Expert Opinion on Pharmacotherapy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ieop20

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To cite this article: Erik Koehne, Ayola Akim Adegnika, Jana Held & Andrea Kreidenweiss (2021): Pharmacotherapy for artemisinin-resistant malaria, Expert Opinion on Pharmacotherapy, DOI: 10.1080/14656566.2021.1959913

To link to this article: https://doi.org/10.1080/14656566.2021.1959913

Published online: 29 Jul 2021.

EXPERT OPINION ON PHARMACOTHERAPY
https://doi.org/10.1080/14656566.2021.1959913

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REVIEW

Pharmacotherapy for artemisinin-resistant malaria

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ABSTRACT

Introduction: Malaria, the most devastating parasitic disease, is currently treated with artemisinin-based combination therapies (ACTs). Unfortunately, some ACTs are unable to rapidly clear *Plasmodium falciparum* parasites from the blood stream and are failing to cure malaria patients; a problem, so far, largely confined to Southeast Asia. There is a fear of resistant *Plasmodium falciparum* emerging in other parts of the world including Sub-Saharan Africa. Strategies for alternative treatments, ideally non-artemisinin based, are needed.

Areas covered: This narrative review gives an overview of approved antimalarials and of some compounds in advanced drug development that could be used when an ACT is failing. The selection was based on a literature search in PubMed and WHO notes for malaria treatment.

Expert opinion: The ACT drug class can still cure malaria in malaria endemic regions. However, the appropriate ACT drug should be chosen considering the background resistance of the partner drug of the local parasite population. Artesunate-pyronaridine, the 'newest' recommended ACT, and atovaquone-proguanil are, so far, effective, and safe treatments for uncomplicated falciparum malaria. Therefore, all available ACTs should be safeguarded from parasite resistance and the development of new antimalarial drug classes needs to be accelerated.

ARTICLE HISTORY
Received 15 January 2021
Accepted 21 July 2021

KEYWORDS
Plasmodium falciparum; malaria; artemisinin-based combination therapies; resistance

1. Introduction

Malaria is caused by protozoan parasites of the genus Plasmodium and is the most important parasitic disease in terms of mortality and morbidity with the World Health Organization (WHO) reporting 229 million cases and 409,000 deaths worldwide in 2019 [1]. *Plasmodium falciparum*, the most life-threatening species of the human pathogenic malaria parasites, has become resistant to many antimalarial drugs. Resistant parasites often emerged for the first time in Southeast Asia including to chloroquine (1950s), sulfadoxine-pyrimethamine (1960s), and mefloquine (1980s), which spread quickly in these regions and eventually appeared in sub-Saharan Africa [2]. Consequently, malaria-attributed mortality increased, especially in Africa, where approximately 90% of malaria deaths occur, predominantly in children under the age of five years [3]. The introduction of artemisinins in the early 1990s marked a turning point in the clinical management and control of malaria.

Artemisinin is a sesquiterpene trioxane lactone naturally produced by the herb *Artemisia annua* having a peroxide bridge essential for its antiplasmodial activity, which is the relevant pharmacophore in all artemisinin-related compounds (here referred to as artemisinins) including artesunate, artemether, and dihydroartemisinin. Artemisinins are highly potent and rapidly kill all life cycle stages of intraerythrocytic *P. falciparum* malaria parasites including young circulating 'ring-stages' (and young sexual forms). This results in a fast, clinical, and parasitological response after treatment start – important for successful therapy of severe malaria complications.

Artemisinins entered clinical testing in the early 1980s and were safe and highly efficacious [4] in patients with uncomplicated malaria in regions where resistance to all other antimalarial drugs had occurred. Artemisinins are metabolized in humans into active dihydroartemisinin that is rapidly eliminated (elimination half-life approx. 1–2 hours [5]) and early reports have shown a high recrudescence rate when an artemisinin was taken as a monotherapy – even with a seven days treatment course, but mainly due to nonadherence to the lengthy regimen [6]. Thus, for the widespread use in uncomplicated malaria it was agreed that artemisinins should always be partnered with a long-acting drug that clears residual parasites to shorten the regimen, ensure an efficacious treatment, and to safeguard artemisinins from parasite resistance [7]. Currently, six artemisinin-based combination therapies (ACTs) are available and recommended by the WHO as treatments for uncomplicated falciparum malaria: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine, dihydroartemisinin-piperaquine, and artesunate-pyronaridine [8]. In addition, other ACT regimens are co-administered, e.g. artemisinin with piperaquine [9] or naphthoquine [9], or arterolane with piperaquine [10]. The WHO policy change started off in 2005 [11] and widespread use of ACTs have since then contributed substantially to the decline in malaria prevalence.

