

Review

Antimalarial drugs – host targets (re)visited

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Every year, forty percent of the world population is at risk of contracting malaria. Hopes for the eradication of this disease during the 20th century were dashed by the ability of *Plasmodium falciparum*, its most deadly causative agent, to develop resistance to available drugs. Efforts to produce an effective vaccine have so far been unsuccessful, enhancing the need to develop novel antimalarial drugs. In this review, we summarize our knowledge concerning existing antimalarials, mechanisms of drug-resistance development, the use of drug combination strategies and the quest for novel anti-plasmodial compounds. We emphasize the potential role of host genes and molecules as novel targets for newly developed drugs. Recent results from our laboratory have shown Hepatocyte Growth Factor/MET signaling to be essential for the establishment of infection in hepatocytes. We discuss the potential use of this pathway in the prophylaxis of malaria infection.

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1 Introduction

Malaria is a major health problem, mainly in Sub-Saharan Africa and in some parts of Asia and South America. Each year there are about 600 million new clinical cases and at least one million individuals, mostly children, die from malaria – a death from malaria every 30 seconds [1]. Over 90% of the deaths occur in Africa. Within the last 10 to 15 years the burden of malaria has been increasing [1], mainly because of the emergence of *P. falciparum* and *P. vivax* variants that are resistant to cheap drugs such as chloroquine, mefloquine, and pyrimethamine [2, 3]. As intensive efforts of malaria vaccination have been largely disappointing, the development of novel antimalarial drugs is crucial. Novel drugs may be variants of existing drugs or

novel compounds that are directed against new targets. The development of variants of existing drugs benefits from the increasing understanding of the molecular mechanisms underlying therapeutic effects, adverse effects and drug resistance, as well as from the availability of very large compound libraries that have previously been generated and screened for antimalarial activity. Computer assisted analyses are increasingly used to define the structures that are related to therapeutic [4–8] or adverse effects [9] of antimalarial drugs. The discovery of new antimalarial drug targets is facilitated by recently developed, large scale research technologies, such as sequencing of the genomes of several *Plasmodium* species [10–14]. The recently completed sequence of the 14 chromosomes of *P. falciparum* clone 3D7 consists of 23 megabases encoding about 5300 genes [11, 12]. Moreover, the sequence of the mammalian host and vector genomes can also prove to be very useful [15–17]. All these data need now to be complemented by functional analyses.

Recent reviews are available on antimalarial drug development in general [18–24] as well as on specific aspects, such as the history of antimalarial drug development [25, 26], the assessment of antimalarial drug efficacy [27], pharmacodynamic properties [28], toxicity [29],

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Abbreviations: ACT, artemisinin-based combination therapy; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; HGF, hepatocyte growth factor; ISIF, infection susceptibility inducing factor; P., *Plasmodium*; PfEMP-1, *P. falciparum* erythrocyte-membrane protein-1; RBC, red blood cell

antimalarial drug resistance [3, 30–34] and clinical trials [35, 36].

In the present review we provide a brief update on currently used antimalarial drugs, related compounds under development, the strategies used to combat resistance as well as a summary on potential novel plasmodial drug targets. We finish by describing recent work in our laboratory, in which we identified a receptor tyrosine kinase of the host as a potential antimalarial drug target, and possible approaches using this knowledge to inhibit malaria infection.

2 Antimalarial drugs and drug resistance

The most extensively used antimalarials are quinolines, antifolate drugs, artemisinins, atovaquone, and antibiotics such as tetracyclines. To counteract the rapid development of resistance some drugs are used in fixed combinations. A few novel drug combinations have been approved and several others are under investigation.

