

# Drugs in Development for Malaria

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**Abstract** The last two decades have seen a surge in anti-malarial drug development with product development partnerships taking a leading role. Resistance of *Plasmodium falciparum* to the artemisinin derivatives, piper-quine and mefloquine in Southeast Asia means new antimalarials are needed with some urgency. There are at least 13 agents in clinical development. Most of these are blood schizonticides for the treatment of uncomplicated falciparum malaria, under evaluation either singly or as part of two-drug combinations. Leading candidates progressing through the pipeline are artefenomel–ferroquine and lumefantrine-KAF156, both in Phase 2b. Treatment of severe malaria continues to rely on two parenteral drugs with ancient forebears: artesunate and quinine, with sevu-parin being evaluated as an adjuvant therapy. Tafenoquine is under review by stringent regulatory authorities for approval as a single-dose treatment for *Plasmodium vivax* relapse prevention. This represents an advance over standard 14-day primaquine regimens; however, the risk of acute haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase deficiency remains. For disease prevention, several of the newer agents show potential but are unlikely to be recommended for use in the main target groups of pregnant women and young children for some years. Latest predictions are that the malaria burden will continue to be high in the coming decades. This fact, coupled with the repeated loss of antimalarials to

resistance, indicates that new antimalarials will be needed for years to come. Failure of the artemisinin-based combinations in Southeast Asia has stimulated a reappraisal of current approaches to combination therapy for malaria with incorporation of three or more drugs in a single treatment under consideration.

## Key Points

Product development partnerships have revived antimalarial drug development, and a variety of target candidate and product profiles have been defined to support control and elimination goals

New agents (arterolane, cipargamin, KAF156) on the horizon show potential to replace failing artemisinin combination therapies as part of novel combinations

The loss of front-line therapies to resistance has stimulated a reappraisal of the current approach to combination therapy for malaria, with consideration of a switch from dual to triple drug combinations

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## 1 Introduction

Malaria is a protozoan parasitic disease transmitted by the female *Anopheles* mosquito, which infects around half a billion people in tropical and sub-tropical countries each year [1]. Six species of *Plasmodium* cause human disease regularly: *P. falciparum*, *P. vivax*, *P. ovale curtisi*, *P. ovale wallikeri*, *P. malariae* and *P. knowlesi*. *P. falciparum* is

responsible for almost 90% of all infections and for most of the 445,000 deaths that occur annually; most are in African children. *P. vivax* and *P. ovale* are relapsing malarias with a dormant hypnozoite stage in the liver which requires targeted therapy to prevent relapse, and *P. knowlesi* is a predominantly zoonotic infection with macaques acting as the natural hosts. The main strategies to control malaria globally are vector control with long-lasting insecticide-treated bed nets, early diagnosis and treatment with artemisinin-based combination therapies (ACTs), and chemoprevention in pregnant women and young children. After decades of research, a malaria vaccine (RTS,S/AS01) received a positive opinion from the European Medicines Agency under Article 58 in 2015; however, it is only moderately efficacious and has an uncertain future [2]. Its introduction is unlikely to lessen the demand for anti-malarial drugs.

The use of ACTs to treat malaria dates back to the early 1990s. At that time the situation in Southeast Asia was bleak; chloroquine, sulfadoxine-pyrimethamine (SP) and mefloquine had been deployed sequentially and fallen to resistance. In 1979, Chinese scientists had published the chemical structure of qinghaosu or artemisinin, a potent antimalarial derived from the sweet wormwood plant *Artemisia annua* [3, 4]. White proposed partnering the fast-acting, highly efficacious, but rapidly eliminated artemisinin derivatives with more slowly eliminated drugs as short-course (3-day) highly effective combination treatments, with the aim of improving cure rates and slowing the development of resistance [5]. ACTs were adopted fairly quickly in Southeast Asia with some produced locally, e.g. Vietnam has produced various dihydroartemisinin (DHA)–piperazine combinations since 1995.

Meanwhile chloroquine- and pyrimethamine-resistant strains of falciparum malaria had spread to Africa leading to an increase in child mortality [6–8]. This crisis renewed the impetus to bring malaria under control, and greater financial resources were made available. An important part of the response was the recognition that new drugs were needed, and that an innovative approach was required to stimulate their development. New product development partnerships (PDPs) were created, such as Medicines for Malaria Venture (MMV, 1999) and the Drugs for Neglected Diseases Initiative (2003). The PDPs harnessed the expertise of the pharmaceutical industry and malaria academics, with financial support coming from a variety of (mainly public and philanthropic) sources. They were given a head start by focusing on the fruits of the labours of Chinese scientists and pharma [9]. By 2006, ACTs had become the recommended treatments for falciparum malaria worldwide [10]. The first ACT produced to recognised international standards of good manufacturing practice (GMP) was artemether–lumefantrine (Coartem®;