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Article highlights

- Artemisinin-based combination therapies are still efficacious against uncomplicated malaria.
- In the Greater Mekong Subregion, malaria treatment options are limited due to *Plasmodium falciparum* resistance against individual artemisinin-based combination therapies.
- Clinical trials in Sub-Saharan Africa and Latin America continue to report high efficacy of first- and second-line artemisinin-based combination therapies.
- Artesunate-pyronaridine and atovaquone-proguanil are important drug combinations for treatment of uncomplicated falciparum malaria.
- Drugs with a new mechanism of action are urgently needed to effectively treat *P. falciparum* infections in the future and to ensure sustainability of malaria elimination efforts.

Monotherapies of artesunate or artemether are reserved only for the parenteral treatment of severe/complicated falciparum malaria [12]. Furthermore, ACTs are also recommended for treatment of infections with *P. vivax*, the dominant Plasmodium species outside of Africa, in areas where chloroquine-resistant strains are circulating. Despite all efforts, artemisinin resistance defined by slow clearing *P. falciparum* emerged and was first reported in 2008 and, since then, is a threat to malaria control.

2. Defining artemisinin-resistant malaria

Antimicrobial drug resistance is usually understood as the occurrence of bacteria, viruses, pathogens, etc. that evade killing or attenuation by a chemotherapeutic drug and continue to survive and propagate in the infected individual, maintaining the presence of the potential disease (based on the assumption of adequate dosing, pharmacokinetics, initial parasite biomass, and accounting for reinfection). The first observations of artemisinin resistance were made in 2008 in Cambodia [13,14] when the time to *P. falciparum* clearance following seven days artesunate monotherapy was prolonged beyond three days and some malaria patients were still parasitemic on day 28 follow-up, although most patients were cured. The WHO defines clinical artemisinin resistance as 'delayed parasite clearance following treatment with an artemisinin-based monotherapy or with an artemisinin-based combination therapy' [8]. This is also labeled as 'artemisinin partial resistance' and is suspected when 10% (or more) of malaria patients present with a parasitemia at day three or have a parasite clearance half-life beyond five hours. However, this clinical phenotype does not necessarily lead to a treatment failure commonly assessed 28 or 42 days after treatment start (timing depends on the half-life of the ACT partner drug). A failure following ACT treatment rather occurs because of an underlying resistance to the ACT partner drug that may or may not be accompanied by a partial artemisinin resistance event in the circulating *P. falciparum* strains [15].

On the molecular level, the delayed clearance phenotype is associated with certain mutations in the *pfkelch13* gene (see chapter 3.2) and *P. falciparum* strains with validated respective mutations are classified as artemisinin resistant parasites – again, not predicting a treatment failure following ACT intake [8].

3. Molecular markers of drug resistance

Slow parasite clearance rates result from decreased artemisinin susceptibility of *P. falciparum* in malaria patients and is correlated with increased survival of young parasite ring stages in *in vitro* assays (0–3 hours ring stage survival assay) [16]. Several candidate resistance genes have been investigated in the past, but evidence is consolidating that mutations in the *P. falciparum kelch 13* propeller domain (*pfkelch13)*, especially the C580Y allele, are of major importance to prevent the antiplasmodial effect of artemisinins [17,18]. Kelch 13 protein is involved in the endocytic uptake of hemoglobin from the host erythrocyte into the parasite at the ring life cycle stage of *P. falciparum* [19,20]. Mutations in *pfkelch13* lead to reduced activity of the protein and result in decreased levels of hemoglobin degradation products required for activation of artemisinins. The identification of a molecular marker for the delayed clearance phenotype following artemisinin exposure is a helpful achievement for identifying and monitoring *P. falciparum* populations since several mutations have been around for quite some time, but gave inconclusive results [21]. Over 200 nonsynonymous mutations in *pfkelch13* have been found throughout South East Asia [22] with the C580Y mutation being one of the most important mutations associated with slow parasite clearance following artemisinin administration and reaching frequencies of up to 91% [23]. Fortunately, most mutations in *pfkelch13* in Sub-Saharan Africa are currently not associated with artemisinin resistance, including A578S, the most prevalent mutation in that region, but *P. falciparum* strains with the C580Y mutation are increasingly detected, fortunately no treatment failure is yet linked to an ACT (Cameroon, Ghana) [24,25]. In 2020, Uwimana et al. reported on a new *pfkelch13* mutation, the R561H, which was found in samples collected in Rwanda from patients treated with either dihydroartemisinin-piperaquine or artemether-lumefantrine [26]. While no reduced efficacy was found for both ACTs, *in vitro* investigations suggest that the R561H mutation can confer artemisinin resistance at similar levels to C580Y. In South America, some *pfkelch13* mutations associated with delayed parasite clearance have been reported [27] as well as in Papua New Guinea [28]. In most South American countries, clinical isolates with the C580Y mutation have not been detected, except in Guyana, which emerged independently from those in Asia [27].