Quinolines are historically among the most important antimalarial drugs ever used. Throughout the 20th century, the immense success and widespread use of chloroquine, the most famous member of this group of drugs, provided well-founded hopes for malaria eradication. Known drugs from this family include amodiaquine, piperazine, primaquine, quinine and mefloquine. The understanding of the mode of action of quinoline-based antimalarials has increased in recent years, but remains incomplete [37–41]. The drugs from this group mostly act during the blood stages of the parasite's lifecycle [39] but some are also believed to target the hepatic stage [42, 43]. Quinolines are thought to inhibit the dimerization of heme and/or prevent the disposal of dimers from the food vacuole to the cytoplasm, where hemozoin is formed [41]. This leads to intra-Plasmodial accumulation of free heme, which becomes highly toxic to the parasite. The most important targets of quinolines seem to be heme and phospholipids, although several other targets have been postulated to be involved in their antimalarial actions such as tyrosine kinases [44], DNA [45], hemoglobin degrading proteases [46] and phospholipases [47]. Unfortunately, as will be discussed in the following section, resistance is now widespread for chloroquine and related compounds and is rapidly increasing for other drugs of this class. For this reason, other drugs of the same kind are in development [48–52]. Moreover, combinations of quinolines with other drugs, have been used [53] and continue to be studied [54, 55] particularly in artemisinin-based combination therapy (ACT) [56, 57].

Antifolates are compounds designed to inhibit the synthesis of folate cofactors that are required for nucleotide synthesis and amino acid metabolism. The most commonly used antifolates are pyrimethamine (2,4-diaminopyrimidine), chloroguanil (proguanil, Paludrine),

and the sulfa-drugs sulfadoxine, sulfalene and dapsone. Antifolates prevent the nuclear division of *Plasmodium* at the schizont stage within erythrocytes and hepatocytes by acting on dihydrofolate reductase (DHFR) [58] or dihydropteroate synthase (DHPS) [59]. Therefore, these drugs act more slowly against *Plasmodium* than the quinoline antimalarials. Antifolate drugs are eliminated slowly, a fact that facilitated the development of resistance. When antifolates are used alone, resistance develops rapidly as a result of mutations in the target enzymes [60]. This problem has accelerated the search for new antifolate drugs [61] and for new antifolate drug combinations [62], such as pyrimethamine-sulfadoxin (FANSIDAR), chloroguanil-dapsone (LAPDAP) and chloroguanil-atovaquone (MALARONE). However, the parasite has already developed resistance to pyrimethamine-sulfadoxin [30, 63] and *P. falciparum* strains with quadruple mutations of the *dhfr-ts* gene, which are highly resistant to pyrimethamine-sulfadoxin, are also resistant to chloroguanil-dapsone [30]. To further counteract the development and spreading of resistance, a triple combination of chloroguanil-dapsone and artesunate is now being developed [64]. A major disadvantage for the use of MALARONE in Africa is the high cost of atovaquone [65].

Atovaquone [66] inhibits electron transport in plasmodial mitochondria [67] and depolarizes the membranes of plasmodial mitochondria [68]. In clinical trials, atovaquone was well tolerated and effective, but recrudescence rates were high due to the rapid development of drug resistant *Plasmodium* strains, resulting from mutations of the cytochrome *c* reductase coding gene [69, 70]. Therefore a fixed combination with chloroguanil has been developed under the tradename MALARONE. Phenyl beta-methoxyacrylates linked to an aromatic ring are thought to interfere with the electron transport in plasmodial mitochondria by inhibiting cytochrome *c* reductase. They represent a novel class of inexpensive antimalarials [71].

