efforts. This is likely a result of polypharmacology and highlights the potential advantages of phenotypic-based drug discovery over traditional target-based approaches focused on a single target.

Multikinase inhibitors

The similarity of the ATP-binding site across the kinase superfamily provides an opportunity for the design of multikinase inhibitors using a merged pharmacophore approach [42]. A range of chemotypes have been reported to inhibit multiple *Plasmodium* kinase targets that may serve as good starting points for multikinase inhibitors (Box 1) [33,43–46]. Although, the advantages of polypharmacology are only likely to be realized if the appropriate target combinations are identified and where a balanced activity profile against the targets is achieved.

Ideal target combinations for designed polypharmacology need to meet a range of criteria, including (i) the potential to yield additive or synergistic antiplasmodium activity, (ii) binding sites with compatible pharmacophoric features, (iii) a design strategy for excluding off-targets and reducing general promiscuity while retaining drug-like properties, and (iv) availability of tools to demonstrate multitarget engagement and to guide drug optimization. Determining the optimal target combination and relative potency against targets to achieve the benefits of polypharmacology – in terms of both efficacy and resistance prevention – is not trivial. Differential expression patterns, cellular locations of targets and substrate (e.g., ATP) concentrations, and binding affinities in the case of competitive inhibitors also add another level of complexity. Kinase inhibitors potently inhibiting validated *Plasmodium* kinase targets PI4Kβ and PKG have been reported, providing exciting prospects for the development of dual PI4Kβ and PKG inhibitors [33,44]. Further work focused on balancing target potency and illustrating the potential benefits of dual PI4Kβ and PKG targeting relative to selective inhibitors is ongoing.

Advances in genetic engineering and omics approaches for *Plasmodium* is leading to a better understanding of parasite biology and essential signaling pathways, which will in turn facilitate more complex approaches to rational drug design [5]. Approaches such as Kinobead technology, cellular thermal-shift assays (CETSAs), or thermal proteomic profiling (TPP), metabolic fingerprinting, and proteomics have enabled the unbiased assessment of protein–inhibitor interactions and the modulation of key processes in the *Plasmodium* parasite [12,33,47–49]. Advances in clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing tools for *Plasmodium* [50] has proved powerful for target validation and demonstrating on-target antiplasmodium activity [19,33,51,52]. Going forward, these tools have the potential to provide key information to aid the identification of target combinations for polypharmacology. These could include kinase target combinations or target combinations across different protein families (Box 1). In addition, advances in computer-aided drug design, mapping of complex signaling

Box 1. Kinase inhibitors showing activity against multiple *Plasmodium* targets

Figure I shows examples of kinase inhibitors that have been shown to inhibit multiple *Plasmodium* targets. Imidazopyridazines with potent antiplasmodium activity have been reported to inhibit several *Plasmodium* kinase targets including CDPK1, PKG, and PI4Kβ. A series of imidazopyridazines that potently inhibit *Pf*CDPK1 could be divided into two classes, represented by compounds 1 and 2, based on their stage specificity in asexual blood-stage parasites [43]. Further investigation identified PKG as the primary efficacious target in asexual blood-stage parasites for the first class of compounds, rather than CDPK1. The second class of compounds was also shown to interact with the chaperone heat shock protein 90 (HSP90), another antimalarial target of interest. Another study showed that several 3,6-diphenylated imidazopyridazine derivatives with potent antiplasmodium activity display potent dual PI4Kβ and PKG inhibition (e.g., Compound 3) [44,65].

Similarly, Compound 4, the human 'mammalian target of rapamycin' (mTOR) inhibitor sapanisertib, currently under clinical investigation for the treatment of cancer, was shown to potently inhibit *Plasmodium* PI4Kβ and PKG in addition to PI3K. Sapanisertib showed potent antiplasmodium activity across the parasite life cycle, providing an opportunity to reposition this compound as a dual PI4Kβ and PKG inhibitor for malaria [33].

