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Advances in Antimalarial Drug Evaluation and New Targets for Antimalarials

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1. Introduction

Parasitic infections due to the protozoa *Plasmodium* are responsible for malaria, a severe disease that still caused about 225 million cases and 781,000 human deaths in 2009, despite the efforts developed during the last decade to fight this disease (Alonso et al., 2011). The international funding allocated to antimalarial strategies has increased regularly since 2003 from about 0.3 billions to 1.7 billion dollars in 2009 (Collier, 2009), allowing many countries to undertake or strengthen effective fights against the parasite, the disease and the vectors. Nonetheless, more than half of the world population still lives in area where there is a risk of malaria transmission. The difficulty in fighting malaria is that five species of *Plasmodium,* namely *P. ovale*, *P. malariae*, *P. vivax*, *P. falciparum* and *P. knowlesi* (until recently considered as a nonhuman primate parasite) transmitted by over 30 species of *Anopheles* female mosquitoes are known to cause human malaria. The most virulent, *P. falciparum*, is responsible for severe clinical malaria and death. Furthermore, an increasing prevalence of resistance of vectors to insecticides, and of parasites to the standard antimalarial drugs has been observed for decades.

Today, the chemotherapeutic arsenal for malaria treatment is limited to three main families of compounds: quinolines, antifolates and artemisinin derivatives. Recommended chemotherapy is based on combinations of existing drugs with artemisinin derivatives (artemisinin combination therapies or ACT), the only antimalarial drug having no clear resistance recorded but for which alarming reports of tolerance in the field indicate it could be just a question of time (Noedl et al., 2008). From 2000 to 2008, the use of ACT combined with vector control allowed to reduce considerably the number of cases of malaria in a dozen African countries, so that nowadays, about 50 % of the total cases of malaria in Africa are found in mainly five countries (Enserink, 2010). However, no new class of antimalarials has been introduced into clinical practice since 1996 due to the intrinsic difficulties in discovering and developing new antimicrobials. A recent review of the global antimalarial drug development (Figure 1), including drugs at various clinical stage development and those expected to enter in phase I studies, showed that the pipeline is rather strong but novelty in terms of drug targets that is required to circumvent resistance is relatively low (Olliaro & Wells, 2009). This situation and the complexity in developing efficient vaccines require an urgent need for new drugs with original mechanisms of actions.

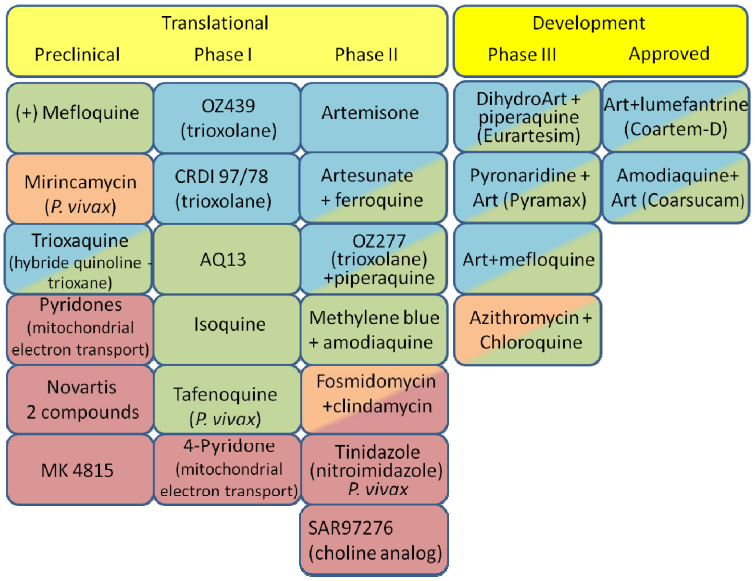


Fig. 1. Global antimalarial drug development pipeline (February 2009), after (Olliaro & Wells, 2009). Artemisinin (Art) derivatives or drugs containing the trioxane ring of artemisinin are illustrated in blue; aminoquinolines and structurally related compounds as well as aryl alcohols are in green; antibiotics are in orange; others drugs having different targets or mechanisms of action are in brown-red.

2. Development of bioassays for antimalarial activity

2.1 Malaria parasite life cycle

P. falciparum has a complex life cycle involving the *Anopheles* vector, where the sexual phase occurs, and humans where the parasite undergoes two phases of extensive asexual proliferation. After the bite by an infected *Anopheles*, the parasites at the sporozoite stage enter the blood and are carried to the liver where they traverse and invade hepatocytes initiating what is called the hepatic or exoerythrocytic phase. During this asymptomatic period, that lasts 5-7 days for *P. falciparum*, the sporozoites develop within the hepatocytes and, after several rounds of mitosis, produce several thousands of new infective forms called the merozoites that are released into the bloodstream and invade the red blood cells. During this intraerythrocytic cycle, which lasts 48 h for *P. falciparum* and causes the malaria disease, the parasite undergoes a successive development into the ring stage (0-20 h) and the trophozoite stage (20-36 h), which then undergoes several mitoses (schizont stage, 36-48 h) that lead to the differentiation of 16-24 infective merozoites. Erythrocyte lysis releases the merozoites into the bloodstream and a new intraerythrocytic cycle can be initiated. For some still not well understood reasons, some merozoites differentiate into male and female gametocytes that are taken up by the *Anopheles* during the blood meal. Gametogenesis resumes in the digestive tract of the mosquito and sexual reproduction occurs forming a zygote that produces, after a meiosis and several mitosis, thousands of haploid sporozoites under the basal lamina, which then migrate to the salivary glands and can be transmitted to

humans during a bite. Different antimalarial bioassays have been developed based on the *in vitro* inhibition of parasite growth or, more recently, on the inhibition of potential parasite targets, allowing the screening of chemical compounds.

2.2 Bioassays against the erythrocytic stage

The intraerythrocytic cycle, being responsible for the symptoms of the disease, is still the main parasite stage against which drugs are tested. Initially, drug screenings were limited to the use of animal malaria models (rodent, chicken or monkey). The development of the continuous culture of *P. falciparum* on human erythrocytes (Trager & Jensen, 1976) was a critical advance, allowing drug evaluation on well established laboratory strains and on fresh isolates from patients. Typically, parasites are maintained on leucocyte-free erythrocytes at 2-5% haematocrit, in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered Roswell Park Memorial Institute (RPMI) medium and supplemented with 5-10% human serum, at 37 °C, under a reduced percentage of oxygen. Hypoxanthine can be added to the culture medium to stimulate parasite growth and to sustain high parasitemia, and it is recommended for the culture of fresh isolates. Standardized protocols have been proposed e.g. (in *Methods in Malaria Research*, http://www.mr4.org/Publications/tabid/326/Default.aspx).

Plasmodium vivax is the most widespread species and, except in equatorial Africa, is responsible for the most prevalent malaria infection of humans, causing 70 to 80 million clinical cases per year. Although *P. vivax* infections are rarely fatal, they remain an important cause of morbidity, particularly in Asia Pacific region. Compared to *P. falciparum*, *P. vivax* can be considered as a neglected disease. Resistance of *P. vivax* to the antimalarial drug chloroquine, the reference drug for treating *P. vivax* infection, has been reported since the 90's. It is therefore of importance to develop tools for monitoring drug resistance and developing new drugs. In contrast to *P. falciparum*, establishment of continuous cultured lines of *P. vivax* has not yet been achieved limiting drug evaluation, particularly high throughput screening. *P. vivax* does not easily grow in culture, requiring removal of leucocytes and enrichment of the growth media. Parasite growth can only be performed for short periods, but maintaining cultures up to 4 weeks can be obtained by supplying reticulocytes from normal blood (Udomsangpetch et al., 2008). Drug assays developed for *P. falciparum* are transposable to fresh and cryopreserved *P. vivax* isolates (Kosaisavee et al., 2006). It is generally assumed that drugs active against *P. falciparum* blood stages will be also active against *P. vivax* blood stages; this has been shown in clinical studies for dihydroartemisinin–piperaquine and for artesunate–pyronaridine (Olliaro & Wells, 2009). Such assumption and the technical constraints to study *P. vivax* explain the limited interest for this species. For the other human malaria parasites, *P. knowlesi* has been adapted to long-term culture on monkey erythrocytes (Kocken et al., 2002) but no continuous cultivation of *P. ovale* and *P. malariae* has been set up.

Standard protocols of drug and resistance evaluation on *P. falciparum* are recommended by the World Health Organisation to facilitate comparison of data. They generally involve evaluation by using Giemsa-stained smears and counting parasitemia or parasite stage distribution in treated and non-treated cultures. These assays require minimal equipment and can be easily applied in the field. However they are time-consuming thereby preventing rapid, large-scale screening of molecules. Several methods have been developed for screening large numbers of compounds in 96-well plates, or even in 384-well plates.

2.2.1 Incorporation of radiolabelled precursors

These assays are based on the incorporation of metabolic precursors by the parasite that reflects its growth. They take advantage of the fact that the red blood cell possesses a reduced basic metabolism. Uptake of [^{3}H]-hypoxanthine, a precursor for nucleic acids, was the first described (Desjardins et al., 1979) and is still the most widely used but other precursors, such as [^{3}H]-amino acids or [^{3}H]-ethanolamine have also been described. Users must keep in mind that addition of hypoxanthine to the medium to sustain parasite development will interfere with the sensitivity of the isotopic assay using [^{3}H]-hypoxanthine, considered as the gold standard antimalarial drug assay. Although very sensitive and reproducible, applicable for high-throughput screening, these assays have several disadvantages. They are costly, involve radioactivity with the constraints associated to the manipulation of radioactive compounds and the treatment of radioactive wastes, and require special equipment (cell harvester, liquid-scintillation counter). They can thus be problematic to set up in locations with poor resources.

2.2.2 Colorimetric assays

Non-radioactive and low-cost alternative assays have been developed but are usually less standardized and often less sensitive than the [^{3}H]-hypoxanthine uptake method. Colorimetric assays include the detection of *Plasmodium* lactate dehydrogenase (pLDH) activity (Makler et al., 1993), by its preferential metabolism of the substrate 3-acetylpyridine adenine dinucleotide which is weakly metabolized by the human LDH, or by the tetrazolium assay (Delhaes et al., 1999). The assays require accessible equipments (spectrophotometers) and good correlations were observed with the radioactive assays. Sensitive immunocapture assays based on monoclonal antibodies directed against pLDH were developed for drug screening (Druilhe et al., 2001, Makler et al., 1998) and were miniaturized for assays in 384-well plates (Bergmann-Leitner et al., 2008). pLDH detection was also the basis of rapid immunoassays for malaria diagnostic from whole blood using strips (OptiMal®) (Palmer et al., 1998). ELISA based on the quantification of the *Plasmodium* histidine-rich protein II (HRP2) was also developed and commercialized (Noedl et al., 2002). Currently, both assays are as sensitive as the isotopic assay. However, reagents are not widely available and their stability in field conditions may be questioned; also the genetic variations encountered within these parasite proteins may lead to a decrease of the assay sensitivity.

2.2.3 Fluorometric and flow cytometry-based assays

Taking advantage that erythrocytes have no or only remnant DNA/RNA, fluorescence-based assays were developed for high-throughput drug assays using DNA-binding fluorophores. Fluorescence correlates parasitemia. The currently used fluorophores are Hoechst, DAPI, SYBRGreen I, PICO green and YOYO, the two former being less used because they exhibit excitation and emission properties not appropriate for current fluorescence plate readers and consumables, which is not the case for the latter. Protocols have been optimized and they propose one-step assays applicable to high-throughput screening and as sensitive as the isotopic and the immunocapture assays on laboratory strains or clinical isolates (Bacon et al., 2007, Baniecki et al., 2007, Bennett et al., 2004). The assays are cost-effective, requiring only a spectrofluorometer, and dyes are readily available

worldwide. These fluorometric assays seem sufficiently sensitive to identify complex chemical mixtures with antiplasmodial activity such as plant extracts (Abiodun et al., 2010). Although several works observed similar results between the isotopic and fluorometric assays, users must have in mind that fluorescence intensity is highly dependent on its physico-chemical environment and that false positive responses due to fluorescence quenching by a chemical compound of the tested sample are not occasional.