Novartis), approved by the US FDA in April 2009 [11]. Artemether–lumefantrine, DHA–piperazine, artesunate–amodiaquine, artesunate–mefloquine and artesunate–sulfadoxine–pyrimethamine are currently the most used combinations. Also available are pyronaridine–artesunate, artemisinin–naphthoquine, and arterolane–piperazine. Arterolane is a newer synthetic peroxide developed in India resembling the artemisinin derivatives. *P. knowlesi* malaria is treated in the same way as falciparum malaria [12]. For uncomplicated disease caused by *Plasmodium vivax*, *ovale* or *malariae* chloroquine remains the first line treatment in most parts of the world; however, there are increasing reports of resistance in *Plasmodium vivax* [13]. In Indonesia where failure rates are high, the ACT, DHA–piperazine, has replaced chloroquine for treatment. Severe malaria continues to be treated by either parenteral artesunate (the drug of choice), or quinine or quinidine (USA only) [14, 15]. A number of adjuvant therapies have been evaluated over the years but have not been shown to improve outcome [16].

Malaria case numbers and deaths have more than halved over the last 15 years and the goal of global elimination is being targeted again. At the same time, a new threat has emerged in Southeast Asia in the form of artemisinin resistance. Evidence that *Plasmodium falciparum* resistant to artemisinin has emerged has accrued steadily since 2008 [17–20]. Contributing factors may have been the continued availability of artesunate monotherapy in the region for many years or falsified or substandard drugs. Artemisinin resistance manifests clinically as delayed parasite clearance and was defined phenotypically using a parasite clearance half-life of  $\geq 5$  h or a high day 3 parasite positivity rate until molecular markers for resistance were reported in 2014: mutations in the kelch gene on chromosome 13 of the parasite [21]. Complete non-response to artemisinin treatment has not been described to date. The blunting of the early parasiticidal effect of the artemisinin derivatives leaves a larger residuum of parasites for the partner drugs to clear. Inevitably resistance to these drugs has now emerged in areas where artemisinin resistance had taken hold and ACTs are failing in Cambodia, Vietnam and Thailand [22–25]. The mismatch of elimination kinetics between the artemisinin derivatives and the drugs it is partnered with was often considered a risk for development of resistance, although to date, resistance to the rapidly eliminated more potent drug (artemisinin) has become apparent first.

In 2018, the situation in Southeast Asia is serious. In Cambodia, treatment has switched back to artesunate–mefloquine from failing DHA–piperazine, which had replaced failing artesunate–mefloquine in 2008. Pyronaridine–artesunate will be introduced as an alternative treatment option in areas of drug resistance this year. Small

numbers of artemisinin-resistant parasites have been found in India and the Americas [26, 27], while in Africa the news is more reassuring so far with no evidence that resistance has become established [28]. However, vigilance is needed, including for the possibility of kelch-independent reduced susceptibility. Triple artemisinin-based combinations, comprising a standard dual ACT with another slowly eliminated antimalarial are being evaluated as a possible holding measure until new agents are available (NCT02453308).

On this backdrop, the development strategy for antimalarial drugs has evolved. Early on, ambitious development goals of single-dose curative (including radical cure) combination treatments were emphasised. There has since been a shift to defining a broader portfolio of target product and candidate profiles suited to different goals of control and elimination, with less emphasis on single-exposure therapy. These include molecules for case management, prevention, transmission blocking and relapse prevention, described in detail in recent reviews [29, 30]. “Resistance-proofing” future compounds in both the selection process and by considering combinations of more than two molecules in new products is a priority.

The process of identifying new antimalarials, dose-finding and evaluation has also evolved over the last 10 years. Phenotypic screening still dominates with target-based methods employing molecular or genome scanning used increasingly [31–34]. There has been a collaborative approach to drug discovery and development with initiatives such as the Malaria Box, an open access resource containing 400 compounds with antimalarial activity made available free of charge until December 2015 [35]. Newer approaches to dose finding include determining the minimum inhibitory concentration of drugs by tracking sub-microscopic parasite densities using ultrasensitive PCR in human challenge models or malaria patients [36, 37].

Chemoprevention for malaria is used in vulnerable groups in endemic areas and in travellers. Strategies targeting pregnant women and young children include intermittent preventive therapy (IPT) with SP for pregnant women and infants, and seasonal malaria chemoprevention (SMC) using SP-amodiaquine in children aged < 5 years living in the Sahel. Administration of a complete treatment course of antimalarials in both IPT and SMC ensures that anyone already harbouring parasites with few or no symptoms receives an effective treatment. Mefloquine and DHA–piperaquine have been evaluated as alternatives for IPT in pregnancy in the face of worsening SP resistance [38–41]. Antimalarials for IPT or SMC ideally have a prolonged protective effect against infection allowing dosing to be adapted to typical antenatal clinic attendance or expanded programme for immunisation schedules.