P. falciparum lineages with *pfkelch13* mutations emerged independently in the Greater Mekong Subregions [29]. In addition, concomitant resistance to the ACT partner drugs established in the local parasite population resulting in treatment failures that has already occurred for artesunate-mefloquine and dihydroartemisinin-piperaquine in some countries of the GMS. Monitoring of drug resistance against ACTs also encompasses partner drug resistance genes. Molecular markers of non-artemisinin drugs include plasmepsin 2/3 (*pfmp2 and pfmp3*) copy number increase for tracking piperaquine resistance [30], mutations in the chloroquine resistance transporter (*pfcrt*) [31] and multi-drug resistant

(*pfmdr1*) [32] gene for amodiaquine, with the later gene also indicating resistance to mefloquine when the gene copy number is increased, and the dihydrofolate reductase (*dhfr*) [33] and dihydropteroate synthase genes (*dhps*) [34] for pyrimethamine and sulfadoxine resistance, respectively. These genes are also important for the monitoring of ACT partner drug efficacy, since resistance may occur in both drugs, causing ACTs to become ineffective as has already occurred for artesunate-mefloquine and dihydroartemisinin-piperaquine.

4. Brief outline of artemisinin resistance development

Occurrence of artemisinin resistance characterized by a slowdown of removal of circulating *P. falciparum* parasites was first confirmed in 2008 by two specifically designed clinical trials where patients with uncomplicated malaria received oral artesunate monotherapy for seven days [13,14]. Time to parasite clearance was prolonged (approximately doubling the time compared to those cured) and some patients still had parasites on day 28 of follow-up in the western Cambodia study sites and later also found on the Thailand-Myanmar border [35]. Since then, the delayed parasite clearance phenotype to artemisinin treatments has spread as well as emerged independently in other regions of Southeast Asia [36] and is now highly prevalent in countries of the Greater Mekong Subregion [35,37,38]. The emergence of *P. falciparum* parasites with decreased susceptibility/resistance to artemisinins and also to the partner drugs, which occurred on the background of preexisting resistance to multiple antimalarial drugs, was devastating news.

The first-line treatment for uncomplicated malaria in many parts of the Greater Mekong Subregion in the 1990s to early 2000s was artesunate-mefloquine [39] and that was also the first ACT to have failed. Since its introduction in early 1990 in Thailand, clinical studies reported a significant decline in artesunate-mefloquine efficacy along the Thai-Myanmar border in 2009 with day 42 treatment failures of 32% [40] and circulating parasite strains concomitantly harboring *pfkelch13* mutations and multiple *pfmdr1* copy numbers [41,42]. For this reason, a change toward the use of dihydroartemisinin-piperaquine for *falciparum* malaria treatment in countries of the Greater Mekong Subregions has occurred. Unfortunately, dihydroartemisinin-piperaquine resistance spread quickly throughout Cambodia starting in 2008 [43–46], to which artesunate-mefloquine was reintroduced as first-line treatment in 2014 [47], and other countries including Thailand and Vietnam, which experienced up to 26% treatment failures in some regions [45,48,49]. Resistance to most ACTs has occurred on the background of preexisting resistance to multiple antimalarial drugs, including those used as partner drugs in ACTs. More details and studies have been excellently summarized in recent reviews [50,51]. According to WHO, artesunate-pyronaridine became recommended as a first-line treatment in Thailand in some regions along the Cambodian border and in Vietnam. Interestingly, in many other countries in Southeast Asia, artemether-lumefantrine is still efficacious and the recommended first-line treatment. In the majority of Sub-Saharan African countries, artemether-lumefantrine and artesunate-amodiaquine are the first-line treatments of uncomplicated *falciparum* malaria, implemented since 2005 [52]. Since then, clinical studies continue to confirm high efficacy of both ACTs [53] with less than 10% treatment failures due to recrudescence and occasional reports of treatment failures in travelers [54]. In South America, the situation is similar and, so far, only one study from Suriname reported an increase in the number of malaria positive cases on day three after artemether-lumefantrine treatment [55].

5. Alternative treatment options

The number of approved medicines to treat falciparum malaria is limited and becomes even smaller if artemisinin-based combinations are excluded. Although, there is quite a list of antiplasmodial substances with proven safety, tolerability, and efficacy, they are usually not used as monotherapy, but rather in combination with other compounds. Another aspect is the global spread of drug resistant *P. falciparum*, e.g. to chloroquine, sulfadoxine, pyrimethamine, mefloquine, and many others that fail to cure malaria patients. The compounds and regimens reviewed here are selected on this background and mainly target the treatment of uncomplicated malaria caused by *P. falciparum* originating from regions with reported treatment failures to an ACT and/or with a delayed parasite-clearance phenotype.

5.1. Non-artemisinin-based malaria medicines in clinical use

Effective drug combinations not involving an artemisinin component are particularly desired for regions where *P. falciparum* strains of the delayed clearance phenotype and resistant to partner drugs are circulating.