Flow cytometry is currently used to study the biology of malaria parasites, but is still little used to evaluate parasite drug susceptibility, certainly because of the high cost of cytometers and a capacity restricted to moderate throughput assays (Grimberg, 2011). However, owing to the large range of fluorescent probes available having different biological or biochemical affinities that can be simultaneously analysed, flow cytometry allows the access to many more information than just parasite proliferation (DNA content). It opens a new way to analyse drug susceptibility by integrating the DNA content and a deeper investigation of biological and biochemical effects of the drug on the parasite. Quantum dot (QD) is a CdSe/ZnS semiconductor crystal widely used for bioimaging applications and diagnostics because of its high photostability, large stokes shift and narrow emission spectra. In a recent work, authors searched for QD chemicals that could label *P. falciparum*-infected erythrocytes and found a polyethylenglycolated cationic QD (PCQD) that specifically label erythrocytes infected by the late stage parasites and not the uninfected erythrocytes and the erythrocytes infected by the early stage parasites (Ku et al., 2011). They used PCQD to quantify the antimalarial effect of chloroquine in 96-well plates by flow cytometry and found the assays as sensitive as the pLDH assay with a comparable cost and its performance enabled high-throughput assays. Innovative advances were also recorded for the development of assays using flow cytometry for evaluating drugs active on the gametocyte stage (see section 2.3).

2.2.4 Interlaboratory variations

Discrepancies in the activity of a same compound are often observed between laboratories. They can be due to the type of antimalarial assay used or to the *P. falciparum* strains used as well as their drug resistance status. However, data may also be influenced by laboratory-specific modifications of screening conditions. One can report:

- The use of asynchronized or synchronized cultures. *P. falciparum* has an asynchronous intraerythrocytic development *in vitro*. The synchronous development that is observed *in vivo* in humans can be restored by specific treatments taking into account the biological properties of the erythrocytes infected by the different parasite stages: e.g. 5% sorbitol treatment or gelatine floatation to specifically lyse or concentrate, respectively, the erythrocytes infected by the trophozoite and schizont stages. Assays on synchronized cultures of early stage parasites aim to be generalized because they reflect the parasite synchronisation observed in humans. Synchronous cultures allow further studies of the drug effects on specific stages of the parasite development.
- The duration of the drug incubation. This can vary from the classical 48 h assays that are widely used, to assays of 24 h or 72 h or of a longer period, especially for evaluating fast or slow-acting drugs. That is particularly illustrated by drugs having a delayed-death effect such as the antibiotics tetracyclines and clindamycin. In the presence of clinically relevant concentrations of these antibiotics, parasites grow normally, divide and invade new erythrocytes. Inhibitory effects are only observed when parasites

develop during the second erythrocytic cycle. This delayed-death effect has been attributed to inhibition of mitochondrion or apicoplast targets (Dahl & Rosenthal, 2008). Activity of such molecules would be underestimated using synchronized cultures and assays of less than 48 h.

- The use of human serum or serum substitutes. Variability of quality of human serum batches as well as the difficulty to have access to non-immune human sera in endemic malaria countries can be serious problems for data reproducibility and has led to the evaluation of numerous serum substitutes or sera of other species to support *P. falciparum* growth in culture (Basco, 2003). Albumax® was proposed as a substitute for human serum and is now used routinely by many laboratories. Two types exist, Albumax® I and II, both are used, depending upon the authors, generally at a concentration of 5 g/l. Their composition is not well characterized, as well as the biochemical differences between both types. They are mainly composed of chromatographically purified fatty acid-rich bovine serum albumin and have a low IgG content. In addition to their high cost, batch-to-batch variability were observed in the past and *Plasmodium* strains must be adapted to culture with Albumax® (Grellier P., personal communication). This latter point can be problematic for evaluating drug resistance of fresh isolates, which have been reported to grow poorly in the presence of Albumax® (Basco, 2004). Furthermore, contrasted results were reported when comparing antimalarial drug efficacy in assays containing Albumax® or human serum. Some authors observed differences in the IC_{50} of antimalarials (Ringwald et al., 1999), others observed no difference (Singh et al., 2007). In our hands and in our search of natural products with antimalarial activity, differences of IC_{50} may be important between both conditions for some chemicals. This can be due in part to the differences in lipid and protein content of the two culture media and to the binding properties of the antimalarial that may influence the pharmacodynamic profile of the drug, as observed for halofantrine, a highly lipophilic drug which significantly associates to triglyceride rich plasma lipoproteins (Humberstone et al., 1998).
- The culture conditions. Initial parasitemia, haematocrit, as well as atmosphere under which cultures are maintained (e.g., a 5% CO_2 atmosphere versus a well-defined atmosphere such as 6% O_2, 3% CO_2, 91% N_2) are all factors that may be the cause of discrepancies. For example, it has been documented that IC_{50} values for chloroquine depend highly on the oxygen pressure during the assay. A *P. falciparum* strain may display chloroquine-resistance (IC_{50} >100nM) at 10% O_2 and behave as a sensitive strain at 21% O_2 (Briolant et al., 2007).

2.3 Bioassays against the gametocyte stages

The emergence of resistance has highlighted the importance to develop drugs against parasite transmission (from human to *Anopheles* and from *Anopheles* to human) that has been recognized to be a priority in the efforts to eradicate malaria. Sexual stages (gametocytes) are being investigated as targets for vaccines but few drugs have been developed against these transmission stages. The only available compounds known to efficiently eliminate gametocytes are 8-aminoquinolines (primaquine and tafenoquine), but their use is restricted due to serious haematological toxicity. They cause haemolysis in patients with G6PD deficiency and, in cases of severe forms of G6PD deficiency, the risks of primaquine treatment might exceed the benefits (Beutler & Duparc, 2007). Difficulties to culture and to

obtain large amounts of gametocytes have limited research in this area. Gametocytes have a quite long maturation period *in vitro* (more than 10 days), gametocytogenesis is stimulated by stress conditions and sexual subpopulation represents a small percentage of infected red blood cells. Furthermore, *Plasmodium* strains have a tendency to lose their capacity to produce gametocytes over time in culture and gametocyte-infected erythrocytes are difficult to separate from erythrocytes infected by asexual stages. Until recently, there had been few significant advances in techniques for producing gametocytes for drug screening. These techniques were difficult to set up and required costly equipments. New protocols have now improved our capability to produce gametocytes *in vitro* in a reproducible way, and to enrich gametocytes with a high degree of purity using MACS® magnetic affinity columns (Baker, 2007). Those improvements associated to the ability to genetically manipulate parasites have allowed the recent setting-up of protocols for evaluating drug effects on sexual conversion and gametocyte maturation with high-throughput capacities. They are based on : 1) the discrimination by flow cytometry of asexual and sexual forms using hydroethine that is taken up by the parasite and metabolized into ethidium, a nucleic acid-binding fluorochrome (Chevalley et al., 2010); 2) the use of transgenic *P. falciparum* parasites expressing a green fluorescent protein chimera of the early sexual blood stage (protein Pfs16) as a marker for commitment to gametocytogenesis; this marker associated to hydroethine allows also to measure the direct activity of drugs against the late-stages gametocytes (Peatey et al., 2009). In a same way, the stage II or later stage marker (PF10_0164) fused to the green fluorescent protein was used associated with the nuclear dye Hoescht 33342 to quantify the drug effects on the asexual stages and on the sexual conversion and the gametocyte maturation in a same assay (Buchholz et al., 2011). In a general way, the application of transfection technology to malaria parasites paves the way to a new generation of assays targeting specific pathways or parasite stages.

2.4 Bioassays for the hepatic stage of *Plasmodium*

Drug development against the liver stage has two main advantages: 1) Such drugs could be used as prophylactic agents by preventing the access of the parasite to the blood and, in consequence, the development of the disease; 2) *P. vivax* and *P. ovale*, unlike *P. falciparum*, have latent forms (hypnozoites) in the liver responsible for relapsing infections, sometimes years after the primary infection. Hypnozoites are unaffected by the drugs acting on the erythrocytic stage. Thus, anti-hypnozoite drugs could be used as anti-relapse agents, limiting then recrudescences.

The number of available drugs that act against the hepatic phase is limited. The 8-aminoquinolines (primaquine and tafenoquine) are the main and the most efficient ones, but have serious haematological toxicity as already mentioned. Evaluation of drugs against the hepatic stage has been mainly performed using *in vivo* models of rodent parasites e.g., *P. berghei* and *P. yoelii*. However, such drug screening is time consuming and costly due to the need to reproduce the complete life-cycle of the parasite to obtain viable sporozoites. Difficulties increase greatly if screening is performed using monkey models for human malaria parasites. The ability to culture *Plasmodium* in hepatocytes has opened new perspectives in the research of drugs active against the hepatic stage (Mazier et al., 1985, Mazier et al., 2004). *In vitro* screenings are usually performed using sporozoites of rodent *Plasmodium* infecting primary rodent hepatocytes or hepatoma cell lines but assays based on

primary culture of human hepatocytes infected with *P. falciparum* sporozoites were also reported. Recently, assay on liver stage of *P. vivax* was established using purified and cryopreserved sporozoites opening new perspectives for development of anti-relapse drugs (Chattopadhyay et al., 2010). The sporozoite development within the hepatocyte into a schizont stage is followed either by Giemsa staining or immunofluorescence assay using specific antibodies or a genetically-manipulated fluorescent parasite. Potential antimalarial drugs acting on the hepatic stage have emerged (Carraz et al., 2006, Mahmoudi et al., 2003, Mahmoudi et al., 2008, Parvanova et al., 2009, Singh et al., 2010, Tasdemir et al., 2010, Yu et al., 2008). A high-throughput *in vitro* screening of drug activity on *Plasmodium* liver stages was developed based on a sophisticated infrared fluorescence scanning system, which allows rapid, automatic counting of infected hepatocytes (Gego et al., 2006). The recent development of bioluminescent parasites allows now a non-invasive real time monitoring and quantitative analysis of liver stage development *in vitro* and *in vivo* in rodents that offers new tools for drug evaluation (Mwakingwe et al., 2009, Ploemen et al., 2009) (see section 4.3.2). Automated visual assay was also set up to follow the extracellular cell death of sporozoites (Hegge et al., 2010). Although promising, such assays are however greatly dependent upon the production of a large number of sporozoites in insects, a limiting step for high-throughput screening.

2.5 Bioassays for parasite targets

Our understanding of malaria parasite biochemistry has considerably increased over the past two decades and has allowed the identification of many potential targets for new drugs, even if half of the *P. falciparum* genes are still in search of a biological function (Florent et al., 2010). Progresses have been made possible thanks to the decrypted genomes of several *Plasmodium* species and other Apicomplexans (see www.eupathdb.org), that have enabled the rapid identification of putative targets that are homologous to validated targets in other organisms. Usually, target validation in *Plasmodium* requires demonstrating that a specific inhibitor, designed or identified as being active against this particular target, indeed shows antimalarial activity *in vitro* and/or *in vivo*. However, such an approach called "chemical-validation" has limits since it is often difficult to demonstrate that the inhibitor kills the parasite by indeed acting specifically on this target and not by inhibiting unrelated biochemical mechanisms. Thus, nowadays, "genetic-validation" of the selected target must also be achieved in parallel i.e., the deletion of the corresponding gene must seriously impair the parasite's growth or ideally prevent its survival.

Recent advances in genetic understanding of *Plasmodium* have greatly increased the ability to genetically validate potential drug targets (Limenitakis & Soldati-Favre, 2011). Methodologies able to transfect rodent *Plasmodium* and *P. falciparum* so as to modify or knock out genes that code for potential target proteins have been available for over a decade. They have been progressively improved and refined to now permit conditional knock out of the gene at a chosen parasite stage that may be different from the transfected stage (Lacroix et al., 2011), conditional elimination of the gene product by fusing a degradation domain and Shield ligand (Dvorin et al., 2010) or compensation of a lethal phenotype resulting from knock out of an essential gene by expression in trans of a wild-type version of this gene (Slavic et al., 2010). Phenotypic analysis of the resultant mutant parasites then allows to study the importance of a particular target in the different phases of

development of the life-cycle of *Plasmodium*. Many putative targets, initially suspected to be essential to a given parasite stage turned out finally dispensable to this stage but essential to others (see few examples such as FabI or falcipain-1 below). Furthermore, access to transcriptomic and proteomic technologies offers new opportunities to study the impact of a drug treatment on the entire parasite metabolism either at the RNA or protein levels. This leads to a better understanding of the mechanism(s) of action of drugs and the biochemical pathways involved in killing the parasites (Sims & Hyde, 2006). Recently, the achievement of random mutagenesis of *P. berghei* and *P. falciparum* genome by PiggyBac transposable element opened the route towards systematic plasmodial gene invalidation, that will obviously speed up the genetic validation process of putative targets (Crabb et al., 2011).

These recent advances have allowed development of bioassays based upon validated targets for drug screening, or targets still in the process of validation (for review see (Grellier et al., 2008, Prabhu & Patravale, 2011, Sahu et al., 2008)) : haem polymerization (O'Neill et al., 2006), pyrimidine, purine, folate (Hyde, 2007), lipid (Wengelnik et al., 2002), shikimate (McRobert et al., 2005), non-mevalonate (Wiesner & Jomaa, 2007) and other apicoplast metabolisms (Sato & Wilson, 2005), mitochondrial electron transport (Mather et al., 2007), redox homeostasis (Bauer et al., 2006), protein prenylation (Van Voorhis et al., 2007), proteases (Wegscheid-Gerlach et al., 2010), kinases (Doerig & Meijer, 2007)... Some of them are detailed below.