Medicinal chemistry optimizing of a thiazole series of potent *Pf*PKG inhibitors led to the identification of thiazole-pyrimidine derivatives, exemplified by Compound 5, that displayed fast-killing kinetics in contrast to the slow-killing phenotype typically observed for PKG inhibitors [45]. This implicated the involvement of other targets. A subsequent Kinobead study identified multiple other putative kinase targets in addition to PKG. The authors concluded that SRPK2 (also known as CLK2) is likely responsible for the fast-killing kinetics, but polypharmacology may be another explanation.

Finally, Compound 6 represents a derivative of human IkappaB kinase (IKK) kinase inhibitor IKK16 that showed potent asexual blood-stage and liver-stage antiplasmodium activity [46]. This compound was shown to inhibit both *Pf*PK6 and *Pf*GSK3 *in vitro*. *Pf*PK6 is genetically essential for asexual blood-stage development, and studies are underway to phenotypically validate this target. Kinome-wide studies have not been reported for Compound 6 so it is still unclear if other *Plasmodium* kinases and or host kinases may be implicated in the mode of action.

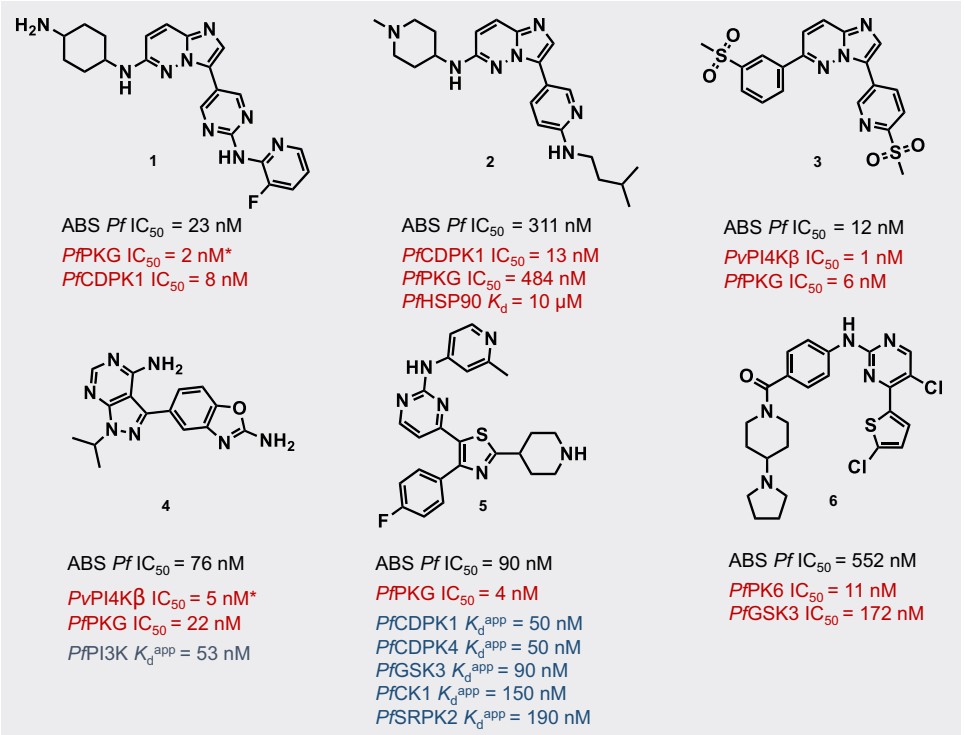

Figure I. Examples of kinase inhibitors with activity against multiple targets. *Indicates the primary target identified in asexual blood-stage parasites through resistance selections and/or chemogenetics approaches. Abbreviations: ABS, asexual blood stage; CDPK1, cyclic-dependent protein kinase 1; CDPK4, cyclic-dependent protein kinase 4; CK1, casein kinase 1; GSK3, glycogen synthase kinase 3; HSP90, heat shock protein 90; PI3K, phosphatidylinositol 3-kinase (also referred to as VPS34); PI4Kβ, phosphatidylinositol 4-kinase beta; PKG, cGMP-dependent protein kinase; SRPK2, serine/arginine protein kinase 2.

pathways, and improved approaches for predicting on-targets and off-targets *in silico*, largely facilitated by artificial intelligence, are enabling designed polypharmacology [53–57].