5.2. Atovaquone-proguanil

One of the few non-ACTs in clinical use is atovaquone-proguanil, a fixed drug combination (250 mg atovaquone plus 100 mg proguanil hydrochloride) applied for oral treatment of uncomplicated *P. falciparum* malaria and for malaria chemoprophylaxis in nonendemic travelers [56]. In addition, a pediatric tablet (62.5 mg atovaquone plus 25 mg proguanil hydrochloride) is available. A full treatment course is a single dose (weight-adjusted) given daily for three consecutive days inhibiting blood schizonts and to a lesser extent hepatic forms. Elimination half-life of atovaquone is two to three days in adults and of proguanil/cycloguanil 12–15 days. Atovaquone is a naphthoquinone and interferes with the mitochondrial cytochrome bc1 complex of *P. falciparum*, while proguanil, via its metabolite cycloguanil, inhibits the dihydrofolate reductase (*pfdhfr*) of the parasite resulting in synergistic drug activity. This is a well-tolerated, safe, and highly efficacious drug combination [57,58] reaching day 28 cure rates of up to 98% in initial clinical trials – also against chloroquine-resistant *P. falciparum*. Interestingly, it is also an effective treatment for *P. ovale* and *P. malariae* infections [59]. Although atovaquone-proguanil was approved in early 2000 (registered drug name Malarone®, GlaxoSmithKline), its use was limited due to

its relatively high cost. With the advent of generic atovaquone-proguanil, this might change within the next years. On the other hand, there is some reserve of widespread use of the drug combination due to the fear of resistant parasites coming up rapidly. On the background of widespread antifolate resistance in malaria endemic areas [60], acquisition of the single point mutation Y268S (or Y268N or Y268C) within the cytochrome b gene could be efficiently selected for and may result in atovaquone-proguanil treatment failures. For a long time, it has been known that atovaquone monotherapy selects for resistant *P. falciparum* rapidly [61]. There are hints that some mutations might be detrimental to the mosquito-stage of the parasite and, thus, could be self-limiting to their propagation [62]. Despite occasional reports of treatment failures, atovaquone-proguanil remains an active antimalarial treatment with little to no reports of cytochrome b mutations causing treatment failures [63]. In a clinical trial conducted in young children with uncomplicated malaria in Cameroon [64], no mutation was found in the cytochrome b sequence of 55 *P. falciparum* isolates analyzed, despite a mediocre efficacy on day 28 of 85% (of note: the study did also assess efficacy of atovaquone-proguanil given together with artesunate, see chapter 4.2.2.). A study by Lin et al. treated 205 participants infected with *P. falciparum* in Cambodia with atovaquone-proguanil (1000 mg/400 mg) with or without 200 mg of artesunate and 15 mg of primaquine on day 1 and reported 14 treatment failures with only one of those samples harboring the cytb Y268 mutation at recrudescence [65]. Atovaquone-proguanil has also been given to (a few) individuals infected with artemisinin resistant *P. falciparum* in Cambodia, where the 28 days cure rate was 100% [66].

5.3. Quinine plus antibiotics

Quinine is the oldest known antimalarial remedy and continues to play a significant role in malaria treatment – particularly in cases where (intravenous) artesunate or artemether fail to improve severe malaria complications, and also in malaria in early pregnancy if there is no alternative option available [67]. Quinine is an aryl amino-alcohol and primarily inhibits *P. falciparum* blood stage schizonts by interfering with the parasite's metabolism of hemin polymerization into hemozoin, the 'malaria pigment.' Despite quinine use for more than 200 years, resistant parasites occur rather occasionally and are more often reported from Southeast Asia [68] and South America [69], and to a lesser extent from Sub-Saharan Africa [70] – although the actual spread and extent is difficult to estimate due to a lack of recent clinical evaluations. Polymorphisms in multiple genes might be required for quinine resistance to occur that is slowing the pace of resistant parasites to develop [71]. Quinine has a short half-life of around 11 hours [72] necessitating an extensive treatment course for uncomplicated malaria with oral quinine (10 mg/kg) every eight hours for seven days. Although good cure rates can be achieved [73], this complex treatment regime plus the limited tolerability (impaired hearing, vertigo, nausea) can result in poor adherence and effectiveness under real life conditions against uncomplicated malaria – as it was shown earlier in a community-based trial in Uganda in children with uncomplicated malaria where day 28 effectiveness of oral quinine was only 64% compared to 96% of artemether-lumefantrine and a low treatment adherence to quinine was noted [74]. Another example is a trial in Gabon in pregnant women where day 28 efficacy was only 60%, attributed to low treatment adherence [75]. Thus, quinine should not be used as monotherapy for uncomplicated malaria treatment, but instead should be co-administered with doxycycline or clindamycin, as recommended by WHO [75].