Databases such as TDR targets (http://tdrtargets.org) gather information on putative targets for several pathogens including *Plasmodium*, providing tools for their prioritization in whole genomes depending on user queries (Aguero et al., 2008). The Protein Data Bank (www.pdb.org) is also a useful resource, providing data on proteins whose 3D-structures have been solved experimentally by using either X-Rays or NMR. As of September 2011, more than 300 entries correspond to plasmodial proteins, knowing that several structures involving different ligands may have been solved for a same protein.

2.5.1 Inhibition of haeme polymerization

Haeme polymerization (Figure 2) is a natural process that occurs in the acidic food vacuole of the parasite where haemoglobin, a major source of amino acids for the parasite, is degraded by specific proteases. Haeme, which is toxic for the parasite, is detoxified by polymerization into a pigment, the haemozoin. Inhibition of haemozoin formation is considered as an attractive target for antimalarial drugs and high-throughput assays are available (Biagini et al., 2003, Deharo et al., 2002, Huy et al., 2007, Ncokazi & Egan, 2005, Rush et al., 2009, Weissbuch & Leiserowitz, 2008). The 4-aminoquinolines such as chloroquine and amodiaquine act by interfering with this polymerization process. Cysteine and aspartic parasite proteases (falcipains and plasmepsins, respectively) involved in the degradation of haemoglobin are also potential targets (Wegscheid-Gerlach et al., 2010) (see section 2.5.2).

2.5.2 Proteases

Although none of the currently marketed antimalarials is targeting plasmodial proteases, this class of enzymes, which is involved in a wide diversity of biological pathways during the parasite life cycle, has been the subject of intense investigations for the last decades

(Wegscheid-Gerlach et al., 2010). The cystein and aspartyl endoproteases involved in the essential pathway of haemoglobin degradation now known as falcipain-2, 2′ and -3 and plasmepsin-I, -II, -III (or HAP, for histo-aspartyl protease) and –IV have first emerged as promising protease targets. Intensive research focused on these enzymes during the last decades has yielded inhibitors active down to the nM levels on the native or recombinant enzymes, inhibiting parasite growth in culture from the nM to μM levels and capable to cure *Plasmodium*-infected mice (McKerrow et al., 2008, Rosenthal, 2010). Gene disruption studies however clearly indicated that all of these enzymes besides falcipain-3 are highly redundant. In fact, falcipain-3 is the only for which gene disruption did not yield viable blood-stage parasites (Liu et al., 2006, Sijwali et al., 2006). The development of inhibitors targeting these enzymes but displaying improved properties in term of activity, selectivity, safety to the host nonetheless pertains, facilitated by their availability under recombinant active form, allowing both high-throughput screenings and experimental determination of their 3D-structures, including for the most recently described plasmepsin I (Bhaumik et al., 2011). A strong argument for maintaining such a development against these haemoglobinases is linked to the proposal to use combined protease inhibitor therapy based on the observation that cystein and aspartyl inhibitors demonstrated synergistic antimalarial effects (Semenov et al., 1998).

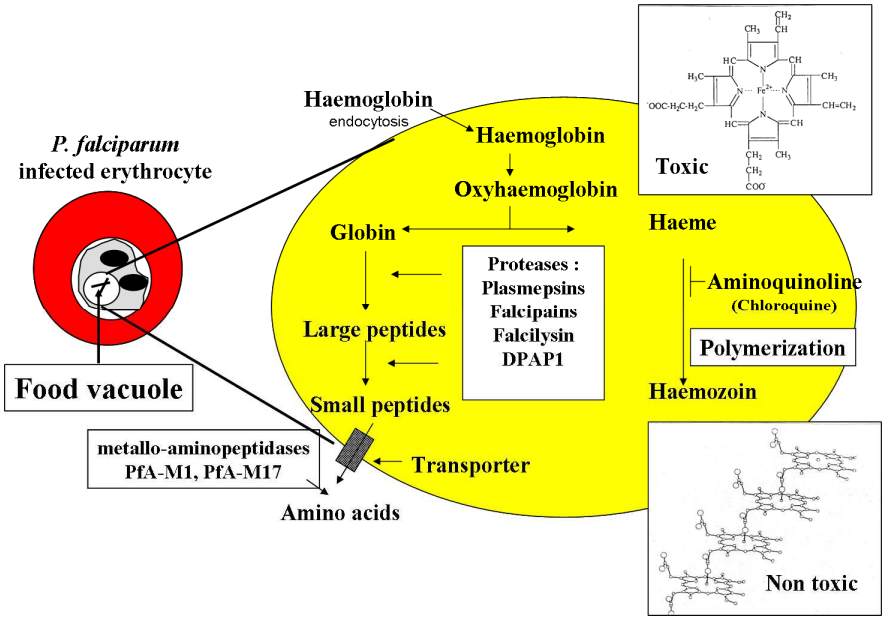


Fig. 2. Mechanisms of haemoglobin degradation and haeme detoxification in *Plasmodium falciparum*-infected red blood cell, after (Mambu & Grellier, 2008)

In parallel, the last decade has seen the emergence as promising targets of several new parasite proteases, among the ~100 that are encoded in the *P. falciparum* genome (Wu et al., 2003). Some are also involved in haemoglobin breakdown such as the cystein protease DPAP1 (Klemba et al., 2004), the metallo-endopeptidase falcilysin displaying however a

dual activity, both able to process peptides in the food vacuole and to mature proteins imported to the apicoplast (Eggleson et al., 1999, Ponpuak et al., 2007), and the metallo-aminopeptidases PfA-M1 and PfA-M17 (McGowan et al., 2009, Skinner-Adams et al., 2009, Trenholme et al., 2010). Some have emerged from studies focusing on other key biological events such as erythrocyte egress/invasion and merozoite surface antigen maturation such as the subtilisin-like proteases -1 and -2 and the cystein proteases DPAP3, SERA-5 and SERA-6 (Blackman, 2008). Other proteases for which essential roles during the parasite asexual development have been demonstrated include Plasmepsin V, involved in maturation of proteins exported to the infected-red blood cell (Boddey et al., 2010, Russo et al., 2010). During these investigations, some proteases were found dispensable for the parasite asexual development in erythrocyte but important in other stages such as gametogenesis or the development of insect stages (Sologub et al., 2011). This is the case for the cystein proteases falcipain-1 and SERA-8. Gene deletion assays were determinant to indicate that falcipain-1, initially believed to be involved in haemoglobin breakdown then in red blood cell invasion, was in fact dispensable for the parasite blood-stage development but important for oocyst production (Eksi et al., 2004). Also, the *P. berghei* ortholog of SERA-8 appears involved in sporozoite egress from oocysts (Aly & Matuschewski, 2005).

2.5.3 Apicoplast-based targets

The apicoplast is a vestigial, non-phostosynthetic, plastid-like organelle inherited from the prokaryotic world by secondary endosymbiosis and found in most apicomplexan parasites including *Plasmodium* that is essential to their biology. In *P. falciparum*, it maintains a 35-kb circular genome and several particular biochemical pathways that are present in bacteria and plants but are absent in humans, thus providing many attractive targets that are extensively investigated for drug development. These pathways include the type II fatty acid biosynthesis pathway, which involves 6 distinct enzymes in *Plasmodium* while in human the type I fatty acid biosynthesis pathway involves a multifunctional enzyme, the 1-deoxy D xylulose 5 phosphate (DOXP) isoprenoid biosynthesis pathway that is mevalonate-independent in the malaria parasite contrary to humans, and apicoplast replication, transcription and translation which involve enzymes of bacterial origins (Dahl & Rosenthal, 2008, Goodman & McFadden, 2007, Grawert et al., 2011, Jayabalasingham et al., 2010). Pioneering works led to the emergence of promising antimalarials such as triclosan (believed to target the NADH-dependent enoyl ACP reductase or FabI enzyme), thiolactomycin (targeting FabH and FabB enzymes) and fosmidomycin (targeting the DOXP reductoisomerase) to name the main ones. Also, antibiotics such as tetracyclines and clindamycin, targeting prokaryotic protein synthesis, or quinolone antibiotics and rifampicin, targeting the prokaryotic DNA and RNA machinery, were shown to inhibit *Plasmodium* growth in culture and *in vivo* (Seeber & Soldati-Favre, 2010).

These results stimulated efforts both to genetically validate these putative targets and to improve inhibitor discovery, by solving 3D structures and producing enzymes under recombinant active forms amenable to medium/high throughput screenings (Freundlich et al., 2007, Sato, 2011). In 2008, the genetic inactivation of FabI in *P. falciparum* and in *P. berghei* produced parasite blood stages that were growing normally and were still affected by triclosan (Yu et al., 2008). The FabI gene deletion, on the other hand, blocked the development of the parasite in the liver. Not long later, the genetic inactivation in murine

models of other enzymes belonging to the FasII metabolism such as FabB/F confirmed that the FasII pathway was dispensable through the entire parasite development to the exception of the hepatic-blood stage transition phase. These results raised the question of the nature of the parasitic target of triclosan in blood stages. Recently five laboratories reported their failed attempts to inhibit rodent *P. berghei* and *P. chabaudi* proliferation in mice by using similar doses of triclosan (Baschong et al., 2011).

Conversely, recent works focusing on the DOXP non-mevalonate isoprenoid pathway confirmed it appears as a relevant target for antimalarial drug development. Key results include the recent failure to delete the DOXP reductoisomerase gene (Odom & Van Voorhis, 2009). The DOXP metabolic pathway is active in all erythrocytic stages but problems with fosmidomycin, which has long been known to inhibit *Plasmodium* growth *in vitro* and *in* mice (Jomaa et al., 1999), emerged. Due to its short half life that may lead to parasite relapses and/or facilitate selection of resistant parasites, analogues with improved half-lives are actively looked for against this enzyme and the other enzymes of this metabolic pathway (Seeber & Soldati-Favre, 2010). The recent discovery that fosmidomycin is little effective against *Theileria*, *Eimeria* and *Toxoplasma* also raised the question of its bioavailability (Seeber & Soldati-Favre, 2010). This led to the proposal that fosmidomycin and its more active derivative FR900098, both active against *Plasmodium* erythrocytic but not hepatocytic stages, would enter the parasite through new permeation pathways called NPPs (Baumeister et al., 2011). Recently also two triose-phosphate transporters present on the apicoplast membranes have been characterized, PfiTPT/PfAPT1 and PfoTPT/PfATP2, and are being considered as putative targets since they are believed to import from the cytosol into the apicoplast key metabolites to fuel the DOXP pathway (Lim & McFadden, 2010).

2.5.4 Kinases

Malarial kinases but also host cell kinases involved in host-parasite interactions are also currently considered as promising targets (Doerig et al., 2010). The *P. falciparum* theroretical kinome was predicted to comprise 85 to 99 enzymes, depending on the stringency of the computational methods used to identify them, and phylogenetic studies have revealed interesting differences with the human kinome that may be exploited for kinase-targeted drug discovery (Leroy & Doerig, 2008). For example, *P. falciparum* possesses kinases and kinase families such as calcium-dependent protein kinases (CDPK) and the apicomplexan specific FIKK kinase families, that are absent in humans. Important advances in this field have been provided by the fact that many *P. falciparum* kinases have been successfully produced as recombinant active enzymes, allowing medium/high troughput studies, many structures are known or can be deduced by modelling (see http://www.thesgc.com) and several chemical libraries, developed for other pathologies such as cancer may be screened on *P. falciparum* putative targets and parasites. For example, PfCDPK1 that has no ortholog in humans was produced as an active recombinant enzyme and used to screen a library of ~20,000 molecules developed against kinases, from which a purine derivative called purfalcamine active to the low nM range on the enzyme was isolated (Kato et al., 2008). This molecule, able to prevent the *P. falciparum* development in culture had however a low *in vivo* activity on *P. berghei*, possibly due to poor pharmacokinetic parameters. Importantly, not only protein kinases but kinases phosphorylating other substrates such as choline kinase and pantothenate kinases could also be targeted (Leroy & Doerig, 2008). Genetic validation

of kinases may be performed by classical gene deletion in *P. falciparum* or rodent *Plasmodium*, but conditional expression systems have also been used as for example using the destabilizing domain and Shield ligand to show the involvement of PfCDPK5 in merozoite egress (Dvorin et al., 2010).

2.5.5 Transporters

Transporters are integral transmembrane proteins that enable the movement of solutes across biological membranes. In *Plasmodium,* they are currently considered as highly promising targets, due to their involvement, as carrier proteins, channels or pumps, in the movement of nutrients, metabolites and ions into and out of the parasite as well as between subcellular compartments within the parasite. They thus regulate essential nutrient uptake into the parasite, homeostasis and disposal of toxic wastes. Host cell transporters modified by the presence of the parasite may also be involved in these biological functions. Of note, malarial transmembrane proteins such as PfCRT, Pgh1, PfNHE1 and possibly PfATP6 are involved in drug-resistance mechanisms.