For travellers, compounds with a shorter protective efficacy, including those requiring daily administration are acceptable. Possession of causal prophylactic activity is advantageous since such agents protect against infection and can be stopped soon after leaving an endemic area. Antimalarials most commonly prescribed to travellers are atovaquone–proguanil, doxycycline and mefloquine, although highly publicised neuropsychiatric side effects following mefloquine make it unpopular among both travellers and prescribers.

Here we review the antimalarials in clinical development and comment on their potential, with an emphasis on those that could succeed failing ACTs for treatment. The prospects for new preventive therapies and agents in the preclinical and discovery phase are also summarised.

## 2 Search Strategy

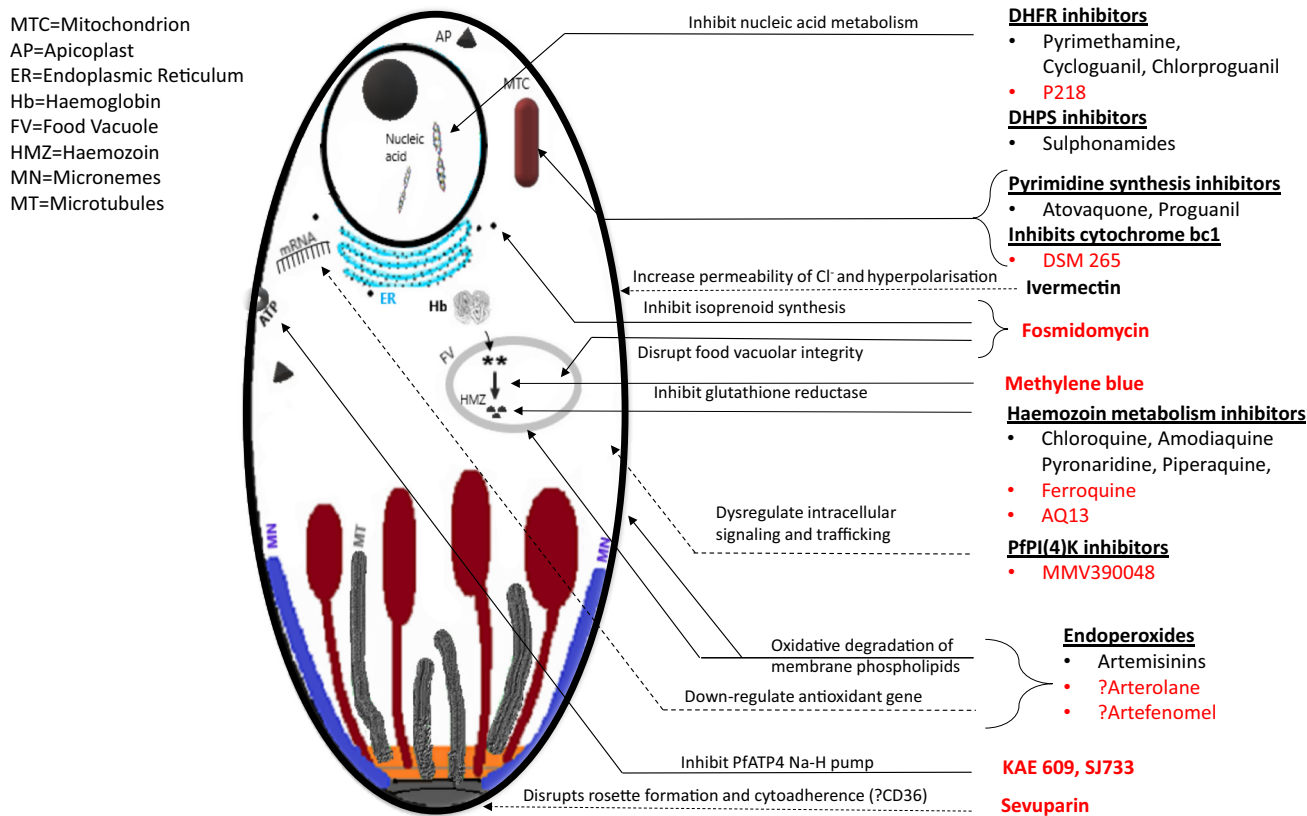
A two-stage approach was used. First a search of recent reviews of new antimalarials and new antimalarials not covered by these was performed in PubMed: (((“new antimalarial\*”[Title/Abstract]) AND review [Publication Type]) AND (“2012/10/01”[Date-Publication]: “3000”[Date-Publication])) AND (“Antimalarials”[MAJR]) AND (“2016/11/01”[Date-Publication]: “3000”[Date-Publication]). The global portfolio of antimalarial medicines on the MMV website was also consulted [42]. Each antimalarial found using this approach was then searched for individually by name(s) in PubMed and Embase databases, for example, ((KAE609 OR KAE 609 OR KAE-609 OR NITD609 OR NITD 609 OR NITD-609 OR cipargamin) AND malaria) and on the ClinicalTrials.gov website (<https://www.clinicaltrials.gov/>).