Combining kinase inhibition with inhibition of beta hemozoin formation

Kinase inhibitors that bind to the ATP-binding site are typically planar by nature and display significant pharmacophoric overlap with inhibitors of **beta hemozoin formation**. Beta hemozoin inhibitors bind to hemozoin crystals, interfering with heme detoxification by biocrystallization in asexual blood-stage parasites, leading to elevated free heme and parasite death. A number of kinase inhibitors display potent beta hemozoin inhibition similar to that observed for chloroquine, including human tyrosine kinase inhibitors lapatinib and nilotinib [58]. This provides a unique opportunity for polypharmacology, combining the targeting of a protein kinase and a nonprotein target.

Repurposing human kinase inhibitors for malaria – better the devil you know than the devil you don't

Drug repurposing offers an attractive route for drug development as it is typically less risky, cheaper, and faster than developing a new drug. This is primarily because pharmacokinetic

(PK) and toxicity data from preclinical and human studies are already available [59]. Many human kinase inhibitors show potent antiplasmodium activity, providing an opportunity to redevelop them as antimalarials. Given that these inhibitors have been designed to potently inhibit human kinase targets, toxicity risks associated with the primary human kinase target/s and off-targets need to be carefully assessed in the context of the new indication, given that toxicity is a function of drug exposure and treatment duration. In cancer, the maximum tolerated dose is generally administered, treatment is typically long-term (months), and severe side effects are accommodated given the terminal nature of the disease. By contrast, malaria treatment is short, and drugs must be well tolerated across a broader population, including children and pregnant women, with minimum adverse side effects to ensure uptake and adherence. To assess the potential of a given drug for repurposing, dose fractionation studies in an *in vivo* model of malaria infection, to determine the PK/pharmacodynamic (PD) drivers responsible for the observed efficacy, coupled with human dose predictions for malaria can be used to evaluate the risk of adverse reactions [60].

In cases where human kinase inhibition is not tolerated, approved human kinase inhibitors can be used as starting points for **drug repositioning**. The advantage here is that the human kinase targets and associated toxicity are already established for the hit compound. In addition, approved kinase inhibitors typically have a narrow spectrum of kinase activity and have already shown some

Key figure

Weighing up the liabilities and opportunities for developing kinase inhibitors for malaria