The recent publication of the theoretical permeome of *P. falciparum* indicated that at least 2.5% of the parasite genome encode transporters, channels and pumps (Martin et al., 2009). Experimental localizations, that have been achieved by using either specific antibodies or epitope-tagged or fluorescent recombinant fusion proteins transfected into parasites, concern few proteins of the parasite surface (PfHT1 or the hexose transporter, PfENT1 or PfNT1, PfMRP, PfAQP), the mitochondrial membrane (ATP/ADP transporter), or the food-vacuole membrane (PfCRT, Pgh1, V-type H+ ATPase and ATP-driven H+ pump). Substrate specificities are also often ill-defined. Even if these could be predicted by orthology for about half of the candidates, experimental validations are still necessary to confirm or discover which they are. Extensive experimental work therefore needs to be done to further exploit such a rich source of potential targets (Staines et al., 2010). Some promising results nonetheless emerged in this field. First, the challenging functional expression of recombinant proteins, which is necessary for the biochemical testing of substrates and inhibitors, has been achieved successfully in *Xenopus laevis* oocytes for several *Plasmodium* transporters. Alternative heterologous systems have also been used such as yeast, *Dictyostelium discoideum* and mammalian cells (Martin et al., 2009). In some cases, cell-free transporter assay systems are used. Recently, recombinant expression of codon-optimized PfHT1 in yeast was performed to permit high-throughput screening of inhibitors (Blume et al., 2010). The P-type ATPases, PfATP4 and PfATP6, and the drug-resistance involved PfCRT and Pgh1 have also been expressed in such heterologous system, opening the road for functional studies (Martin et al., 2009). The number of known 3D-structures remains however extremely low, limited to PfAQP although a model has been proposed for PfHT1 based on the structure of *E. coli* permease (Staines et al., 2010).

Transfection methods have been also critical in this field, in particular to assess the role of PfCRT and Pgh1 in drug resistance, by transfecting *P. falciparum* resistant alleles into sensitive parasites and vice-versa, or by transfecting these alleles in various heterologous expression systems such as yeast (Martin et al., 2009). Gene deletion studies have been also undertaken, but so far for a limited number of candidates. While several genes could be deleted with none or limited impact on parasite asexual growth such as those encoding PfENT1, PfMRP, the *P. berghei* orthologue of the PfAQP, PfKch1 (PfK1) and PfACα, the

deletion of genes encoding PfHT1, PfCRT and Pgh1 could not be achieved, suggesting that the two latter ones play essential functions in the parasite biology beside being involved in drug-resistance (Sanchez et al., 2010). Such experiments performed using rodent malaria models further indicated that some candidates, apparently dispensable for the asexual development, turned out important for the development of other parasite stages in insects or liver (Martin et al., 2009, Staines et al., 2004). Among all these candidates, PfHT1 is the only malarial transporter that has been validated both chemically and genetically (Staines et al., 2010). Gene deletion studies and D-glucose derivatives used as inhibitors confirmed the essential role of the hexose transporters for the asexual parasite development and other parasite stages (Blume et al., 2010, Slavic et al., 2011).

3. Renew of phenotypic screening approaches

Advanced antimalarial drug discovery programs revealed four general strategies to discover new drugs (Wu et al., 2011) : 1) To start from specific, validated or not, malaria targets to find new hits (e.g. haem polymerization, enzymes of the pyrimidine synthesis pathway); 2) To synthesize new analogs from known antimalarial pharmacophores (e.g. synthetic endoperoxides (Trioxolanes) or hybrid molecules having two pharmacophores (Trioxaquines)); 3) To start from drugs developed for other diseases whose similar targets are present in malaria parasites (as illustrated by the piggy-back approach undertaken against parasite protein farnesyltransferases using anti-cancer agents targeting protein prenylation developed by pharmaceutical companies); 4) To screen chemicals on whole cell-based assays to identify hits that kill parasite for further optimizations. Most of the current antimalarials were originated from this last phenotypic drug discovery approach. The revolution in molecular biology led to a switch to target-based approaches for drug discovery in pharmaceutical industries. However, this approach failed to deliver the expected results, especially for antimicrobials (Keller et al., 2011, Payne et al., 2007, Sams-Dodd, 2005). In the recent years, the access to large chemical libraries and the improvements of whole cell-based high throughput screening assays led to a renaissance of the phenotypic approach with the forward chemical genetic strategy. Chemical genetics is the study of biological systems using small molecules as tools (O'Connor et al., 2011). Forward chemical genetic uses small molecules to modulate gene-product function leading to a phenotype of interest (parasite killing for example), and the target must be further identified. In contrast, reverse chemical genetic screens specific gene products with libraries of small molecules to identify ligands, which are then tested on cells for phenotypic changes. Over the last few years, reports demonstrated the power of chemical genetic for antimalarial drug discovery. The GlaxoSmithKline (GSK)'s chemical library constituted of nearly 2 million chemical entities was tested upon drug sensitive and multidrug resistant *P. falciparum* strains. More than 8,000 compounds, clustered into 416 molecular frameworks, showed potent antiplasmodial activity. Analysis of historical GSK data suggested that the main target classes affected by these compounds are malaria kinases, proteases and G-protein coupled receptors (Gamo et al., 2010). In a similar study, a library of nearly 310,000 chemicals, designed to cover a large diversity of bioactive compounds, was screened upon drug sensitive and multidrug resistant *P. falciparum* strains (Guiguemde et al., 2010). Amongst hits, 172 were cross-validated by three laboratories using distinct assays. A reverse chemical genetic approach was undertaken with the validated set of compounds using 66 malarial target assays and identified 19 new inhibitors of 4 validated targets. Preliminary

pharmacokinetic profiling found most of them suitable for further development. One lead was further evaluated *in vivo* on *P. yoelii*-infected mice and displayed significant antimalarial activity. In another study, the screening of a focused library of about 12,000 natural and synthetic chemicals led to identification of spiroindolone compounds as appropriate candidates for further development (Rottmann et al., 2010). Optimization studies produced NITD609, which is developed by Novartis.

4. *In vivo* antimalarial drug evaluation

Plasmodium species that infect humans are essentially unable to infect non-primate animal models. Historically, *in vivo* evaluation of antimalarial compounds began with the use of avian parasites in birds and simian parasites in monkeys. The discovery by Ignace Vincke and Marcel Lips in 1948 at Keyberg in the then Belgian Congo, of a rodent malaria parasite (*Plasmodium berghei*) which readily infected laboratory mice and rats, greatly facilitated *in vivo* drug screening management. Since then, several other species and subspecies of rodent parasites have been described and employed. With the discovery in the early 90's that the owl monkey (*Aotus trivirgatus*) is susceptible to infection with the human parasites *P. falciparum* and *P. vivax*, the simian models of malaria have regained interest. Infection with *P. falciparum* is now well characterized in both *Aotus* and *Saimiri* monkeys (Collins, 1992), and primate models, because they provide a clear prediction of drug efficacy and pharmacokinetics in humans, are a logical transition to clinical studies. However, there are obvious limitations to their use, and any primary screen dependent upon monkeys appears both wasteful in terms of animal conservation, drug consumption, and ethics. So, the first steps of *in vivo* drug screening typically begins with the use of mouse models of malaria with the rodent parasites *P. berghei*, *P. yoelii*, *P. chabaudi* and *P. vinckei*, that have been validated through the evaluation of several antimalarial drugs.

4.1 Rodent models of malaria

Individual parasite species and strains have been well characterized, including duration of cycle, synchronicity, drug sensitivity and course of infection in genetically defined mouse strains. *P. berghei, P. vinckei* and few strains of *P. yoelii* and *P. chabaudi* cause lethal infections in mice, whereas *P. yoelii, P. chabaudi,* and *P. vinckei petterei* are usually cleared after the initial acute parasitaemia or after a subsequent low-grade chronic parasitaemia.

P. berghei - The strains K173, ANKA, NK65 and SP11 are the most frequently used for experimental work. The affinity for immature erythrocytes (reticulocytes) varies according to the strain. ANKA invades preferentially reticulocytes, while NK65 invades indifferently reticulocytes and mature erythrocytes, i.e. normocytes. Both strains are lethal, but ANKA kills the mouse with a low parasitaemia (it rarely exceeds 20%), whereas infection with NK65 induces a continuous increase in parasitaemia until the mouse dies. The erythrocytic cycle is asynchronous and its duration is about 21 h. Schizogony lasts 50 h in hepatocytes. Susceptible mice infected with *P. berghei* ANKA die within two weeks after infection with severe neurological symptoms and cerebral microvascular abnormalities. These are common pathologies to both murine and human infections that make of *P. berghei* ANKA a useful experimental model of cerebral malaria. Resistant mice do not show any neurological symptom but die during the third or fourth week post-infection (p.i.) with high parasitaemia.

P. vinckei - Among four subspecies recognized (*P. vinckei vinckei*, *P. vinckei petteri*, *P. vinckei lentum* and *P. vinckei brucechwatti*), *P. v. vinckei* and *P. v. petteri* have been used in some studies for the identification of new antimalarial drugs. Both subspecies preferentially invade normocytes. Schizogony in erythrocytes has a duration time of 24 h and is synchronous. Schizogony in hepatocytes lasts for 60 h or more.

P. yoelii – Three subspecies are recognized: *P. yoelii yoelii*, *P. yoelii killicki* and *P. yoelii nigeriensis*. *P. yoelii* shows a preference for reticulocytes, but it may also invade normocytes, depending on the virulence of the strain. Duration of the schizogonic cycle is 18 h in erythrocytes. Schizogony is about 45 h long in hepatocytes. The erythrocytic cycle is asynchronous. The parasitaemia of *P. y. yoelii* 17X and 265BY depends mainly on the production of reticulocytes by the host, whereas *P. y. nigeriensis* grows very rapidly by invading all available erythrocytes. Of note, *P. yoelii* is more resistant to chloroquine than are the other species (Beaute-Lafitte et al., 1994). Gametocyte production is constant throughout the infection.

P. chabaudi – Two subspecies of *P. chabaudi* have been identified: *P. chabaudi chabaudi* and *P. chabaudi adami*. This species exhibits many similarities to *P. falciparum*, including analogous blood-stage antigens, invasion of reticulocytes and normocytes, suppression of B- and T-cell responses, and parasite sequestration in liver and spleen which induces parasite withdrawal from the peripheral circulation. The schizogonic cycle of *P. chabaudi* is 24 h long in erythrocytes and is synchronous. It runs 54-58 h in liver. In resistant mice, a rapid parasite multiplication during the first week p.i. is followed by parasite elimination by the fourth week p.i., whereas susceptible mice usually die during the second week p.i.

4.2 Designing an experimental mouse model

Many inbred and outbred strains of mice are available to design a mouse model. Considering that every species and every line or clone of a particular *Plasmodium* species exhibits particular characteristics, the resulting infection may vary greatly within the different laboratory strains of mice. As an example, blood-induced infections of the K173 (N) of *P. berghei* follow a fulminating course in many strains of mouse, but are slow in NMRI mice, and infections with *P. y. yoelii* 17X in Swiss mice are lethal, whereas they resolve in CBA/Ca mice. Scientists should use the most appropriate model for their particular research question and take advantage of their particular knowledge about compounds to be tested, especially in terms of molecular and/or biological targets. A rather rich presentation of experimental malaria infections in different mouse strains, comprising some models for cerebral malaria, can be found in (Sanni et al., 2002).

The difference in the degree of infection, lethality and synchronicity between the rodent plasmodia enlarges the number of possible assays for drug evaluation. For example, parasites that generate high parasitaemia and synchronous infections, like *P. chabaudi* and *P. vinckei*, are more appropriate to study compounds exhibiting stage selectivity. Also, it is important to note that the drug sensitivity of a given rodent *Plasmodium* species may not reflect that of the other rodent species. This can be illustrated by *P. berghei* exhibiting less sensitivity than *P. chabaudi* and *P. vinckei* to iron chelators and lipid biosynthesis inhibitors (Peters & Robinson, 1999, Wengelnik et al., 2002). Drug sensitivity may also not mirror that of *P. falciparum*, as shown in the case of cysteine protease inhibitors, owing to the fact

that the enzyme active site is different between rodent plasmodia and *P. falciparum*. This situation has led to question the use of these models in lead optimization (Singh et al., 2002).