## 3 Drugs in Clinical Development

There are around 13 new antimalarial drugs in clinical development, nine of which are in Phase 2 [31]. The majority are blood schizonticides intended for the treatment of uncomplicated *P. falciparum* (Fig. 1). The chances of successful progress through the antimalarial development pipeline from Phase 2 to registration have been estimated to range from 34% (2a) to 60% (2b) [30].

### 3.1 New Drugs in Clinical Development for Treatment

An ideal antimalarial treatment would be a potent inhibitor of parasite multiplication, short-course or single-dose, well tolerated in pregnant women and young children, have a good safety profile, be affordable and have a low



**Fig. 1** Schematic representation of intra-erythrocytic trophozoite showing sites of action of newer antimalarials. Agents in red are still in development

propensity for resistance to develop. An expanded list of criteria used to assess antimalarials is shown in Table 1 with illustrative examples from drugs in use. Several new agents have been studied in Phase 2a before being paired with partner drugs for evaluation as a combination product (Table 2). The positive experience of using ACTs has led to a preference for at least one partner in a new combination to reduce the parasite biomass rapidly. Encouragingly, this is the case with the ozonides (e.g. OZ439), imidazolopiperazines (KAF 156) and the spiroindolone cipargamin (KAE 609), all potent inhibitors of parasite multiplication (Fig. 2).

Antimalarials with a synthetic peroxide scaffold, e.g. arterolane, artefenomel, were prioritised for development initially because the advantageous properties of the artemisinin derivatives were replicated (rapid onset, strong parasitocidal effect), but with modifications to remove some of the disadvantages (reliance on agricultural supply, short duration of action, reproductive toxicity in animals) [43]. The establishment of artemisinin resistance raises the concern of cross-resistance due to chemical similarities between the two groups of compounds. By contrast, cipargamin and KAF 156 are structurally unrelated to the artemisinin derivatives.

*Artefenomel-ferroquine* is a combination of a fast- and long-acting synthetic ozonide, artefenomel (previously OZ439), which has an elimination half-life of 46–62 h [44, 45], and ferroquine (FQ), a 4-aminoquinoline. The goal is to create a single-dose curative treatment, and a multicentre dose-finding Phase 2b study (NCT02497612) is underway. An advantage of this product is that neither of the constituent drugs has been deployed as monotherapy previously. FQ retains activity against chloroquine- and piperaquine-resistant parasites in vitro and has a long elimination half-life of 16 days. It is only moderately efficacious as monotherapy but when combined with artesunate (daily dose of 4 or 6 mg/kg FQ plus artesunate 4 mg/kg for 3 days) the polymerase chain reaction (PCR)-corrected efficacy at 28 days for the treatment of uncomplicated falciparum malaria was 99% (95% CI 93–100) [46]. The potential for cross resistance of artemisinin-resistant parasites to artefenomel has been investigated. Evidence from in vitro ring-stage survival assays showed cross resistance only for parasites carrying the *kelch13* I543T mutation, and not the more well-known C580Y mutation [47]. In a clinical study close to the Thailand-Myanmar border in 2015, median parasite clearance following treatment of 19 patients with *kelch13* mutant