Artemisinins have a broad spectrum of activity against all parasite phases within erythrocytes, in particular younger ring forms [72], and suppress gametocyte transmission [73]. Artemisinins selectively accumulate in *P. falciparum*-infected erythrocytes [74] and, although their mode of action is still controversial, they are thought to kill parasites via free radicals generated by activation of artemisinins by ferrous heme or exogenous free iron [75]. However, the hypothesis of heme-dependent activation is not consistent with the finding that these drugs do not localize to the food vacuole, where heme is abundant, but to parasite membranes [76], nor with the observation that they affect parasite stages that do not contain haemozoin, such as younger ring forms [72] and gametocytes [73]. Recent studies indicate that the inhibition of *PfATP6*, the only parasite gene that shows homology to SERCA-type Ca^{2+} -dependent ATPases, could be a possible mechanism for artemisinin action [77]. Conceivably, artemisinins

have complex antiplasmodial effects that involve several targets. Artemisinins act more rapidly against *Plasmodium* than any other known antimalarial drug [78, 79]. Used as first line antimalarial drugs, artemisinins have a high rate of treatment failure, in particular in patients with high levels of parasitemia at admission [80]. Still, resistance of *P. falciparum* to artemisinins has not yet emerged [81, 82], not even in China where artemisinin-containing plant extracts have been used for 2000 years. This is at least in part attributable to the efficacy and the very short half life of these drugs [28]. Disturbingly, a recent study found an association between recrudescence and decreased sensitivity to dihydroartemisinin, suggesting that the emergence of resistance to artemisinins is a serious possibility [83]. Major problems with artemisinins are their expensive isolation [84] and poor solubility [85]. To overcome these problems a lot of work has focused on the development of semisynthetic artemisinin derivatives and fully synthetic analogs [24, 54, 86–90]. Artemisinin derivatives currently in use are artemether, arteether, artesunate and dihydroartemisinin. These drugs are now widely used in combination with various other antimalarials for the treatment of both uncomplicated and severe malaria. However, several studies have associated neurotoxicity to artemisinins, particularly ototoxicity, an observation that may limit the use of these drugs, particularly in children [91–99]. Efforts over the last ten years have been focussed on generating synthetic compounds with prolonged half lives and reduced neurotoxicity [54, 90, 100].

Finally, some antibiotics have also been used as antimalarials. A variety of antibiotics are bacteriostatic by inhibiting RNA translation. Many of these antimicrobial agents are also effective against parasites that carry a circular plastid, known as apicoplast, which contains elements resembling prokaryotic transcription and translation systems. The most common ones are the prokaryotic translation inhibitors tetracycline [101–103], doxycycline [104], and clindamycin [53]. These drugs act slowly and are mainly used in combination with quinine or other fast acting antimalarials.

3 Fighting resistance

Because of the rapid emergence of drug resistance, cheap and effective treatment for malaria with a single drug is no longer an option for most countries in Africa. Drug resistance is defined as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject [105]. *P. falciparum*, the parasite that causes the most severe form of malaria, is now resistant to the antimalarial drug chloroquine in nearly all the areas where the disease is rife. Resistance to antifolates and an-

tifolate combinations develops rapidly raising and serious doubts that these drugs may ever provide a satisfying solution in endemic areas. So far, the only antimalarial drugs to which *Plasmodium* has not developed resistance are the artemisinins, but on the other hand, these present several drawbacks such as their price and the fact that they have to be used in combinations with other drugs because of the high failure rates observed when used alone as first line drugs.

The rapid emergence and spread of resistance emphasizes the need for early detection of drug-resistant *Plasmodium* strains and a variety of methods have been developed to address this issue [27]. A combination of *in vitro* and *in vivo* tests has been recommended to detect resistance against artemisinins as early as possible [106, 107]. Some of the more recently developed *in vitro* assays [108] may also be useful for the development of novel drugs. Resistance to drugs may be counteracted by combining them with compounds that revert resistance [109–113]. In view of their cost and the questionable efficacy, drug resistance converters are unlikely to make an important contribution to the solution of the drug resistance problem.