Given this, the following criteria must be considered when selecting a mouse model. On the host side, innate factors like peculiarities in pharmacodynamics of the drug; innate immunity; variation between individuals of the same species (strain, age, sex and immune status); environmental factors (temperature and stress, nutrition, intercurrent infections). On the parasite side: variation within a single *Plasmodium* species (variation of sensitivity among different clones, geographic strains, time since isolation of "wild" strain); variation among different *Plasmodium* species; mode and intensity of infection. Also, attention should be paid to drug-dependent factors like the mode of formulation, the route of administration or the drug dosage regimen.

4.3 Current *in vivo* antimalarial tests using rodent models of malaria

4.3.1 Erythrocytic stages of malaria parasites

The most widely used initial test is the "four-day suppressive test", which commonly uses *P. berghei* or *P. chabaudi*. The efficacy of four daily doses of compounds is measured by comparison of mouse survival time and blood parasitaemia on day four p.i. in treated and untreated mice. Compounds identified as being active in those assays can subsequently be progressed through various secondary tests, such as 1) the "dose ranging, full four-day test", in which compounds are tested at a minimum of four different doses to determine effective dose values and get information on oral bioavailability and relative potency of the tested compounds; 2) the "onset/recrudescence" test, in which mice are administered a single dose on day 3 after infection and parasitaemia is monitored daily. Compounds can also be tested for prophylactic activity: the compound is administered prior to infection, and parasitaemia is followed daily.

4.3.2 Other stages of malaria parasites

Several tissue schizontocidal tests (the test of Gregory and Peters (1970) using sporozoites of *P. yoelii nigeriensis*, the test of Rane and Kinnamon with *P. yoelii yoelii*, and the Hill test for causal prophylactic compounds with residual action) have been reported in details (Peters, 1987). Tests that apply to the screening of drugs having gametocidal or sporontocidal action can be found at the same source. A technique such as real time PCR allows quantification of the parasite charge in liver and thus, the inhibitory effect of the molecule tested (Carraz et al., 2006). Recently, the use of a transgenic *P. berghei* parasite expressing the bioluminescent reporter protein luciferase to visualize and quantify parasite development in liver cells in live mice using real-time luminescence imaging was reported (Ploemen et al., 2009). The applicability of real time imaging to assess parasite drug sensitivity in the liver was demonstrated by analysing the effect of primaquine and tafenoquine *in vivo*. The methodology is relatively simple and offers the possibility to analyse liver development in live mice without surgery. It opens up new possibilities for research on *Plasmodium* liver infections and for evaluating the effect of drugs on the liver stage of *Plasmodium*.

4.4 Humanized mice models of malaria

Immunocompromized mice, developed and used in other research contexts, have been used for some years to elaborate new mouse models for human malaria. By grafting them with either human erythrocytes or human hepatocytes, these animals can support, respectively, the asexual blood cycle or the hepatic phase of the human parasite *P. falciparum*. So, drug efficacy and pharmacokinetics can be assessed in an *in vivo* setting against the true parasite target during its blood or hepatic stage development.

4.4.1 The *P. falciparum*-human erythrocyte mouse models

In 2001, the feasibility of evaluating *in vivo* responses to antimalarial drugs in humanised mice models was demonstrated using the *P. falciparum*-human erythrocyte-BXN model, in which Beige Xid Nude (BXN) mice, genetically deficient in T- and B-cell functions and chemically controlled for response of cells involved in innate defences, were grafted with human erythrocytes infected with *P. falciparum* (Moreno et al., 2001). The mice received human erythrocytes infected with drug (chloroquine/quinine/mefloquine) resistant or sensitive strains of *P. falciparum*, and the blood schizonticidal effects of various antimalarial drugs were assessed. Parasite clearance and parasite reduction rate in the mouse model were shown to parallel those reported in humans infected with *P. falciparum*. Since then, improvements of the *P. falciparum* humanized mouse model have been made using NSG mice with improved genetic deficiency of innate immunity. High reproducibility of human erythrocytes grafting and parasite survival could be achieved, along with the possible adjustment of parasitaemia over a range of 1-40% for several weeks (Arnold et al., 2011). In the near future, a model harbouring a hematopoietic stem cell line capable of producing erythroblasts/cytes should replace current models with the advantage of producing reticulocytes to also support *P. vivax*. To date, we are aware of only one work dealing with drug testing in humanised mouse model of malaria, i.e. the one about trioxaquine selection for drug development (Cosledan et al., 2008). The scarcity of reports exemplifies the serious limitations to the use of such malaria models in drug discovery: 1) in most of the models, parasitaemia remains stable only in a minor subset of mice whereas it is rapidly cleared in the majority of the animals, 2) the use of toxic reagents to minimize the mouse innate defence is susceptible to affect the efficacy of antimalarials or effector cells, 3) management of those experimental models is cumbersome and requires specific facilities.

4.4.2 The *P. falciparum*-human hepatocyte mouse models

There have been many attempts to develop laboratory models supporting the survival of human hepatocytes, given that *in vivo* only human cells or those of higher primates are receptive to *P. falciparum*. Invasion by and development of *P. falciparum* in human hepatocyte-transplanted animals was demonstrated in 2006, by combining 1) the use of mice with two genetic deficiencies (uPA-SCID mice) affecting hepatocytes and T and B lymphocytes, and 2) the pharmacological control of their non-adaptive defences, which improved the results of xenotransplantation into mice (Morosan et al., 2006). In these mice, *P. falciparum* sporozoites delivered intravenously infected the transplanted human hepatocytes and developed into liver schizonts up to a size that was comparable to that found in humans and chimpanzees. Alternative approaches to elaborate human hepatocyte-

engrafted mouse models have been reported recently. One used the *Fah–/–Rag2–/–Il2rg–/–* mice, the liver of which was very efficiently repopulated (up to approximately 95% of the murine liver) by human hepatocytes under selection pressure by the drug NTBC (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione) (Bissig et al., 2010). The suitability of this model for antiviral drug testing after successful infection of the chimeric liver by hepatitis B and C viruses was demonstrated, legitimating to believe in future successful infection by human *Plasmodium* species. Also, very recently, researchers at the MIT developed artificial humanised mouse livers engineered by growing human hepatocytes and human liver endothelial cells with mouse fibroblasts in a three-dimensional polymeric scaffold, and implanted them into mice (Chen et al., 2011). The ectopic livers responded to drugs in a way very similar to the way a human liver does.

4.5 Comments

Obviously those recent experimental models are not yet elaborated enough to meet the requirements for examining the large number of compounds involved in initial drug development. However, they pave the way for more efficient testing of drugs designed for humans. Ultimately, the combination of the immunocompromised mouse model for the liver stage and the model for the blood stage should open access to the complete cycle of the *P. falciparum* development in a small laboratory host, thereby providing an opportunity that should have numerous applications not only in the field of drug testing, pharmacokinetics and toxicology, but also in vaccine development and to investigate some of the biological and physiological aspects of human malaria.

Whatever will be the future of these new models, it seems however that exploration of drug potency using the "classical" rodent models might already be improved by considering some points of interest that we would like to list hereafter. 1) Because rodent malaria species can differ significantly in sensitivity to certain classes of compounds, as illustrated above with iron chelators and lipid biosynthesis inhibitors, it may be recommended to test new compounds on different models. 2) Also, the aspect of synergy between drugs should be considered and examination of therapies in conjunction with antimalarials typically given to treat patients with malaria should be favoured. 3) Notions of chronobiology may help in the selection of an experimental model and in the interpretation of chemotherapy experiments. Indeed, specific characteristics such as the duration of the schizogonic cycle, the time of schizogony or the synchronicity/asynchronicity of the chosen *Plasmodium* species and strain may influence its responsiveness to a drug depending on the time of administration and half-life of the drug (Beaute-Lafitte et al., 1994). This can be easily understood in the case of active compounds that preferentially affect a particular period of the parasite life cycle. Drug administration could be planed such that the peak level of the drug in the blood will be reached at the time the sensitive stage of the parasite is present in the circulation. It is assumed that such timing should increase the efficacy of drug treatment and minimize the dose to be injected. 4) Finally, it is remarkable that most of the drug testing performed in laboratories and promoted as potential therapy is carried out before the onset of malaria symptoms, whereas drug administration to treat human malaria is initiated after the onset of symptoms. Primary screening would gain advantage if new compounds were tested also in this configuration.

5. Conclusions

This last decade, the international mobilization dedicated to antimalarial strategies has regularly increased, allowing many countries to undertake or strengthen effective fight against the parasite, the disease and the vectors, and leading to a slowing-down, even a decline in some place, of malaria all over the world, thanks to the usage of impregnated bed nets and the setting-up of artemisinin combination therapies. However, the resistance of the insect vectors to insecticides and of the parasites to the current antimalarial drugs, especially to artemisinin derivatives, is still increasing and problematic since no new class of antimalarials has been introduced since 1996. The current global antimalarial drug development shows that the pipeline of antimalarials is rather strong in term of initiatives but also rather weak in term of novelty of mechanism of action that is necessary to circumvent resistance. This situation results in an urgent need for new drugs with original mechanisms of action. This last decade has also seen a considerable increase in our understanding of malaria parasite biochemistry that has allowed the identification of many potential targets for new drugs such as apicoplast metabolisms, proteases, kinases, transporters… That has been made possible thanks to the decrypted genomes of several *Plasmodium* species, to our ability to genetically validate potential drug targets and to the access to the transcriptomic and proteomic technologies that offer new opportunities to study the impact of drugs on the entire parasite metabolism.

These advances associated to the setting-up of high-throughput screening platforms on whole-cells or on specific parasite targets, and to the access to large chemical libraries with broad chemical diversity have seen the recent emergence of new potential antimalarial drugs with original molecular frameworks and mechanisms of action, that are auspicious for the future of antimalarial drug development. We are however facing important challenges in the next decade to propose efficient global antimalarial drug development. This will require :

1. Ability to propose efficient heterologous expression and folding systems to produce recombinant active proteins for targets, in order to set up high-throughput screening assays or to obtain 3D-dimensional structure elucidations using X-ray crystallography for drug design.
2. Development of researches on *P. vivax*, which can be considered as a neglected disease when compared to the efforts developed for *P. falciparum* whereas *P. vivax* infection is more widespread and remains an important cause of morbidity.
3. Strengthening researches on drugs acting on the liver parasite stages, including hypnozoites, and the parasite transmission stages, in order to propose an antimalarial drug strategy not only acting on the disease by itself due to the intraerythrocytic parasite development, but also acting on the disease transmission and the disease relapse in the case of *P. vivax*.

Finally, it must be kept in mind that for any promising molecule that will be selected, administration to patients will be the acid test. That is why development of small, easy to manage *in vivo* models as close to humans as possible remains a really challenging part of any therapeutic molecule development. Efforts are currently made to achieve these goals.

6. References

Abiodun, O.O., Gbotosho, G.O., Ajaiyeoba, E.O., Happi, C.T., Hofer, S., Wittlin, S., Sowunmi, A., Brun, R. & Oduola, A.M. (2010). Comparison of SYBR Green I-, PicoGreen-, and [3H]-hypoxanthine-based assays for in vitro antimalarial screening of plants from Nigerian ethnomedicine. *Parasitol Res*, 106, 4, pp. 933-939

Aguero, F., Al-Lazikani, B., Aslett, M., Berriman, M., Buckner, F.S., Campbell, R.K., Carmona, S., Carruthers, I.M., Chan, A.W., Chen, F., Crowther, G.J., Doyle, M.A., Hertz-Fowler, C., Hopkins, A.L., Mcallister, G., Nwaka, S., Overington, J.P., Pain, A., Paolini, G.V., Pieper, U., Ralph, S.A., Riechers, A., Roos, D.S., Sali, A., Shanmugam, D., Suzuki, T., Van Voorhis, W.C. & Verlinde, C.L. (2008). Genomic-scale prioritization of drug targets: the TDR Targets database. *Nat Rev Drug Discov*, 7, 11, pp. 900-907

Alonso, P.L., Brown, G., Arevalo-Herrera, M., Binka, F., Chitnis, C., Collins, F., Doumbo, O.K., Greenwood, B., Hall, B.F., Levine, M.M., Mendis, K., Newman, R.D., Plowe, C.V., Rodriguez, M.H., Sinden, R., Slutsker, L. & Tanner, M. (2011). A research agenda to underpin malaria eradication. *PLoS Med*, 8, 1, pp. e1000406

Aly, A.S. & Matuschewski, K. (2005). A malarial cysteine protease is necessary for *Plasmodium* sporozoite egress from oocysts. *J Exp Med*, 202, 2, pp. 225-230

Arnold, L., Tyagi, R.K., Meija, P., Swetman, C., Gleeson, J., Perignon, J.L. & Druilhe, P. (2011). Further improvements of the *P. falciparum* humanized mouse model. *PLoS One*, 6, 3, pp. e18045

Bacon, D.J., Latour, C., Lucas, C., Colina, O., Ringwald, P. & Picot, S. (2007). Comparison of a SYBR green I-based assay with a histidine-rich protein II enzyme-linked immunosorbent assay for in vitro antimalarial drug efficacy testing and application to clinical isolates. *Antimicrob Agents Chemother*, 51, 4, pp. 1172-1178