Tetracyclines are broad-spectrum antibiotics and their activity against *P. falciparum* infections has been recognized already in the 1950s. Doxycycline is a synthetically derived tetracycline and was used early on for the prevention of *P. falciparum* infections by travelers also in regions with chloroquine or multidrug-resistant parasites. Administered together with more rapidly acting quinine, doxycycline is also used for the treatment of uncomplicated falciparum malaria. The drug is rapidly absorbed after oral intake, and the half-life is about nine to 22 hours. Doxycycline is a slow-acting drug inhibiting blood schizonts by acting on the apicoplast [75], a Plasmodium-specific organelle containing a circular DNA. The common treatment course for uncomplicated falciparum malaria is seven days 100 mg doxycycline given twice per day plus 10 mg/kg quinine once per day and reaches treatment efficacy between 90% and 100% [76,77]. A clinical trial in Gabon reported a 91% day 28 cure rate of a short quinine-doxycycline treatment course (quinine: 12 mg/kg per dose, three doses, one dose every 12 hours; doxycycline: 2 mg/kg per dose, six doses, one dose every 12 hours). This is an interesting finding as WHO now refrains from recommending quinine plus doxycycline administration due to low adherence to the long treatment schedule leading to treatment failures [67]. Clinical doxycycline resistance is not yet confirmed, but from *in vitro* work indications toward parasites with reduced susceptibility are present [78]. Doxycycline is generally well-tolerated, but gastrointestinal problems and photosensitivity are common side effects. It should not be given to children below the age of eight years, because it can incorporate into teeth and bones, and to pregnant women. In contrast, clindamycin, a lincosamide antibiotic, is WHO recommended for the treatment of uncomplicated malaria in early pregnancy if co-administered with quinine. As a monotherapy, clindamycin is highly active against *P. falciparum*, but similarly to other antibiotics, it is slow acting and requires an intensive treatment course (twice daily, for minimum of five days) [79]. The recommended treatment for uncomplicated malaria during the first three months of pregnancy is clindamycin plus quinine given for seven days. A three-day treatment regimen of oral quinine plus clindamycin (quinine, 15 mg/kg, and clindamycin, 7 mg/kg, per dose) given twice daily for three days to Gabonese children (3–12 years old) with uncomplicated falciparum malaria resulted in a 94% day 28 cure rate [80]. Clindamycin is a well-tolerated and safe drug, but diarrhea can occur. The drug is rapidly absorbed after oral intake and the half-life is two to four hours. Due to the short half-life of clindamycin and quinine, accurate and complete treatment administration is essential for a successful cure [79].

5.4. Artemisinin-based regimens

Parasites exerting the delayed clearance phenotype are usually still removed from the blood stream of the malaria patient if parasites are susceptible to the ACT partner drug and/or stringent adherence to the recommended treatment schedule (excluding particular host factors such as splenectomy, etc.). Despite alarming findings of *P. falciparum* strains that can tolerate artemisinins for some time and a better understanding of the related mechanism [19], ACTs are still highly efficacious, well-tolerated, safe, and their use is well justified.

5.4.1. Approved drugs

None of the six WHO recommended ACTs is neither failing to cure *P. falciparum* malaria in all the malaria endemic regions nor in the resistance 'hotspots' Cambodia, Laos, Thailand, Vietnam, and China-Yunnan. Thus, although dihydroartemisinin-piperaquine and artesunate-mefloquine can result in treatment failures in these countries [41,81], other ACTs like artesunate-amodiaquine [82] or artesunate-pyronaridine [83] are still effective. It has also been shown, e.g. in Cambodia for artesunate-mefloquine that a change of the recommended first-line ACT can revert its activity after some time [84]. The ACTs are still fundamental for malaria treatment, but the recommended ACT drug should not include a partner drug to which resistance exists in the region. In light of this, the 'new' ACT artesunate-pyronaridine is currently explicitly recommended in the GMS for the use where ACT treatment failures occur [85].

5.5. Artesunate-pyronaridine

Artesunate-pyronaridine is the sixth ACT recommended by WHO for the treatment of uncomplicated malaria. The fixed-dose drug combination with the product name Pyramax® (pyronaridine-artesunate) was developed by Medicines for Malaria Venture (Geneva, Switzerland), a not-for-profit organization, and Shin Poong Pharmaceuticals Co. (Seoul, South Korea). Pyramax was recently granted a *positive scientific opinion* by the European Medicines Agency applying article 58 procedures [86]. Pyramax is given once daily for three days as tablets to adults (180 mg pyronaridine tetraphosphate and 80 mg artesunate) or as oral suspension of granules (60 mg pyronaridine tetraphosphate and 20 mg artesunate) to young children. Artesunate is the rapid acting, semisynthetic artemisinin derivate with a short half-life of about one to two hours and mainly inhibits the early blood stage rings of the parasites. Pyronaridine, the long-acting partner drug with a half-life of up to 17 days [87], is a benzo-naphthyridine derivative originally synthesized and developed in China in the 1970s. The drug interferes with the parasite´s food vacuole inhibiting blood stage schizonts, but a detailed understanding of the molecular mechanism of the drug´s activity is lacking (as is the case for most of the antimalarial compounds) [88]. Early clinical trials in Southeast Asia and Africa showed that pyronaridine is safe, well-tolerated and highly efficacious against uncomplicated malaria including cases with infections of chloroquine resistant *P. falciparum* [89,90]. Pyronaridine was mainly used in China before the development toward a fixed artesunate-pyronaridine drug combination started in 2002. Although *P. falciparum* strains from China were found with a decreased *in vitro* sensitivity toward pyronaridine [91], clinical resistance has not yet been reported in humans. This circumstance might be owed to the, so far, relatively little clinical use of the monotherapy not posing a relevant selection pressure to circulating parasites. An early dose-finding study done in Gabon in children with uncomplicated malaria reported a day 28 efficacy of 100% at all dose levels including good safety and tolerability data [92]. These excellent day 28 efficacy data were also supported by other studies done in Asia and in Africa [93]. Initial doubts of hepatic safety in repeatedly treated children [94] were removed by two phase 4 clinical trials that reported only transient mild elevations in transaminases if observed, and efficacies reaching almost 100% for day 28 and day 42, (clinicaltrials.gov: NCT03201770). A recent meta-analysis of clinical trials confirmed that artesunate-pyronaridine was safe and 95% efficacious for the treatment of uncomplicated *P. falciparum* malaria in adults and children [95].