**Table 1** Criteria used to assess antimalarials

Individual antimalarial drugs	Examples
Anti-parasitic activity	
Stage-specificity, e.g. blood schizonticide (treatment), gametocytocide or sporontocide (transmission-blocking), hypnozoiticide (relapse prevention), hepatic schizonticide (causal prophylaxis)	The artemisinin derivatives have the broadest stage-specificity of action of all registered antimalarials (trophozoites, including young rings, gametocytes, with the exception of Stage V) Only the 8-aminoquinolines (primaquine, tafenoquine) are active against hypnozoites for relapse prevention in vivax or ovale malaria
Pharmacokinetics	
Speed of action (including dependence on co-factors, e.g. for absorption)	Lumefantrine AUC is the principal determinant of cure following artemether–lumefantrine treatment (absorption is enhanced by coadministration with fat)
Speed of elimination	Slowly eliminated drugs (e.g. piperaquine) have the added advantage of a longer post-treatment prophylactic effect and hence fewer episodes of malaria in high-transmission areas [115]
Pharmacodynamics	
Parasitocidal effect: relates to asexual stage-specific activity	The most potent antimalarials have the greatest inhibitory effect on parasite multiplication. The average parasite biomass in an adult with uncomplicated falciparum malaria is $> 10^{12}$ . Artesunate reduces the biomass by $\sim 10^4$ per asexual life-cycle (48 h) in sensitive infections. Thus, if used alone $\geq 6$ -day treatment (3 cycles) must be given to have the best chance of cure [5]
Safety/toxicity	
Pregnant women and children	ACTs were contraindicated in the first trimester of pregnancy due to concerns of embryotoxicity in animals and a lack of safety data in humans until recently
Repeated dosing	Patients in high transmission areas may have multiple episodes of malaria per year. Repeated dosing of artesunate-pyronaridine was not recommended initially due to safety concerns (signal of hepatotoxicity). This caution has since been lifted
Propensity for resistance to develop	Atovaquone–proguanil is extremely vulnerable to resistance development due to rapid selection of cytochrome b mutations
Cost	Artemether–lumefantrine costs US\$0.38–1.3 per treatment [116]
<b>Antimalarial combinations</b>	
<b>Formulation</b>	
Co-formulations, tablet burden	All leading ACTs now exist as fixed-dose combinations with the exception of AS-SP
Paediatric formulations	Most leading ACTs have paediatric dosage forms
<b>Posology</b>	
Dose number and frequency	Most antimalarials are given as 3-day treatments. Artemether lumefantrine has the highest dose frequency (6 doses)
<b>Matched pharmacokinetics</b>	
Elimination half-lives	For the ACTs in use there is a mismatch of the elimination kinetics of artemisinin derivatives and partner drugs, which leaves the slowly eliminated drug unprotected
<b>Drug–drug interactions</b>	
	Triple ACTs under evaluation exploit inverse resistance selection properties of different partner drug pairings, e.g. amodiaquine and lumefantrine Global recommendations to add a single low dose of primaquine to ACTs in areas targeting elimination necessitate study of potential drug–drug interactions with any new treatment

parasites (including C580Y) compared to wild type was 5.5 vs 4.4 h;  $p = 0.34$  [48]. Artefenomel was originally paired with piperaquine, and a Phase 2 study found parasite clearance times were longer in patients with parasites

carrying mutations in *kelch13* [49]. More clinical data are needed to resolve this question; however, this is challenging to provide since artemisinin-resistant malaria in Southeast Asia is now typically concentrated in remote

**Table 2** New agents in clinical development or under review by stringent regulatory authorities

Name and development partners	Type/target	Activity spectrum	First in human	In vivo or in vitro efficacy data	Safety	Vision for development
<i>Under review by stringent regulatory authorities</i>						
Tafenoquine GSK, MMV, US Army	3-phenoxy-substituted 8-aminoquinoline, i.e. synthetic analogue of primaquine	PV blood and tissue schizonticide, gametocytocidal	FIH study in HAVs reported in 1998 defined long half-life (14 days) and good tolerability [117]	Relapse-free efficacy at 6 months of chloroquine (CQ) + 300 mg tafenoquine (89.2% (77–95%) compared to 37.5% (23–52) for CQ alone [118]	Acute haemolytic anaemia in G6PD deficiency, elevated creatinine, methaemoglobinemia	Single-dose relapse prevention for PV (given in combination with blood schizonticide)
<i>Phase 2</i>						
Artefenomel (formerly OZ439)	Synthetic endoperoxide; several theories on mechanism of action [119]	Blood schizonticide for falciparum and vivax	Study of artefenomel in HAVs demonstrated a terminal half-life of 2.5–3.0 h; exposure increased by co-administration with food [120]. Evaluation in human induced blood-stage malaria model predicted an MIC of 4.1 ng/mL [121]	Artefenomel-piperazine, $PC_{1/2}$ (min-max) in Vietnam was 6.1 (1.1–12.7) vs 3.5 (1.2–7.7) h in Africa and D-28 ACPR were 66.2% (95% CI 54.6–76.6) vs 74.5% (95% CI 7.81–79.7) [49]	Dose independent QTc prolongation, mild transaminitis [45, 122, 123]	Single dose combination treatment [49] (NCT02497612)
MMV (+ ferroquine)			FQ FIH data in HAVs were not published in a primary report [122]			
KAF156	Imidazolopiperazine; unknown mechanism of action	Multi-stage (liver, blood and transmission-blocking) [124, 125]	KAF 156 FIH study in male HAVs published in 2014 with PK [126]	Median (IQR) $PC_{1/2}$ 3.5 h (3.4–3.8) multiple-dose and 3.4 (3.2–4.1) single dose. Single-dose D-28 ACPR 67% [50]	Post-treatment mild transaminitis. Asymptomatic bradycardia [50]	Single dose multi-stage treatment (with lumefantrine)
Cipargamin (formerly KAE609)	Spiroindolone PfATP4 inhibitor [64, 127]	Blood schizonticide for falciparum and vivax	Single- and multiple-dose cohorts studied. Dose-related gastrointestinal and genitourinary adverse events reported [128]	Median (IQR) $PC_{1/2}$ 0.9 h (0.78–1.07) in PF [63]. D28 ACPR 50% at 30 mg, 60% at 20 mg, 29% at 10 mg, 14% at 15 mg stat dosing [129]. In vivo MIC estimated as 0.126 ng/mL [37]	Hepatotoxicity concerns-dose-escalation safety study ongoing (NCT03334747)	To combine with longer-acting partner drug not prone to PfATP4 mutation
MMV, Novartis						
Fosmidomycin (+ piperazine)	Inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase in isoprenoid biosynthesis pathway	Uncomplicated PF blood schizonticide	PK study of parenteral and oral fosmidomycin reported in 1982 [62]	PCT 49 h in small study of 5 days monotherapy and D14 cure rate of 89% [130]. With piperazine D28 efficacy 100% [60]	Mild post-treatment transaminitis [130]	Combination therapy in areas with no piperazine resistance
JOMAA						