The current most important strategy used to fight resistance is the use of drug combinations. This strategy has been used in the past through the utilization of fixed combinations such as pyrimethamine-sulfadoxine or chloroguanil-atoquavone in combination with quinoline compounds. More recently, a variety of novel strategies have been implemented and several others are under investigation (for review see publication of the World Health Organization – WHO/CDS/RBM/2001.35). The combination of fast acting artemisinins with other slower acting antimalarials, is currently the most widely used strategy. Artemisinins have several advantages as drug combination partners. First, they act faster than any other known antimalarial drugs [28]. Second, the rapid action and high effectiveness of artemisinins reduce the probability of resistance development [3], as indicated by the fact that resistance against artemisinins has not yet been observed. Third, artemisinins also affect gametocytes and, therefore, reduce infectivity [114, 115]. However, the artemisinin-based combination therapy (ACT) strategy is clearly unsatisfactory in areas where resistance rates against the partner drug are high [84], meaning that this strategy should mainly be used in areas where resistance against the partner drug has not yet emerged. Thus, despite the fact that ACT is likely to delay the future emergence of drug resistant strains, it does not solve the problem of existing antimalarial drug resistance. Moreover, the higher cost of the combination of a new drug with artemisinins is likely to prevent a universal application of the ACT strategy [116].

4 Novel plasmoidal targets and emerging drug candidates

The urgent need for a replacement drug having the advantages and efficacy that once characterized chloroquine, is a driving priority for malaria research. Novel approaches to antimalarial drug development are reviewed in recent publications [18, 20, 23, 24]. Metabolic pathways in *Plasmodium*, in particular those that do not occur or that are quite different in humans, provide important novel antimalarial drug targets (Table 1). The apicoplasts of *Plasmodium* and several other parasites provide a rich source of such targets. These apicoplasts are reminiscent of the chloroplasts of plants, probably originated from the engulfment of an organism of the red algal lineage [117]. The apicoplast has its own genome, containing a small number of genes (35 kb of circular DNA), but involves about 400 proteins that are encoded by nuclear genes and targeted to the organelle via a secretory pathway. DNA replication and protein synthesis in apicoplasts share features with these processes in prokaryotes. Moreover, apicoplasts have a variety of typical plastid functions such as fatty acid, isoprenoid and heme synthesis [118, 119]. These features make the molecular processes that take place in the apicoplast a rich source for novel antimalarial drug targets [118, 120]. As mentioned above, various compounds previously developed as antibacterial agents or as herbicides interfere with apicoplast functions. Several of these compounds are already used for malaria prophylaxis and therapy, while others are being evaluated as antimalarial drug candidates and/or provide leads for the development of novel drugs. An overview on metabolic pathways in *Plasmodium* is available on the internet (<http://sites.huji.ac.il/malaria/>).

Table 1. Metabolic pathways providing new possibilities for drug targets

Pathway	References
Apicoplast DNA replication	[1, 120–123]
Pyrimidine synthesis	[124–129]
Protein synthesis in the apicoplast	[130–134]
Polyamines	[135–140]
Lipid synthesis and metabolisms	[141]
Fatty acid synthesis in the apicoplast	[142–146]
Phospholipid synthesis	[147–151]
Glycosphingolipid synthesis	[152–155]
Glycosylphosphatidylinositol anchor synthesis	[156]
Isoprenoid synthesis and protein prenylation	[157–163]
Shikimate pathway	[164]
Lactate dehydrogenase	[165, 166]
Proteases	[20, 167–169]
Protein kinases	[170–173]
Cyclin dependent kinases (CDKs)	[174]
Mitogen activated protein kinases (MAPKs)	[174, 175]
Cyclic nucleotide signaling	[176, 177]
Cyclophilins and calcineurin	[178–180]
Transport systems	[181–185]