5.5.1. Artemisinin-based regimens in development

Until new antimalarial drugs become available, a temporary strategy is to combine an ACT with an additional partner drug resulting in triple or even quadruple artemisinin-based combination therapies to prolong the lifespan of available antimalarials and to provide a future treatment to patients where existing regimens are failing [96]. The extended drug combinations should ensure rapid reduction of parasite biomass to improve disease course, remove, ultimately, remaining parasites to achieve cure, and mutually protect the compounds from parasite resistance development. Multidrug therapies are common in other diseases such as tuberculosis where a four-drug regimen is standard. However, drug–drug interactions potentially resulting in modified efficacies and causing adverse events cannot be predicted from the individual approved drug, but must be carefully investigated in clinical trials before this regimen can become recommended and eventually enter treatment guidelines. This would include monitoring compliance of patients to such treatment regimens with multiple pills to swallow to reach weight adjusted doses and assess treatment efficiency. Recent examples of currently investigated triple combination therapies are dihydroartemisinin-piperaquine plus mefloquine and artemether-lumefantrine plus amodiaquine, which turned out to be efficacious, and generally well-tolerated and safe, also in children (only artemether-lumefantrine plus amodiaquine was tested in the pediatric cohort) in a multicenter trial mainly done in the GMS [96]. Interestingly, in Vietnam, Thailand, and Cambodia, regions with known high rates of dihydroartemisinin-piperaquine treatment failures, the addition of mefloquine increased efficacies from 48% to 98% [45]. In this line, the most efficacious ACT artesunate-pyronaridine (one tablet) is combined with the most efficacious non-ACT (one tablet) and is currently assessed in two clinical trials (NCT03726593, PACTR202010540737215) in adults in the GMS and in children

in sub-Saharan Africa to understand the combined efficacy, pharmacokinetic properties, and safety – particularly important for administration in the vulnerable pediatric cohort.

5.5.2. Artemisinin-based combinations for severe malaria

Severe malaria is accounting for the majority of the annual 409,000 fatal *P. falciparum* infections per year – mainly occurring in Sub Saharan Africa. Treatment is based on parenteral artesunate (or artemether) administration for a minimum of 24 hours followed by three days ACT when oral administration is tolerated. For children below 6 years of age with suspected severe malaria living in remote areas, far from the next health care facility, a single dose of artesunate suppositories is recommended, to bridge the time until the next health post is reached and parenteral treatment can be started [67,97]. The loss of artesunate (artemether) would be disastrous to the clinical management of life-threatening malaria complications; with the majority of victims being children. As comparably effective drugs are not within reach, artesunate needs to be safeguarded from parasite resistance by all means and alternative regimens should be prepared. Quinine is only a limited substitute, due to its poor tolerability. A current strategy in development is the combination of artesunate with two antibiotics, clindamycin and fosmidomycin. Clindamycin is well known for its antiplasmodial activity and is available as a parenteral formulation (see chapter 4.1.). Fosmidomycin inhibits the nonmevalonate pathway of isoprenoid biosynthesis in *P. falciparum* [98] and has been studied in combination with either of the drugs, and also in children [99]. It is a rapidly acting blood schizonticide with a short plasma half-life of approximately two hours and acts synergistically with clindamycin [100]. Cure rates above 90% have been achieved in children with uncomplicated malaria in Gabon given together with clindamycin [101,102] as well as when given together with artesunate [103] in regimens of two doses for three days. Fosmidomycin is a promising partner drug candidate with a good safety profile and is well-tolerated. The intended future dosing is a parenteral, three-day regimen. In addition, the triple combination will also target bacterial coinfections often concomitantly occurring in febrile African children. Clindamycin is an inhibitor of gram-positive bacteria and fosmidomycin of gram-negative bacteria.