Table 2 continued

Name and development partners	Type/target	Activity spectrum	First in human	In vivo or in vitro efficacy data	Safety	Vision for development
DSM265 Takeda, MMV, UT Southwestern	Dihydroorotate dehydrogenase inhibitor	Uncomplicated PF schizonticide, liver stage activity; no gametocytocidal activity; no data in PV	Favourable early safety, tolerability, pharmacokinetic, and activity data published in HAVs and human challenge study in 2017 [66]	Parasite reduction ratio at 48 h in the human challenge study measured 1.55 (95% CI 1.42–1.67), which was lower than that following a 10 mg/kg dose of mefloquine (2.34)	Mild, e.g. headache [66]	Prevention (travellers' market) and partner drug in combination therapy
AQ-13 Immtech and Tulane University	Modified 4-aminoquinoline	Blood schizonticide for falciparum and vivax	HAV study published in 2007 showed a similar tolerability and PK profile to chloroquine [70]	Mean PCT 47.3 h (43.5–51.1) vs 32.5 (28.0–37.0) for artemether–lumefantrine (AL); by day 42, 0/28 vs 2/33 recrudescences in AL arm [69]	No grade 2–4 adverse events [69]	Partner drug in combination therapy
Methylene Blue University of Heidelberg	Phenothiazine derivative. Prevents haem polymerisation by inhibiting <i>P. falciparum</i> glutathione reductase	PF schizonticide and potent gametocytocidal agent (male and female Stage V gametocytes)	First description of methylene blue to treat malaria was in 1891 [131]	D28 ACPR 72–84% in semi-immune adults [132]; 95% in combination with amodiaquine [133]	Increased vomiting when combined with artesunate–amodiaquine [82]	Part of a triple combination with ACTs + transmission blocking
Sevuparin (DF02) Dilaforette-Karolinska Institute	Anti-adhesive polysaccharide derived from heparin with eliminated antithrombin binding site [87]	Blocks merozoite invasion and sequestration [87]	Well tolerated in HAVs [87]	$\geq 100$ µg/mL inhibits cytoadherence [86]. No significant difference in parasite clearance between sevuparin–atovaquone–proguanil (AP) vs AP alone [87]	No adverse event leading to study withdrawal. No bleeding AEs [87]	Adjunctive treatment for severe malaria
MMV 390048 MMV and University of Cape Town	Aminopyridine PPI (4) K inhibitor [71]	Blood schizonticide inhibits gametocytogenesis and oocyst formation (preclinical) [71]	FIH (NCT02230579; NCT02554799), PK (NCT02783820) and human challenge studies (NCT02281344) are complete (data not published)	Phase 2a study (NCT02880241) recruitment suspended for interim data analysis	Not published	Part of single-dose treatment, transmission-blocking, chemoprevention [71, 134]
<i>Phase 1</i>						
P218 MMV, Janssen (Biotec Thailand)	PF-dihydrofolate reductase (DHFR) inhibitor	Blood schizonticide but inactive across all gametocyte stages [135]	Safety, tolerability and PK study in HAVs completed; not published (NCT02885506)	In vitro IC <sub>50</sub> (SD) for quadruple DHFR mutant is 56 nM (20) compared to > 100,000 for pyrimethamine [136]	–	Chemoprevention
CDRI 97/78 Ipeca	Trioxane	Blood schizonticide	Well-tolerated in HAVs with half-life of 11.85 ± 1.94 h [137]	–	AEs were few and not severe [137]	Part of a combination therapy