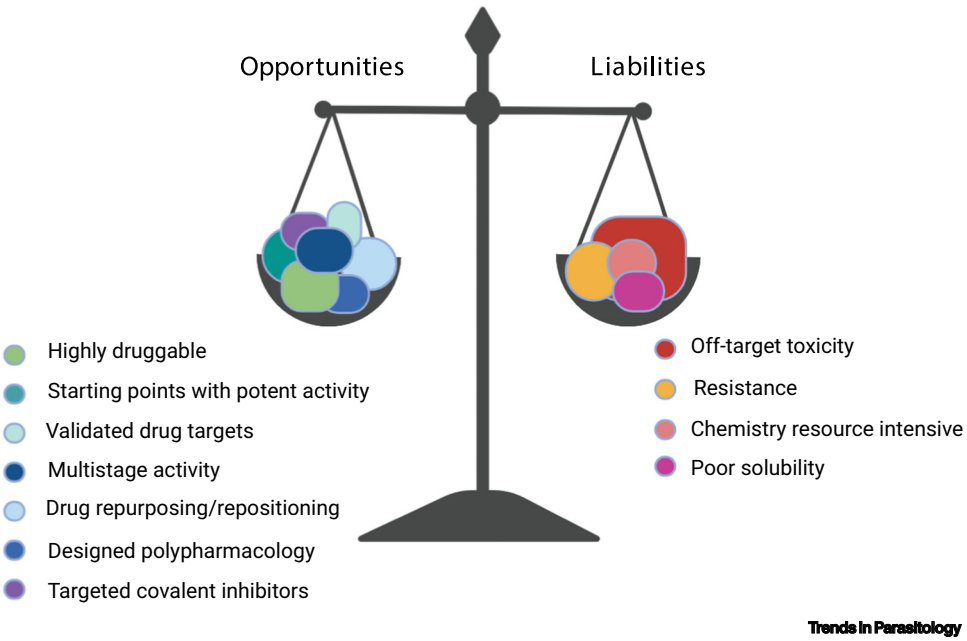

Figure 2. Kinase inhibitors for malaria provide exciting prospects for drug development, including the potential for the development of compounds with multistage activity and the opportunity for designed polypharmacology. However, targeting kinases also comes with significant challenges, including the risk of adverse reactions due to the inhibition of off-target human kinases and the risk of resistance. These opportunities and liabilities need to be critically assessed for any given kinase-focused malaria drug discovery program. This figure was created using BioRender.

level of safety in humans. Once the target in *Plasmodium* has been identified, target-based optimization efforts can focus on improving potency for the *Plasmodium* target and antiplasmodium activity, while dialing out potency for the human kinase targets that are associated with a toxicity risk. It is worth noting that host-directed therapy targeting human kinases that have been shown to serve as host dependency factors for parasite development has been proposed as a treatment strategy (either primary or adjunctive) for malaria [61–63]. This emphasizes that 'off-target' human kinase activity may even be advantageous in terms of malaria treatment outcomes and resistance generation in certain instances, providing further opportunities for polypharmacology.

Concluding remarks

Despite challenges, targeting kinases for malaria provides exciting opportunities for drug discovery (Figure 2, Key figure). Several promising targets have been validated, and efforts to phenotypically validate other promising targets are underway (see Outstanding questions). Kinase inhibitors exhibiting polypharmacology offer the potential benefit of synergy and reduced risk of acquired resistance. Synergistic effects may mean that a drug only needs to moderately inhibit the individual targets to achieve the desired efficacy. This is in contrast with designing small molecules that need to strongly inhibit or tightly bind to a single target. However, suitable target combinations still need to be identified and the potential benefits of *Plasmodium* kinase inhibitors displaying polypharmacology need to be demonstrated experimentally (see Outstanding questions). In addition to multikinase inhibitors, dual kinase-hemozoin inhibitors offer a potential attractive approach for drug development from the perspective of resistance risk as hemozoin is immutable as a nonprotein target. Polypharmacology has the potential to address liabilities of individual targets and reduce the complexity of combination drug regimens. It is also worth noting that covalent kinase inhibitors have been used effectively in oncology and provide another novel strategy for developing selective kinase inhibitors for malaria with the potential benefit of prolonged target engagement.

Outstanding questions

Are there other high-priority *Plasmodium* kinase targets that can be identified and phenotypically validated with the potential to yield potent and selective kinase inhibitors for malaria?

Can kinase target combinations (or other target combinations) be identified for designed polypharmacology with the potential to reduce resistance risk and improve efficacy relative to compounds acting on individual targets? Can artificial intelligence play a role in facilitating this?

Can the simultaneous knockdown of two targets be used to help identify promising target pairs for polypharmacology and to demonstrate intracellular multitarget engagement?

Can we generate *in vitro* and *in vivo* data showing the advantages of multikinase inhibitors with respect to resistance generation?

Can human kinase inhibitors be identified with sufficient efficacy and safety margins to be repurposed for malaria?

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Declaration of interests

The authors declare no competing financial interests.

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