Most drug development focuses on targeting *Plasmodium*. It is actually an important requirement of traditional anti-microbial chemotherapy not to interfere with functions of the host. However, *P. falciparum* is a highly variable parasite with immense recombination abilities. For example, *P. falciparum* erythrocyte-membrane protein-1 (PfEMP-1) is the major parasite ligand involved in cytoadherence and has been considered a potential target for vaccine development [186, 187] as well as for anti-adhesion therapies [186, 188, 189]. However, PfEMP1 is an extremely variable protein, encoded by a large and diverse gene family called *var* [190, 191]. Each parasite genome is estimated to contain 50–150 *var* genes, encoding 200–350 kDa proteins [192]. Although each parasite within an RBC expresses a single *var* gene, other *var* genes in its repertoire can be expressed up to a rate of 2% per parasite growth cycle [193]. This outstanding capacity for antigenic variation constitutes a major virulence factor in malaria and represents a potential limitation for PfEMP-1-based drugs. Other parasite-based drugs exhibit, to different extents, similar potential drawbacks. For these reasons, host-based therapies should not be disregarded and, instead, deserve renewed attention. As the knowledge of the genome of *P. falciparum* opened new avenues for the design of drugs directed against molecules of the parasite, the completion of the human genome project allows researchers to systematically evaluate host factors that may be targets for antimalarial drugs. Targeting host components has the major advantage that drug resistance cannot result from alterations of the drug target, as recently mentioned [194]. Resistance is even less likely to occur if the targeted host component remains outside the microorganism. It is perfectly reasonable to conceive that the development of resistance to a drug is easier to achieve for *Plasmodium* if the drug targets the parasite's own molecules than if it targets host molecules. Indeed, chloroquine remained for years an extremely effective drug and it took *Plasmodium* several decades to develop ways of evading its action. As detailed above, chloroquine acts by interacting with heme molecules. Heme is neither a parasite-derived molecule nor a protein that can mutate under drug pressure, leaving *Plasmodium* with the difficult task of evolving mechanisms that prevent chloroquine-heme interactions or overcome the damage that results from these complexes [195].

It seems clear that the struggle against malaria is an uphill battle, with *Plasmodium* constantly evolving ways of averting the weapons launched upon it. Indeed, this is a very resourceful parasite and all strategies should be considered when attempting to fight it. Because the vertebrate host is an essential part of *Plasmodium*'s lifecycle, acting upon the host's molecules should clearly be one of those strategies. Recently we have identified the human hepatocyte growth factor (HGF) and its receptor (c-MET) as novel targets for antimalarial drug development [196].

5 Are HGF and its receptor c-MET possible targets for malaria prophylaxis?

After *Plasmodium* sporozoites are injected into the mammalian host skin by infected mosquitoes, they migrate to the liver. Once there, sporozoites traverse the cytosol of several hepatocytes before reaching a final one that they will infect by formation of a parasitophorous vacuole [197, 198]. This final invasion is indispensable for the differentiation of sporozoites into the next infective stage [197]. Each sporozoite can give rise to as many as 30 000 merozoites that are released into the blood stream, initiating the pathologic blood stage of infection.

When studying the molecular mechanisms underlying the passage of *Plasmodium* we realized that hepatocytes wounded by sporozoite migration release one or more infection susceptibility inducing factors (ISIF) that render neighbouring hepatocytes susceptible to infection. Subsequent work has shown that one of the ISIF released is hepatocyte growth factor (HGF) and that sporozoite development in hepatocytes depends on the activation of the HGF receptor, also known as the protooncogene c-MET [196]. These findings suggest that sporozoite development in the liver might be impaired by compounds that inhibit the expression of either HGF or c-MET and/or compounds that interfere with c-MET functions at the level of the plasma membrane or inhibit HGF-mediated signal transduction.

Considering our results, compounds that increase HGF levels may be expected to increase infection in the liver. Indeed, malaria occurs more frequently in hepatitis B virus carriers, who have elevated HGF levels in the blood [199, 200]. HGF levels may also be increased by HGF degradation inhibitors such as dextran sulfate, heparan sulfate, dermatan sulfate, keratan sulfate, chondroitin, or chondroitin sulfate [201]. HGF activity is tightly controlled by posttranslational modifications. It is secreted as a single chain polypeptide (Pro-HGF) that binds to proteoglycans in the vicinity of the producer cells. Activation of the single chain precursor into the biologically active heterodimer by proteolytic cleavage between Arg494 and Val495 is a tightly controlled process [202].