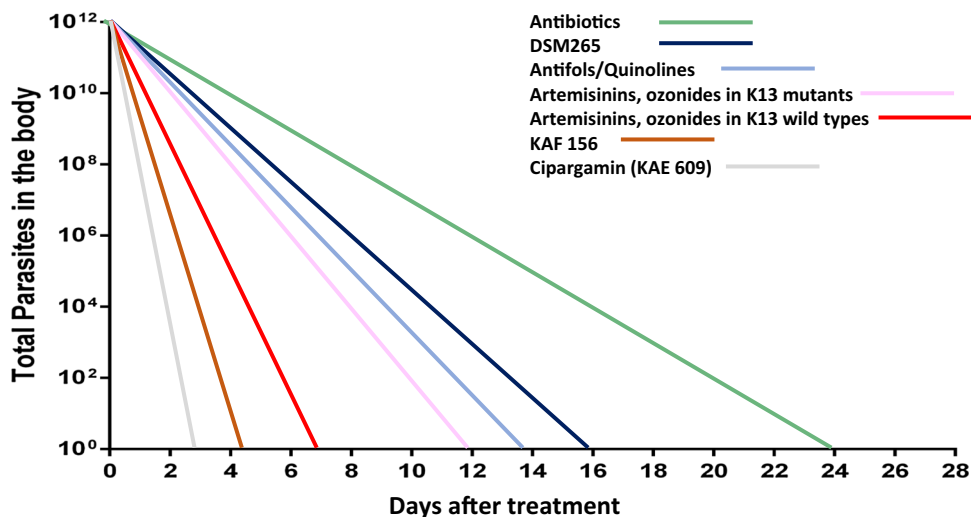
Table 2 continued

Name and development partners	Type/target	Activity spectrum	First in human	In vivo or in vitro efficacy data	Safety	Vision for development
M5717 (formerly DDD107498)	Quinoline-4-carboxamide inhibits <i>P. falciparum</i> translation elongation factor-2 required for protein synthesis [72]	Multi-stage (liver, blood and transmission blocking)	FIH study of single ascending dose, multiple ascending dose and malaria challenge model in healthy subjects is recruiting (NCT03261401)	More potent than artesunate in ex vivo study: median EC50 0.81 nM (range 0.29–3.29 nM) [72]	–	Single-dose treatment costing US\$1
MMV, Merck, University of Dundee						
SJ733	Dihydroisoquinoline inhibiting PfATP4	Blood schizonticide for falciparum and vivax, inhibits gametocytogenesis [138, 139]	Dose-escalation study in HAVs (NCT02661373) is due to complete recruitment in July 2018. A human challenge (early induced blood stage malaria infection) study is complete (NCT02867059)	EC <sub>50</sub> range 10–60 nM. Effective dose and exposure (AUC <sub>ED90</sub> ) superior to artesunate in animal models [139]	No toxicity in mouse model with a dose ~ 43-fold higher than effective dose [139]	Part of single dose treatment
MMV, University of Kentucky, Eisai						
ACT-451840	Phenylalanine-based compound; unknown mechanism of action [140]	Blood schizonticide	Single ascending-dose study in HAVs. Exposure increased with food. Half-life ~ 34 h [141]	Minimal parasitocidal concentration was 1.5 ng/mL [141]	No adverse event leading to withdrawal [141]	Combination therapy, e.g. with lumefantrine or mefloquine [140]
Actelion Pharmaceuticals [140]						

ACPR adequate clinical and parasitological response, AE adverse event, ALT alanine aminotransferase, FIH first in human, HAV healthy adult volunteer, MMV Medicines for Malaria Venture, PCT parasite clearance time, PF *Plasmodium falciparum*, PC<sub>1/2</sub> parasite clearance half-life, PK pharmacokinetic, PV *Plasmodium vivax*



**Fig. 2** Graph representing comparative parasite clearance rates after treatment with different antimalarial classes. Adapted from White NJ [150] with permission



areas making conducting Phase 3 trials of new drugs in these populations less feasible than previously.

*Lumefantrine-KAF156* combines an aryl-amino alcohol, already in widespread use in the artemether–lumefantrine (LUM) combination, with the highly potent imidazolo-piperazine KAF156, which has multistage activity and an elimination half-life of  $48.7 \pm 7.9$  h [50]. Regimens comprising different single daily doses given for one, two or three days are being evaluated in a Phase 2b study in West Africa (NCT03167242). A new formulation of lumefantrine, LUM-SDF (Lumefantrine Solid Dispersion Formulation) that can be given once daily is being studied in this trial [51]. This has an advance over the artemether–lumefantrine co-formulation in widespread use, which is administered twice daily, since lumefantrine absorption has been shown to be fat-dependent and dose-limited [52]. The prospects for LUM-KAF156 in areas where artemether–lumefantrine has been used for a long time, or in areas of multidrug resistance are unclear [53, 54]. Piperaquine was assessed as a potential partner for KAF156 initially, and a drug–drug interaction study in healthy volunteers was performed with reassuring findings; however, development of this combination is not being pursued currently [55].