5.6. Outlook: Non-artemisinin drugs in development

Of utmost importance, to be able to combat artemisinin and ACT-resistant falciparum malaria in the upcoming years, is the development of malaria drugs unrelated to artemisinins and with a different antiplasmodial mechanism. The drug development pipeline, although scarce in the number of new chemical classes, includes promising candidates in the advanced phases of clinical drug development [104] such as ganaplacide (KAF156) [105], cipargamin (KAE609), ferroquine, and others. The webpage of Medicines for Malaria Venture, the lead not-for profit organization for the development of new malaria drugs, should be consulted for an excellent and up-to-date overview of ongoing drug projects [104].

Insights into the international, collaborative efforts of the development of new malaria drugs adhering to stringent gate stages for efficacy and safety is provided here via the example of the development program for ferroquine-artefenomel, a drug combination aiming for a single-exposure radical cure treatment as well as for ganaplacide and cipargamin.

5.7. Artefenomel

Artefenomel (OZ439) is a synthetic second-generation trioxolane that contains a similar peroxide pharmacophore as artemisinin [106], showing a similar rapid onset of action. A first generation compound called arterolane (OZ277) is marketed in India in combination with piperaquine (Synriam^{TM}) and has gained market approval for several African countries [107]. In comparison to its predecessor, artefenomel shows improved pharmacologic properties and [108] displays a longer half-life (approximately three to five days) when compared to classical artemisinin derivatives. Its favorable features gave hope that artefenomel could be developed as a single-dose cure for uncomplicated malaria in combination with a strong partner drug.

A single dose regimen of artefenomel (800 mg) partnered to piperaquine (three different doses either 640 mg, 960 mg, or 1440 mg) was tested and given to malaria patients in a phase 2b study [109]. This regimen could not meet the required level of 95% efficacy as the adequate clinical and parasitological response on day 28 was only 71%, 68%, and 79% in the respective dose groups [109]. One problem, particularly in young children, was a high dosing volume and a high rate of vomiting that might explain a lower drug exposure in the youngest children, leading to treatment failures. As an alternative combination partner, ferroquine was identified. Two studies on a single dose cure by artefenomel plus ferroquine combination concluded recently (clinicaltrials.gov: NCT02497612, NCT03660839). Results posted on the clinicaltrials.gov webpage on the phase 2b efficacy and safety study of a single dose regimen in adults and children state that all treatment arms did not show the required level of efficacy during the preplanned interim analysis, and that, therefore, the study was terminated early (NCT02497612). Recruitment in Asia was stopped even earlier in August 2018 due to lack of efficacy. Study results are not yet published in peer reviewed journals, but the MMV homepage already states that the close out of the 2b program is currently running and a development plan for ferroquine with alternative partner drugs needs to be elaborated.

5.8. Ferroquine

Ferroquine (SSR97193) was discovered by CNRS Lille and has been identified as a lead compound in 1997, but has not yet entered the market [110]. It is a 4-aminoquinoline analogue like chloroquine containing an additional ferrocenyl group in its side chain [111]. *In vitro*, it shows high activity against chloroquine-sensitive and chloroquine-resistant *P. falciparum* and *P. vivax* isolates from different endemic areas [112,113]. Ferroquine accumulates in the digestive vacuole of the parasite interfering with the hemozoin formation, a mode of action similar to that of chloroquine [114]. In addition, it is described to produce hydroxyl radicals that damage the parasite. The

major active metabolite of ferroquine is a N-monodemethyl derivative (SSR97213) that shows an equal activity as its mother compound *in vitro*. Ferroquine was more than 95% efficacious in the three-day dose regimen together with artesunate at 2 mg/kg, 4 mg/kg, and 6 mg/kg when evaluated on day 28 [115], but development has been stopped in favor of a non-artemisinin-based combination therapy. In the following, it is/was in development together with artefenomel as a single dose cure regimen, in a non-ACT based combination therapy. Results of the phase 2 studies are not yet published, but posted in a clinical trials registry (clinical trials.gov: NCT02497612, NCT03660839 [116]), revealing that this combination will not be further pursued, but that a different partner compound will be identified. Ferroquine is the long-acting partner drug in any combination regimen, with a terminal half-life of around 30 days, and its metabolite of even up to 40 days, therefore presenting a promising candidate for post treatment prophylaxis. Adverse reactions that should be monitored when ferroquine is given include effects on cardiac functions as QTc prolongation and possible effects on ECG morphology [115,117], side effects known also for the other compounds of the 4-aminoquinoline class [118].