Thus, molecules involved in HGF activation are potential targets for drug candidates that modulate the generation of these active heterodimers.

Another possible level of intervention could be the binding of HGF to its receptor, c-MET. c-MET is a heterodimer that consists of a β -subunit, which is highly glycosylated and entirely extracellular, and an α -subunit with a large extracellular region and an intracellular tyrosine kinase domain. c-MET is a member of a superfamily of receptor tyrosine kinases (RTKs). Some HGF isoforms, generated by differential splicing of primary HGF transcripts, also bind with high affinity to c-MET [203–205]. These HGF variants may act either as partial HGF agonists or as HGF antagonists, depending on the cell context, the pres-

ence or absence of heparin, and the HGF function analyzed. Thus, the HGF variant NK2 antagonizes many of the responses to HGF, but shares with HGF the ability to dissociate (scatter) cells, a response that facilitates metastasis [206]. NK4, another HGF variant, is a pure HGF antagonist [207–209]. Like the isolated HGF α chain, NK4 binds to c-MET but does not induce its autophosphorylation unless an isolated HGF β -chain is added. Single chain HGF variants similar to NK4, which have been engineered to be resistant against proteolytic cleavage, have been developed at the Biotech company Genentech [210, 211]. HGF–MET binding is also inhibited by a naturally occurring, soluble form of c-MET, a genetically engineered MET–IgG fusion protein, certain anti-MET antibodies [212], and c-MET selective aptamers [213]. Angiostatin, a fragment of plasminogen that contains 3–4 kringle domains, is a more recently discovered c-MET antagonist. The anti-angiogenic activity of angiostatin is at least in part due to its ability to neutralize the effects of HGF [214]. Angiostatin, which has 47% sequence homology with HGF, binds to c-MET and prevents HGF mediated signaling in endothelial cells and smooth muscle cells. It inhibits the proliferation of these cells in response to HGF but not in response to other growth factors such as vascular endothelial cell growth factor (VEGF) or basic fibroblast growth factor (bFGF), which act through other protein tyrosine kinase receptors. Thus, angiostatin functions as a selective c-MET antagonist.

Further downstream, modelling of the cytoplasmic domain of the c-MET suggests that the C-terminal tail gets into contact with the catalytic pocket and thereby acts as an intramolecular modulator of the receptor. Peptides that correspond to sequences in the C-tail of c-MET have been designed and such a peptide was able to block ligand-induced autophosphorylation as well as downstream c-MET signaling [215].

Various natural and synthetic compounds inhibit protein kinases by blocking the ATP pocket of the enzymes. At high concentrations, these compounds have a broad spectrum of activities against proteins using ATP. At lower concentration selectivity for tyrosine kinases is observed with several of such compounds, including genistein [216], tyrphostins, and herbimycin A.

Recently some progress has been made in the development of more selective tyrosine kinase inhibitors, although extensive analyses with a large panel of kinases reveal that none of the known tyrosine kinase inhibitors is specific for one particular kinase. Prominent examples are the c-abl selective drug GLEVEEC® [217] and the EGF receptor selective drug IRESSA® [218], which have been approved for the treatment of chronic myeloid leukemia and non small cell lung cancer, respectively. Since c-MET is involved in a variety of cancers, intensive efforts are underway to develop c-MET selective inhibitors. A promising lead compound is the indrocarbazole K252a, which is a general inhibitor of most protein kinases, but inhibits

c-MET mediated signals at nanomolar concentrations [219]. Results from our group have shown *in vitro* inhibition of malaria infection by K252a [196]. A screening program for HGF receptor selective inhibitors revealed SU11274, a sulfonamide, which inhibited cell growth driven by a constitutively active TPR–HGF receptor fusion protein, but did not affect a variety of other tyrosine kinases [220].