Baker, D.A. (2007). Malaria gametocytogenesis. *Mol Biochem Parasitol*, 172, 2, pp. 57-65

Baniecki, M.L., Wirth, D.F. & Clardy, J. (2007). High-throughput *Plasmodium falciparum* growth assay for malaria drug discovery. *Antimicrob Agents Chemother*, 51, 2, pp. 716-723

Baschong, W., Wittlin, S., Inglis, K.A., Fairlamb, A.H., Croft, S.L., Kumar, T.R., Fidock, D.A. & Brun, R. (2011). Triclosan is minimally effective in rodent malaria models. *Nat Med*, 17, 1, pp. 33-34

Basco, L.K. (2003). Molecular epidemiology of malaria in Cameroon. XV. Experimental studies on serum substitutes and supplements and alternative culture media for in vitro drug sensitivity assays using fresh isolates of *Plasmodium falciparum*. *Am J Trop Med Hyg*, 69, 2, pp. 168-173

Basco, L.K. (2004). Molecular epidemiology of malaria in cameroon. XX. Experimental studies on various factors of in vitro drug sensitivity assays using fresh isolates of *Plasmodium falciparum*. *Am J Trop Med Hyg*, 70, 5, pp. 474-480

Bauer, H., Fritz-Wolf, K., Winzer, A., Kuhner, S., Little, S., Yardley, V., Vezin, H., Palfey, B., Schirmer, R.H. & Davioud-Charvet, E. (2006). A fluoro analogue of the menadione derivative 6-[2'-(3'-methyl)-1',4'-naphthoquinolyl]hexanoic acid is a suicide substrate of glutathione reductase. Crystal structure of the alkylated human enzyme. *J Am Chem Soc*, 128, 33, pp. 10784-10794

Baumeister, S., Wiesner, J., Reichenberg, A., Hintz, M., Bietz, S., Harb, O.S., Roos, D.S., Kordes, M., Friesen, J., Matuschewski, K., Lingelbach, K., Jomaa, H. & Seeber, F. (2011). Fosmidomycin uptake into *Plasmodium* and *Babesia*-infected erythrocytes is facilitated by parasite-induced new permeability pathways. *PLoS One*, 6, 5, pp. e19334

Beaute-Lafitte, A., Altemayer-Caillard, V., Chabaud, A.G. & Landau, I. (1994). *Plasmodium yoelii nigeriens*is: biological mechanisms of resistance to chloroquine. *Parasite*, 1, 3, pp. 227-233

Bennett, T.N., Paguio, M., Gligorijevic, B., Seudieu, C., Kosar, A.D., Davidson, E. & Roepe, P.D. (2004). Novel, rapid, and inexpensive cell-based quantification of antimalarial drug efficacy. *Antimicrob Agents Chemother*, 48, 5, pp. 1807-1810

Bergmann-Leitner, E.S., Duncan, E.H., Burge, J.R., Spring, M. & Angov, E. (2008). Miniaturization of a high-throughput pLDH-based *Plasmodium falciparum* growth inhibition assay for small volume samples from preclinical and clinical vaccine trials. *Am J Trop Med Hyg*, 78, 3, pp. 468-471

Beutler, E. & Duparc, S. (2007). Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am J Trop Med Hyg*, 77, 4, pp. 779-789

Bhaumik, P., Horimoto, Y., Xiao, H., Miura, T., Hidaka, K., Kiso, Y., Wlodawer, A., Yada, R.Y. & Gustchina, A. (2011). Crystal structures of the free and inhibited forms of plasmepsin I (PMI) from *Plasmodium falciparum*. *J Struct Biol*, 175, 1, pp. 73-84

Biagini, G.A., O'neill, P.M., Nzila, A., Ward, S.A. & Bray, P.G. (2003). Antimalarial chemotherapy: young guns or back to the future? *Trends Parasitol*, 19, 11, pp. 479-487

Bissig, K.D., Wieland, S.F., Tran, P., Isogawa, M., Le, T.T., Chisari, F.V. & Verma, I.M. (2010). Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. *J Clin Invest*, 120, 3, pp. 924-930

Blackman, M.J. (2008). Malarial proteases and host cell egress: an 'emerging' cascade. *Cell Microbiol*, 10, 10, pp. 1925-1934

Blume, M., Hliscs, M., Rodriguez-Contreras, D., Sanchez, M., Landfear, S., Lucius, R., Matuschewski, K. & Gupta, N. (2010). A constitutive pan-hexose permease for the *Plasmodium* life cycle and transgenic models for screening of antimalarial sugar analogs. *FASEB J*, 25, 4, pp. 1218-1229

Boddey, J.A., Hodder, A.N., Gunther, S., Gilson, P.R., Patsiouras, H., Kapp, E.A., Pearce, J.A., De Koning-Ward, T.F., Simpson, R.J., Crabb, B.S. & Cowman, A.F. (2010). An aspartyl protease directs malaria effector proteins to the host cell. *Nature*, 463, 7281, pp. 627-631

Briolant, S., Parola, P., Fusai, T., Madamet-Torrentino, M., Baret, E., Mosnier, J., Delmont, J.P., Parzy, D., Minodier, P., Rogier, C. & Pradines, B. (2007). Influence of oxygen on asexual blood cycle and susceptibility of *Plasmodium falciparum* to chloroquine: requirement of a standardized in vitro assay. *Malar J*, 6, pp. 44

Buchholz, K., Burke, T.A., Williamson, K.C., Wiegand, R.C., Wirth, D.F. & Marti, M. (2011). A high-throughput screen targeting malaria transmission stages opens new avenues for drug development. *J Infect Dis*, 203, 10, pp. 1445-1453

Carraz, M., Jossang, A., Franetich, J.F., Siau, A., Ciceron, L., Hannoun, L., Sauerwein, R., Frappier, F., Rasoanaivo, P., Snounou, G. & Mazier, D. (2006). A plant-derived morphinan as a novel lead compound active against malaria liver stages. *PLoS Med*, 3, 12, pp. e513

Chattopadhyay, R., Velmurugan, S., Chakiath, C., Andrews Donkor, L., Milhous, W., Barnwell, J.W., Collins, W.E. & Hoffman, S.L. (2010). Establishment of an in vitro assay for assessing the effects of drugs on the liver stages of *Plasmodium vivax* malaria. *PLoS One*, 5, 12, pp. e14275

Chen, A.A., Thomas, D.K., Ong, L.L., Schwartz, R.E., Golub, T.R. & Bhatia, S.N. (2011). Humanized mice with ectopic artificial liver tissues. *Proc Natl Acad Sci U S A*, 108, 29, pp. 11842-11847

Chevalley, S., Coste, A., Lopez, A., Pipy, B. & Valentin, A. (2010). Flow cytometry for the evaluation of anti-plasmodial activity of drugs on *Plasmodium falciparum* gametocytes. *Malar J*, 9, pp. 49

Collier, R. (2009). WHO reports progress in malaria control. *CMAJ*, 182, 2, pp. E105-106

Collins, W.E. (1992). South American monkeys in the development and testing of malarial vaccines-a review. *Mem Inst Oswaldo Cruz*, 87 Suppl 3, pp. 401-406

Cosledan, F., Fraisse, L., Pellet, A., Guillou, F., Mordmuller, B., Kremsner, P.G., Moreno, A., Mazier, D., Maffrand, J.P. & Meunier, B. (2008). Selection of a trioxaquine as an antimalarial drug candidate. *Proc Natl Acad Sci U S A*, 105, 45, pp. 17579-17584

Crabb, B.S., De Koning-Ward, T.F. & Gilson, P.R. (2011). Toward forward genetic screens in malaria-causing parasites using the piggyBac transposon. *BMC Biol*, 9, pp. 21

Dahl, E.L. & Rosenthal, P.J. (2008). Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics. *Trends Parasitol*, 24, 6, pp. 279-284

Deharo, E., Garcia, R.N., Oporto, P., Gimenez, A., Sauvain, M., Jullian, V. & Ginsburg, H. (2002). A non-radiolabelled ferriprotoporphyrin IX biomineralisation inhibition test for the high throughput screening of antimalarial compounds. *Exp Parasitol*, 100, 4, pp. 252-256

Delhaes, L., Lazaro, J.E., Gay, F., Thellier, M. & Danis, M. (1999). The microculture tetrazolium assay (MTA): another colorimetric method of testing *Plasmodium falciparum* chemosensitivity. *Ann Trop Med Parasitol*, 93, 1, pp. 31-40

Desjardins, R.E., Canfield, C.J., Haynes, J.D. & Chulay, J.D. (1979). Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob Agents Chemother*, 16, 6, pp. 710-718

Doerig, C. & Meijer, L. (2007). Antimalarial drug discovery: targeting protein kinases. *Expert Opin Ther Targets*, 11, 3, pp. 279-290

Doerig, C., Abdi, A., Bland, N., Eschenlauer, S., Dorin-Semblat, D., Fennell, C., Halbert, J., Holland, Z., Nivez, M.P., Semblat, J.P., Sicard, A. & Reininger, L. (2010). Malaria: targeting parasite and host cell kinomes. *Biochim Biophys Acta*, 1804, 3, pp. 604-612

Druilhe, P., Moreno, A., Blanc, C., Brasseur, P.H. & Jacquier, P. (2001). A colorimetric in vitro drug sensitivity assay for *Plasmodium falciparum* based on a highly sensitive double-site lactate dehydrogenase antigen-capture enzyme-linked immunosorbent assay. *Am J Trop Med Hyg*, 64, 5-6, pp. 233-241

Dvorin, J.D., Martyn, D.C., Patel, S.D., Grimley, J.S., Collins, C.R., Hopp, C.S., Bright, A.T., Westenberger, S., Winzeler, E., Blackman, M.J., Baker, D.A., Wandless, T.J. & Duraisingh, M.T. (2010). A plant-like kinase in *Plasmodium falciparum* regulates parasite egress from erythrocytes. *Science*, 328, 5980, pp. 910-912

Eggleson, K.K., Duffin, K.L. & Goldberg, D.E. (1999). Identification and characterization of falcilysin, a metallopeptidase involved in hemoglobin catabolism within the malaria parasite *Plasmodium falciparum*. *J Biol Chem*, 274, 45, pp. 32411-32417.

Eksi, S., Czesny, B., Greenbaum, D.C., Bogyo, M. & Williamson, K.C. (2004). Targeted disruption of *Plasmodium falciparum* cysteine protease, falcipain 1, reduces oocyst production, not erythrocytic stage growth. *Mol Microbiol*, 53, 1, pp. 243-250

Enserink, M. (2010). Redrawing Africa's malaria map. *Science*, 328, 5980, pp. 842

Florent, I., Marechal, E., Gascuel, O. & Brehelin, L. (2010). Bioinformatic strategies to provide functional clues to the unknown genes in *Plasmodium falciparum* genome. *Parasite*, 17, 4, pp. 273-283

Freundlich, J.S., Wang, F., Tsai, H.C., Kuo, M., Shieh, H.M., Anderson, J.W., Nkrumah, L.J., Valderramos, J.C., Yu, M., Kumar, T.R., Valderramos, S.G., Jacobs, W.R., Jr., Schiehser, G.A., Jacobus, D.P., Fidock, D.A. & Sacchettini, J.C. (2007). X-ray structural analysis of *Plasmodium falciparum* enoyl acyl carrier protein reductase as a pathway toward the optimization of triclosan antimalarial efficacy. *J Biol Chem*, 282, 35, pp. 25436-25444

Gamo, F.J., Sanz, L.M., Vidal, J., De Cozar, C., Alvarez, E., Lavandera, J.L., Vanderwall, D.E., Green, D.V., Kumar, V., Hasan, S., Brown, J.R., Peishoff, C.E., Cardon, L.R. & Garcia-Bustos, J.F. (2010). Thousands of chemical starting points for antimalarial lead identification. *Nature*, 465, 7296, pp. 305-310

Gego, A., Silvie, O., Franetich, J.F., Farhati, K., Hannoun, L., Luty, A.J., Sauerwein, R.W., Boucheix, C., Rubinstein, E. & Mazier, D. (2006). New approach for high-throughput screening of drug activity on *Plasmodium* liver stages. *Antimicrob Agents Chemother*, 50, 4, pp. 1586-1589

Goodman, C.D. & Mcfadden, G.I. (2007). Fatty acid biosynthesis as a drug target in apicomplexan parasites. *Curr Drug Targets*, 8, 1, pp. 15-30

Grawert, T., Groll, M., Rohdich, F., Bacher, A. & Eisenreich, W. (2011). Biochemistry of the non-mevalonate isoprenoid pathway. *Cell Mol Life Sci*, DOI: 10.1007/s00018-011-0753-z