5.9. Ganaplacide and cipargamin

Ganaplacide (KAF156), an imidazolpiperazine, and cipargamin (KAE609), a spiroindolone, are new antimalarial compounds currently in clinical development for the treatment of uncomplicated malaria and are currently in ongoing phase 2 trials. Ganaplacide and cipargamin were shown to be effective *in vitro* against *P. falciparum* with IC50s of 6–17.4 nM and 0.5 to 1.4 nM, respectively [119,120]. Currently, data from four published clinical trials exist for ganaplacide including a malaria challenge study [121–124]. Ganaplacide was well-tolerated when given at a daily dose of 400 mg for 3 days and a single dose of 800 mg, and had an overall 28-day cure rate of 67% in a small number of adults with uncomplicated *P. vivax* or *P. falciparum* malaria after single-dose administration [122]. Ganaplacide is currently developed in combination with lumefantrine [125] for a short treatment course. Data from seven clinical trials show cipargamin to be well-tolerated in healthy volunteers and in patients, however, there are some safety concerns in regard to liver function test abnormalities [126] A recently published malaria challenge trial reports antiplasmodial activity of a single dose, however liver safety signals appeared [127]. In a phase 2 trial done in Thailand, twenty-one patients with uncomplicated *P. falciparum* and *P. vivax* malaria were treated with a dose of 30 mg per day for three days and showed a parasite clearance half-life of less than 1 hour, that is even faster than artesunate [122,128]. Cipargamin is a promising candidate for a once-daily regimen.

6. Expert opinion

Parasites resistant to antimalarial treatments are a threat to the clinical management of falciparum malaria, but are also a public health concern with the overall aim of eliminating and even eradicating this deadly disease. Even though there are a few alternative antimalarial drugs available and several candidates are in the drug development pipeline, there is great fear the current treatment options could be lost with emerging and spreading artemisinin and ACT resistant parasites. All state-of-the-art treatments – for both uncomplicated and complicated falciparum malaria – are built on the same most potent current drug class, namely the artemisinins, the rapidly acting, but short-lasting primary drug component in artemisinin-based therapies, and currently no comparably promising compound is within reach.

The report of artemisinin resistant *P. falciparum* parasites in Cambodia in 2008 was devastating news and the increase in treatment failures following ACT intake in Greater Mekong countries is worrisome. Although this is rather attributed to parasites resistant to the long-acting partner drugs – such as mefloquine, piperaquine, amodiaquine, lumefantrine, sulfadoxine/pyrimethamine – failures to cure have been documented in artemisinin monotherapy clinical trials. Still a puzzling phenotype is that of slow clearing *P. falciparum* strains following an artemisinin-based treatment circulating in the GMS that is associated with some mutations in the *pfkelch13* gene. The GMS is known to be a recurrent source of drug resistant *P. falciparum*, most mutations originated there for the first time. Although the factors and mechanisms behind this are difficult to decipher, a considerable contributor is the enormous drug pressure on a relatively small parasite population in a host population with only a low-level immunity allowing for a faster spread and selection of resistant *P. falciparum*. Reassuringly, since the first reports on artemisinin resistance, falciparum malaria cases and deaths in countries of the Greater Mekong were successfully reduced in the past 10 years by more than 90%, resulting in 9,000 *P. falciparum* cases in 2020 (until October 2020) and 10 deaths. This is a tremendous achievement in a region with more than approximately 300 million inhabitants and malaria elimination in this region is within reach [129]. Clearly, in Sub-Saharan Africa with 215 million cases and 384.000 deaths in 2019 and a population of more than 1.1 billion people, some success has been achieved in controlling malaria, but this region is far away from malaria elimination, except for South Africa, and Botswana. Mutations in the *pfkelch13* gene (R561H, R622I, etc.) have been identified and emerged independently in some East African countries. Despite some mutations leading to increased survival of those strains in *in vitro* testing, favorably, neither prolonged clearance nor failures to cure malaria were detected following treatment with ACTs. However, this potential threat requires regular and thorough monitoring to be able to promptly adapt treatment policies that could recommend a different ACT or other strategies although alternatives are limited.

New malaria drugs with antiplasmodial mechanisms different from those of available medicines are urgently needed. They can be found in the current drug pipeline, but are not close to market approval, and failure rates of completely novel drug classes in development are high. To bridge this lack and to safeguard available treatments from resistance, combination of multiple drugs is a current approach under investigation. The addition of drugs with prolonged plasma half-lives to existing ACTs aim to ensure effective malaria treatment and to

reciprocally safeguard the long-acting drugs from selection of parasites with mutations conferring a survival benefit. These drug combinations should be carefully studied for adverse events and tolerability resulting from unknown drug-drug interactions. Such complex regimens could be of some value in areas with decreasing *P. falciparum* transmission and with parasites under high selection pressure. Ideally, one component should additionally exert gametocidal activity to contribute to elimination efforts and avoid the spread of multiresistant parasites. Another option under discussion is to prolong the three days ACT course as artesunate monotherapy for seven days is known to be efficacious given stringent adherence to the treatment schedule. Although this might be a relatively efficient approach to approval, adverse events like postartesunate delayed hemolysis could become a problem [130].

Unfortunately, synthetic endoperoxides like artefenomel or arterolane which share the similar antiplasmodial pharmacophore with artemisinins do not meet the expectation that arose from the first studies. New promising compounds are in development, but they still have to go the hard road of clinical development and it will take some time until they will (hopefully) be available. In this respect, it is reassuring that existing ACTs are still effective, provided that resistance to the combination partner is taken into account.

Funding

The authors are funded by the University Hospital Tübingen, Germany.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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