Upon binding to its receptor, HGF activates a variety of signal transduction pathways, which may be different in different cell types and in different contexts [221]. Different pathways mediate diverse responses such as cell proliferation, spreading and migration, morphogenesis, as well anti-apoptotic and immunoregulatory effects. Hepatocyte infection by sporozoites is associated with actin reorganization [196] and host cell apoptosis inhibition [222, 223]. HGF/MET signaling seems to be involved in both these features, which normally are mediated by PI3K activation [224]. We therefore postulated that hepatocyte infection by sporozoites involves the MET mediated activation of PI3K. Inhibition of the PI3K pathway in fact leads to a strong reduction of the infection level and correlates with the level of apoptosis in infected cells [222]. Further experiments are required to confirm the putative role of PI3K activation in the infection of hepatocytes by sporozoites and to identify additional proteins involved in the transduction of HGF-induced signals that could serve as targets for protection against malaria infection.

6 Host liver molecules as targets for prophylaxis

In the above sections, we have briefly described a large variety of proteins and small molecular weight compounds that interfere with HGF production or HGF/MET signaling. Proteins are unsuitable for malaria prophylaxis since they are expensive and require parenteral applications. K525a, a small molecular weight compound, is an inhibitor of HGF-mediated signal transduction. Moreover, compounds that selectively inhibit the enzymatic activity of c-MET are being developed for cancer therapy [220]. Much progress has recently been made in the development of specific protein tyrosine kinase inhibitors. Indeed, protein tyrosine kinases have become the second most important drug targets after G protein coupled receptors [225]. The use of tyrosine kinase inhibitors against malaria have the additional advantage that *Plasmodium* have no tyrosine kinases and thus resistance is even less likely to occur [226]. We have shown that the c-MET inhibitor K525a inhibited the hepatocyte infection by *Plasmodium* sporozoites, which requires HGF [196]. Much work remains to be done in order to understand the usefulness of selective HGF/MET antagonists for malaria prophylaxis. We believe that genistein might be a very promising lead. Our preliminary results suggest that genistein has an effect against liver stages of malaria infection both

in vitro and *in vivo* (our unpublished results). This might be due not only to its effect on HGF/MET signaling but also on other host tyrosine kinases important for parasite development. At first sight, this compound appears not to qualify as an agent for malaria prophylaxis. It is a broad spectrum tyrosine kinase inhibitor, has estrogenic and a variety of other biological activities [227]. However, genistein has an advantage over other tyrosine kinase inhibitors since it is found in soy foods that have been ingested by several Asian populations for centuries without any obvious adverse effects (for safety information on genistein supplementation see COT Report – Phytoestrogens and Health, 2003, published by the Food and Standard Agency in the UK). Moreover, genistein is thought to be responsible for the low incidence of diseases such as prostate cancer and postmenopausal osteoporosis in consumers of a high-soy diet [228, 229]. Because of these putative health effects, a variety of genistein food supplements is now commercially available. A large number of clinical trials have addressed the utility of genistein as a prophylactic agent. Thus, the groundwork for testing the utility of genistein for malaria prophylaxis in field studies has already been done.

In the host liver each parasite gives rise to 10 – 30 000 new parasites in 2 – 7 days, depending on the parasite species. Despite this amazing multiplication rate, not much is known about its molecular requirements. Since sporozoite multiplication in the liver is not associated with any signs of pathology, this first stage of malaria infection is not a target for therapy but represents the most appealing stage for prophylaxis. If infection can be blocked at this stage, there will be no pathology and therefore no disease. The major concern of this strategy is drug resistance, if the drug target is parasite-derived, and toxicity, if the drug target is host-derived. With increasing knowledge of the molecular mechanism underlying parasite development in the liver it may become possible to design drugs that do not interfere with normal liver functions but do prevent infection.

7 References

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