Grellier, P., Depoix, D., Schrevel, J. & Florent, I. (2008). Discovery of new targets for antimalarial chemotherapy. *Parasite*, 15, 3, pp. 219-225

Grimberg, B.T. (2011). Methodology and application of flow cytometry for investigation of human malaria parasites. *J Immunol Methods*, 367, 1-2, pp. 1-16

Guiguemde, W.A., Shelat, A.A., Bouck, D., Duffy, S., Crowther, G.J., Davis, P.H., Smithson, D.C., Connelly, M., Clark, J., Zhu, F., Jimenez-Diaz, M.B., Martinez, M.S., Wilson, E.B., Tripathi, A.K., Gut, J., Sharlow, E.R., Bathurst, I., El Mazouni, F., Fowble, J.W., Forquer, I., Mcginley, P.L., Castro, S., Angulo-Barturen, I., Ferrer, S., Rosenthal, P.J., Derisi, J.L., Sullivan, D.J., Lazo, J.S., Roos, D.S., Riscoe, M.K., Phillips, M.A., Rathod, P.K., Van Voorhis, W.C., Avery, V.M. & Guy, R.K. (2010). Chemical genetics of *Plasmodium falciparum*. *Nature*, 465, 7296, pp. 311-315

Hegge, S., Kudryashev, M., Barniol, L. & Frischknecht, F. (2010). Key factors regulating *Plasmodium berghei* sporozoite survival and transformation revealed by an automated visual assay. *FASEB J*, 24, 12, pp. 5003-5012

Humberstone, A.J., Cowman, A.F., Horton, J. & Charman, W.N. (1998). Effect of altered serum lipid concentrations on the IC50 of halofantrine against *Plasmodium falciparum*. *J Pharm Sci*, 87, 2, pp. 256-258

Huy, N.T., Uyen, D.T., Maeda, A., Trang, D.T., Oida, T., Harada, S. & Kamei, K. (2007). Simple colorimetric inhibition assay of heme crystallization for high-throughput screening of antimalarial compounds. *Antimicrob Agents Chemother*, 51, 1, pp. 350-353

Hyde, J.E. (2007). Targeting purine and pyrimidine metabolism in human apicomplexan parasites. *Curr Drug Targets*, 8, 1, pp. 31-47

Jayabalasingham, B., Menard, R. & Fidock, D.A. (2010). Recent insights into fatty acid acquisition and metabolism in malarial parasites. *F1000 Biol Rep*, 2, pp. 24

Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H.K., Soldati, D. & Beck, E. (1999). Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science*, 285, 5433, pp. 1573-1576.

Kato, N., Sakata, T., Breton, G., Le Roch, K.G., Nagle, A., Andersen, C., Bursulaya, B., Henson, K., Johnson, J., Kumar, K.A., Marr, F., Mason, D., Mcnamara, C., Plouffe, D., Ramachandran, V., Spooner, M., Tuntland, T., Zhou, Y., Peters, E.C., Chatterjee, A., Schultz, P.G., Ward, G.E., Gray, N., Harper, J. & Winzeler, E.A. (2008). Gene expression signatures and small-molecule compounds link a protein kinase to *Plasmodium falciparum* motility. *Nat Chem Biol*, 4, 6, pp. 347-356

Keller, T.H., Shi, P.Y. & Wang, Q.Y. (2011). Anti-infectives: can cellular screening deliver? *Curr Opin Chem Biol*, 15, 4, pp. 529-533

Klemba, M., Gluzman, I. & Goldberg, D.E. (2004). A *Plasmodium falciparum* dipeptidyl aminopeptidase I participates in vacuolar hemoglobin degradation. *J Biol Chem*, 279, 41, pp. 43000-43007

Kocken, C.H., Ozwara, H., Van Der Wel, A., Beetsma, A.L., Mwenda, J.M. & Thomas, A.W. (2002). *Plasmodium knowlesi* provides a rapid in vitro and in vivo transfection system that enables double-crossover gene knockout studies. *Infect Immun*, 70, 2, pp. 655-660

Kosaisavee, V., Suwanarusk, R., Nosten, F., Kyle, D.E., Barrends, M., Jones, J., Price, R., Russell, B. & Lek-Uthai, U. (2006). *Plasmodium vivax*: isotopic, PicoGreen, and microscopic assays for measuring chloroquine sensitivity in fresh and cryopreserved isolates. *Exp Parasitol*, 114, 1, pp. 34-39

Ku, M.J., Dossin, F.M., Choi, Y., Moraes, C.B., Ryu, J., Song, R. & Freitas-Junior, L.H. (2011). Quantum dots: a new tool for anti-malarial drug assays. *Malar J*, 10, pp. 118

Lacroix, C., Giovannini, D., Combe, A., Bargieri, D.Y., Spath, S., Panchal, D., Tawk, L., Thiberge, S., Carvalho, T.G., Barale, J.C., Bhanot, P. & Menard, R. (2011). FLP/FRT-mediated conditional mutagenesis in pre-erythrocytic stages of *Plasmodium berghei*. *Nat Protoc*, 6, 9, pp. 1412-1428

Leroy, D. & Doerig, C. (2008). Drugging the *Plasmodium* kinome: the benefits of academia-industry synergy. *Trends Pharmacol Sci*, 29, 5, pp. 241-249

Lim, L. & Mcfadden, G.I. (2010). The evolution, metabolism and functions of the apicoplast. *Philos Trans R Soc Lond B Biol Sci*, 365, 1541, pp. 749-763

Limenitakis, J. & Soldati-Favre, D. (2011). Functional genetics in Apicomplexa: potentials and limits. *FEBS Lett*, 585, 11, pp. 1579-1588

Liu, J., Istvan, E.S., Gluzman, I.Y., Gross, J. & Goldberg, D.E. (2006). *Plasmodium falciparum* ensures its amino acid supply with multiple acquisition pathways and redundant proteolytic enzyme systems. *Proc Natl Acad Sci U S A*, 103, 23, pp. 8840-8845

Mahmoudi, N., Ciceron, L., Franetich, J.F., Farhati, K., Silvie, O., Eling, W., Sauerwein, R., Danis, M., Mazier, D. & Derouin, F. (2003). In vitro activities of 25 quinolones and fluoroquinolones against liver and blood stage *Plasmodium* spp. *Antimicrob Agents Chemother*, 47, 8, pp. 2636-2639

Mahmoudi, N., Garcia-Domenech, R., Galvez, J., Farhati, K., Franetich, J.F., Sauerwein, R., Hannoun, L., Derouin, F., Danis, M. & Mazier, D. (2008). New active drugs against liver stages of *Plasmodium* predicted by molecular topology. *Antimicrob Agents Chemother*, 52, 4, pp. 1215-1220

Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L. & Hinrichs, D.J. (1993). Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *Am J Trop Med Hyg*, 48, 6, pp. 739-741

Makler, M.T., Piper, R.C. & Milhous, W.K. (1998). Lactate dehydrogenase and the diagnosis of malaria. *Parasitol Today*, 14, 9, pp. 376-377

Mambu, L. & Grellier, P. (2008). Antimalarial compounds of traditionally used medicinal plants, In: *Bioactive natural products, detection, isolation, and structural determination*, 2nd edition, Molyneux, R.J. & Colgate, S.M., pp. 491, CRC Press

Martin, R.E., Ginsburg, H. & Kirk, K. (2009). Membrane transport proteins of the malaria parasite. *Mol Microbiol*, 74, 3, pp. 519-528

Mather, M.W., Henry, K.W. & Vaidya, A.B. (2007). Mitochondrial drug targets in apicomplexan parasites. *Curr Drug Targets*, 8, 1, pp. 49-60

Mazier, D., Beaudoin, R.L., Mellouk, S., Druilhe, P., Texier, B., Trosper, J., Miltgen, F., Landau, I., Paul, C., Brandicourt, O. & Et Al. (1985). Complete development of hepatic stages of *Plasmodium falciparum* in vitro. *Science*, 227, 4685, pp. 440-442

Mazier, D., Franetich, J.F., Carraz, M., Silvie, O. & Pino, P. (2004). Models for studying effects of herbal antimalarials at different stages of the *Plasmodium* life cycle, In: *Traditional medicinal plants and malaria, Willcox, M., Bodeker, G. & Rasoanaivo, P.*, pp. 271, CRC Press, Boca raton

Mcgowan, S., Porter, C.J., Lowther, J., Stack, C.M., Golding, S.J., Skinner-Adams, T.S., Trenholme, K.R., Teuscher, F., Donnelly, S.M., Grembecka, J., Mucha, A., Kafarski, P., Degori, R., Buckle, A.M., Gardiner, D.L., Whisstock, J.C. & Dalton, J.P. (2009). Structural basis for the inhibition of the essential *Plasmodium falciparum* M1 neutral aminopeptidase. *Proc Natl Acad Sci U S A*, 106, 8, pp. 2537-2542

Mckerrow, J.H., Rosenthal, P.J., Swenerton, R. & Doyle, P. (2008). Development of protease inhibitors for protozoan infections. *Curr Opin Infect Dis*, 21, 6, pp. 668-672

Mcrobert, L., Jiang, S., Stead, A. & Mcconkey, G.A. (2005). *Plasmodium falciparum*: interaction of shikimate analogues with antimalarial drugs. *Exp Parasitol*, 111, 3, pp. 178-181

Moreno, A., Badell, E., Van Rooijen, N. & Druilhe, P. (2001). Human malaria in immunocompromised mice: new in vivo model for chemotherapy studies. *Antimicrob Agents Chemother*, 45, 6, pp. 1847-1853

Morosan, S., Hez-Deroubaix, S., Lunel, F., Renia, L., Giannini, C., Van Rooijen, N., Battaglia, S., Blanc, C., Eling, W., Sauerwein, R., Hannoun, L., Belghiti, J., Brechot, C., Kremsdorf, D. & Druilhe, P. (2006). Liver-stage development of *Plasmodium falciparum*, in a humanized mouse model. *J Infect Dis*, 193, 7, pp. 996-1004

Mwakingwe, A., Ting, L.M., Hochman, S., Chen, J., Sinnis, P. & Kim, K. (2009). Noninvasive real-time monitoring of liver-stage development of bioluminescent *Plasmodium* parasites. *J Infect Dis*, 200, 9, pp. 1470-1478

Ncokazi, K.K. & Egan, T.J. (2005). A colorimetric high-throughput beta-hematin inhibition screening assay for use in the search for antimalarial compounds. *Anal Biochem*, 338, pp. 306-319

Noedl, H., Wernsdorfer, W.H., Miller, R.S. & Wongsrichanalai, C. (2002). Histidine-rich protein II: a novel approach to malaria drug sensitivity testing. *Antimicrob Agents Chemother*, 46, 6, pp. 1658-1664

Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D. & Fukuda, M.M. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med*, 359, 24, pp. 2619-2620

O'connor, C.J., Laraia, L. & Spring, D.R. (2011). Chemical genetics. *Chem Soc Rev*, 40, 8, pp. 4332-4345

O'neill, P.M., Ward, S.A., Berry, N.G., Jeyadevan, J.P., Biagini, G.A., Asadollaly, E., Park, B.K. & Bray, P.G. (2006). A medicinal chemistry perspective on 4-aminoquinoline antimalarial drugs. *Curr Top Med Chem*, 6, 5, pp. 479-507

Odom, A.R. & Van Voorhis, W.C. (2009). Functional genetic analysis of the *Plasmodium falciparum* deoxyxylulose 5-phosphate reductoisomerase gene. *Mol Biochem Parasitol*, 170, 2, pp. 108-111

Olliaro, P. & Wells, T.N. (2009). The global portfolio of new antimalarial medicines under development. *Clin Pharmacol Ther*, 85, 6, pp. 584-595

Palmer, C.J., Lindo, J.F., Klaskala, W.I., Quesada, J.A., Kaminsky, R., Baum, M.K. & Ager, A.L. (1998). Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin Microbiol*, 36, 1, pp. 203-206

Parvanova, I., Epiphanio, S., Fauq, A., Golde, T.E., Prudencio, M. & Mota, M.M. (2009). A small molecule inhibitor of signal peptide peptidase inhibits *Plasmodium* development in the liver and decreases malaria severity. *PLoS One*, 4, 4, pp. e5078

Payne, D.J., Gwynn, M.N., Holmes, D.J. & Pompliano, D.L. (2007). Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov*, 6, 1, pp. 29-40

Peatey, C.L., Skinner-Adams, T.S., Dixon, M.W., Mccarthy, J.S., Gardiner, D.L. & Trenholme, K.R. (2009). Effect of antimalarial drugs on *Plasmodium falciparum* gametocytes. *J Infect Dis*, 200, 10, pp. 1518-1521

Peters, W. (1987). Techniques of drug evaluation I: primary screening, In : *Chemotherapy and drug resistance in Malaria*, Vol. 1, Jovanovich , H.B., pp 97, Academic Press, Oval road, London

Peters, W. & Robinson, B.L. (1999). The chemotherapy of rodent malaria. LVI. Studies on the development of resistance to natural and synthetic endoperoxides. *Ann Trop Med Parasitol*, 93, 4, pp. 325-329

Ploemen, I.H., Prudencio, M., Douradinha, B.G., Ramesar, J., Fonager, J., Van Gemert, G.J., Luty, A.J., Hermsen, C.C., Sauerwein, R.W., Baptista, F.G., Mota, M.M., Waters, A.P., Que, I., Lowik, C.W., Khan, S.M., Janse, C.J. & Franke-Fayard, B.M. (2009). Visualisation and quantitative analysis of the rodent malaria liver stage by real time imaging. *PLoS One*, 4, 11, pp. e7881

Ponpuak, M., Klemba, M., Park, M., Gluzman, I.Y., Lamppa, G.K. & Goldberg, D.E. (2007). A role for falcilysin in transit peptide degradation in the *Plasmodium falciparum* apicoplast. *Mol Microbiol*, 63, 2, pp. 314-334

Prabhu, P. & Patravale, V. (2011). Novel Targets for Malaria Therapy. *Curr Drug Targets*, pp.

Ringwald, P., Meche, F.S., Bickii, J. & Basco, L.K. (1999). In vitro culture and drug sensitivity assay of *Plasmodium falciparum* with nonserum substitute and acute-phase sera. *J Clin Microbiol*, 37, 3, pp. 700-705

Rosenthal, P.J. (2010). Falcipains and other cysteine proteases of malaria parasites, In: *Falcipains and other cysteine proteases of malaria parasites*, *Advances in Experimental Medicine and Biology, vol.712,* Robinson, W. and Dalton, JP., pp. 30, Landes Biosciences and Springer Science+Business Media, LCC, 233 Spring Street, New York, New York, USA.

Rottmann, M., Mcnamara, C., Yeung, B.K., Lee, M.C., Zou, B., Russell, B., Seitz, P., Plouffe, D.M., Dharia, N.V., Tan, J., Cohen, S.B., Spencer, K.R., Gonzalez-Paez, G.E., Lakshminarayana, S.B., Goh, A., Suwanarusk, R., Jegla, T., Schmitt, E.K., Beck, H.P., Brun, R., Nosten, F., Renia, L., Dartois, V., Keller, T.H., Fidock, D.A., Winzeler, E.A. & Diagana, T.T. (2010). Spiroindolones, a potent compound class for the treatment of malaria. *Science*, 329, 5996, pp. 1175-1180

Rush, M.A., Baniecki, M.L., Mazitschek, R., Cortese, J.F., Wiegand, R., Clardy, J. & Wirth, D.F. (2009). Colorimetric high-throughput screen for detection of heme crystallization inhibitors. *Antimicrob Agents Chemother*, 53, 6, pp. 2564-2568

Russo, I., Babbitt, S., Muralidharan, V., Butler, T., Oksman, A. & Goldberg, D.E. (2010). Plasmepsin V licenses *Plasmodium* proteins for export into the host erythrocyte. *Nature*, 463, 7281, pp. 632-636

Sahu, N.K., Sahu, S. & Kohli, D.V. (2008). Novel molecular targets for antimalarial drug development. *Chem Biol Drug Des*, 71, 4, pp. 287-297

Sams-Dodd, F. (2005). Target-based drug discovery: is something wrong? *Drug Discov Today*, 10, 2, pp. 139-147

Sanni, L.A., Fonseca, L.F. & Langhome, J. (2002). Mouse models for erythrocytic-stage malaria, In: *Methods in molecular medicine, Vol. 72: Malaria methods and protocols*, Doolan, D.L., pp 57, Humana Press, Inc., Totowa, NJ

Sanchez, C.P., Dave, A., Stein, W.D. & Lanzer, M. (2010). Transporters as mediators of drug resistance in *Plasmodium falciparum*. *Int J Parasitol*, 40, 10, pp. 1109-1118

Sato, S. & Wilson, R.J. (2005). The plastid of *Plasmodium* spp.: a target for inhibitors. *Curr Top Microbiol Immunol*, 295, pp. 251-273

Sato, S. (2011). The apicomplexan plastid and its evolution. *Cell Mol Life Sci*, 68, 8, pp. 1285-1296

Seeber, F. & Soldati-Favre, D. (2010). Metabolic pathways in the apicoplast of apicomplexa. *Int Rev Cell Mol Biol*, 281, pp. 161-228

Semenov, A., Olson, J.E. & Rosenthal, P.J. (1998). Antimalarial synergy of cysteine and aspartic protease inhibitors. *Antimicrob Agents Chemother*, 42, 9, pp. 2254-2258

Sijwali, P.S., Koo, J., Singh, N. & Rosenthal, P.J. (2006). Gene disruptions demonstrate independent roles for the four falcipain cysteine proteases of *Plasmodium falciparum*. *Mol Biochem Parasitol*, 150, 1, pp. 96-106

Sims, P.F. & Hyde, J.E. (2006). Proteomics of the human malaria parasite *Plasmodium falciparum*. *Expert Rev Proteomics*, 3, 1, pp. 87-95

Singh, A., Shenai, B.R., Choe, Y., Gut, J., Sijwali, P.S., Craik, C.S. & Rosenthal, P.J. (2002). Critical role of amino acid 23 in mediating activity and specificity of vinckepain-2, a papain-family cysteine protease of rodent malaria parasites. *Biochem J*, 368, 1, pp. 273-281

Singh, A.P., Zhang, Y., No, J.H., Docampo, R., Nussenzweig, V. & Oldfield, E. (2010). Lipophilic bisphosphonates are potent inhibitors of *Plasmodium* liver-stage growth. *Antimicrob Agents Chemother*, 54, 7, pp. 2987-2993

Singh, K., Agarwal, A., Khan, S.I., Walker, L.A. & Tekwani, B.L. (2007). Growth, drug susceptibility, and gene expression profiling of *Plasmodium falciparum* cultured in medium supplemented with human serum or lipid-rich bovine serum albumin [corrected]. *J Biomol Screen*, 12, 8, pp. 1109-1114

Skinner-Adams, T.S., Stack, C.M., Trenholme, K.R., Brown, C.L., Grembecka, J., Lowther, J., Mucha, A., Drag, M., Kafarski, P., Mcgowan, S., Whisstock, J.C., Gardiner, D.L. & Dalton, J.P. (2009). *Plasmodium falciparum* neutral aminopeptidases: new targets for anti-malarials. *Trends Biochem Sci*, 35, 1, pp. 53-61

Slavic, K., Straschil, U., Reininger, L., Doerig, C., Morin, C., Tewari, R. & Krishna, S. (2010). Life cycle studies of the hexose transporter of *Plasmodium* species and genetic validation of their essentiality. *Mol Microbiol*, 75, 6, pp. 1402-1413

Slavic, K., Krishna, S., Derbyshire, E.T. & Staines, H.M. (2011). Plasmodial sugar transporters as anti-malarial drug targets and comparisons with other protozoa. *Malar J*, 10, pp. 165

Sologub, L., Kuehn, A., Kern, S., Przyborski, J., Schillig, R. & Pradel, G. (2011). Malaria proteases mediate inside-out egress of gametocytes from red blood cells following parasite transmission to the mosquito. *Cell Microbiol*, 13, 6, pp. 897-912

Staines, H.M., Powell, T., Thomas, S.L. & Ellory, J.C. (2004). *Plasmodium falciparum*-induced channels. *Int J Parasitol*, 34, 6, pp. 665-673

Staines, H.M., Derbyshire, E.T., Slavic, K., Tattersall, A., Vial, H. & Krishna, S. (2010). Exploiting the therapeutic potential of *Plasmodium falciparum* solute transporters. *Trends Parasitol*, 26, 6, pp. 284-296

Tasdemir, D., Sanabria, D., Lauinger, I.L., Tarun, A., Herman, R., Perozzo, R., Zloh, M., Kappe, S.H., Brun, R. & Carballeira, N.M. (2010). 2-Hexadecynoic acid inhibits plasmodial FAS-II enzymes and arrests erythrocytic and liver stage *Plasmodium* infections. *Bioorg Med Chem*, 18, 21, pp. 7475-7485

Trager, W. & Jensen, J.B. (1976). Human malaria parasites in continuous culture. *Science*, 193, 4254, pp. 673-675

Trenholme, K.R., Brown, C.L., Skinner-Adams, T.S., Stack, C., Lowther, J., To, J., Robinson, M.W., Donnelly, S.M., Dalton, J.P. & Gardiner, D.L. (2010). Aminopeptidases of malaria parasites: new targets for chemotherapy. *Infect Disord Drug Targets*, 10, 3, pp. 217-225

Udomsangpetch, R., Kaneko, O., Chotivanich, K. & Sattabongkot, J. (2008). Cultivation of *Plasmodium vivax*. *Trends Parasitol*, 24, 2, pp. 85-88

Van Voorhis, W.C., Rivas, K.L., Bendale, P., Nallan, L., Horney, C., Barrett, L.K., Bauer, K.D., Smart, B.P., Ankala, S., Hucke, O., Verlinde, C.L., Chakrabarti, D., Strickland, C., Yokoyama, K., Buckner, F.S., Hamilton, A.D., Williams, D.K., Lombardo, L.J., Floyd, D. & Gelb, M.H. (2007). Efficacy, Pharmacokinetics, and Metabolism of Tetrahydroquinoline Inhibitors of *Plasmodium falciparum* Protein Farnesyltransferase. *Antimicrob Agents Chemother*, 51, 10, pp. 3659-3671

Wegscheid-Gerlach, C., Gerber, H.D. & Diederich, W.E. (2010). Proteases of *Plasmodium falciparum* as potential drug targets and inhibitors thereof. *Curr Top Med Chem*, 10, 3, pp. 346-367

Weissbuch, I. & Leiserowitz, L. (2008). Interplay between malaria, crystalline hemozoin formation, and antimalarial drug action and design. *Chem Rev*, 108, 11, pp. 4899-4914

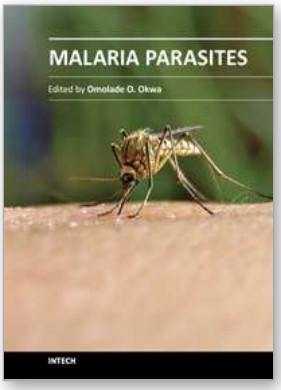
Wengelnik, K., Vidal, V., Ancelin, M.L., Cathiard, A.M., Morgat, J.L., Kocken, C.H., Calas, M., Herrera, S., Thomas, A.W. & Vial, H.J. (2002). A class of potent antimalarials and their specific accumulation in infected erythrocytes. *Science*, 295, 5558, pp. 1311-1314

Wiesner, J. & Jomaa, H. (2007). Isoprenoid biosynthesis of the apicoplast as drug target. *Curr Drug Targets*, 8, 1, pp. 3-13

Wu, T., Nagle, A.S. & Chatterjee, A.K. (2011). Road towards new antimalarials - overview of the strategies and their chemical progress. *Curr Med Chem*, 18, 6, pp. 853-871

Wu, Y., Wang, X., Liu, X. & Wang, Y. (2003). Data-mining approaches reveal hidden families of proteases in the genome of malaria parasite. *Genome Res*, 13, 4, pp. 601-616.

Yu, M., Kumar, T.R., Nkrumah, L.J., Coppi, A., Retzlaff, S., Li, C.D., Kelly, B.J., Moura, P.A., Lakshmanan, V., Freundlich, J.S., Valderramos, J.C., Vilcheze, C., Siedner, M., Tsai, J.H., Falkard, B., Sidhu, A.B., Purcell, L.A., Gratraud, P., Kremer, L., Waters, A.P., Schiehser, G., Jacobus, D.P., Janse, C.J., Ager, A., Jacobs, W.R., Jr., Sacchettini, J.C., Heussler, V., Sinnis, P. & Fidock, D.A. (2008). The fatty acid biosynthesis enzyme FabI plays a key role in the development of liver-stage malarial parasites. *Cell Host Microbe*, 4, 6, pp. 567-578



Malaria Parasites

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Malaria is a global disease in the world today but most common in the poorest countries of the world, with 90% of deaths occurring in sub-Saharan Africa. This book provides information on global efforts made by scientist which cuts across the continents of the world. Concerted efforts such as symbiont based malaria control; new applications in avian malaria studies; development of humanized mice to study P.falciparium (the most virulent species of malaria parasite); and current issues in laboratory diagnosis will support the prompt treatment of malaria. Research is ultimately gaining more grounds in the quest to provide vaccine for the prevention of malaria. The book features research aimed to bring a lasting solution to the malaria problem and what we should be doing now to face malaria, which is definitely useful for health policies in the twenty first century.

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