*Fosmidomycin–piperaquine* Fosmidomycin was developed as an antibacterial drug in the 1980s. This inhibitor of isoprenoid biosynthesis has a good safety profile but has shown variable efficacy both as monotherapy or when partnered with clindamycin [56–58]. Most recently, it has been partnered with piperaquine, a bisquinoline developed in the 1960s, which already forms part of a leading ACT in combination with dihydroartemisinin [59]. A small Phase 2b trial in Ghanaian patients of all ages showed high efficacy of fosmidomycin–piperaquine [60]. Of note, the dose of piperaquine is 16 mg/kg bodyweight, which has been

demonstrated to be sub-optimal in the standard DHA–piperaquine ACT in children less than 5 years of age [61]. This is likely to be of relevance for the combination with fosmidomycin, a less potent drug than DHA with an elimination half-life of around 2 h [62]. Unfortunately, piperaquine resistance has now been reported from Cambodia, Thailand and Vietnam, effectively ruling this combination out as a successor for the failing ACTs in Southeast Asia [25].

A feature of the combinations in Phase 2 is that they all contain one partner which may have compromised efficacy in areas of multidrug resistance. This is a potential setback for the new treatments and may stimulate new combinations of the more potent drug classes with partners not in widespread use such as pyronaridine and methylene blue.

*Cipargamin*, a spiroindolone and potent, long-acting (elimination half-life  $18.5 \pm 4.7$  h [63, 64]) blood schizonticide is still under evaluation as a single agent. A dose-escalation safety study is recruiting in Mali (NCT03334747) with a special focus on hepatotoxicity after signals in earlier studies [63]. If cipargamin is shown to be well-tolerated, it has potential to become part of a single-dose treatment. A Phase 1 drug–drug interaction study of cipargamin and piperaquine showed no increase in exposure to either drug and no safety concerns [65].

*DSM-265* is a long-acting (elimination half-life 86–118 h in experimental malaria infection) dihydroorotate dehydrogenase inhibitor with blood and liver stage activity in Phase 2 development for treatment and prevention. It is a less potent inhibitor of parasite multiplication than some of the newer classes, thus is likely to be partnered with a more rapidly effective agent [66, 67]. In vitro studies suggest a relatively low barrier to resistance selection, so measures to protect this drug, such as

matching with a partner with similar elimination kinetics and only deploying as part of a fixed-dose combination will be important [68].

*AQ-13* is a substituted 4-aminoquinoline, which has already been in clinical development for more than 10 years. A small proof-of-concept study reported in 2017 showed it to be non-inferior to artemether–lumefantrine in symptomatic adult males in Mali [69]. In terms of activity and potency it is likely to be similar to ferroquine. The mean (95% CI) elimination half-life was reported as 3.9 (2.4–5.3) days in malaria patients in the Mali study [69] compared to an earlier finding of 14.3 days [6.2–39.3] in healthy adult volunteers [70].

*MMV 390048* is an aminopyridine *Plasmodium* phosphatidylinositol 4-kinase inhibitor currently in Phase 2a (NCT02880241). This is a multistage antimalarial with potential for chemoprevention [71]. Another treatment prospect that has moved into clinical development recently is *DDD 107498*, also known as *M5717*, an inhibitor of peptide elongation factor 2. This is a multiple-stage antimalarial with activity against blood stages, pre-erythrocytic stages, and male and female mature gametocytes [72, 73].

### 3.2 New Drugs in Clinical Development for Prevention

There is a paucity of new agents in development for chemoprevention alone. DSM-265 shows potential as a prophylactic agent for travellers, although doses studied so far in a human challenge model have not conferred seven days of protection from malaria infection, which would be required for weekly dosing [74].

Pregnant women and infants are typically excluded from studies of new antimalarials, despite bearing the brunt of most malaria-associated morbidity and mortality. As a result, they are the last to receive them since it takes time for sufficient efficacy and safety data to accumulate post-registration. Ideally, drugs used for prevention should be different to those deployed for treatment, but currently this is not the case and adequate data on treatment of malaria in pregnancy with a new agent is required before it is considered acceptable to assess use for prevention. SP continued to be used for prevention after it had been abandoned as a treatment in some countries since it retains some protective effect in the absence of high-grade resistance. Antimalarial underdosing in pregnant women and young children has been recognised belatedly for several drugs, e.g. DHA–piperquine, SP, artemether–lumefantrine, and this is a risk for the new agents, none of which have yet been evaluated in pregnancy [75–77].

### 3.3 New Drugs in Clinical Development for Relapse Prevention (*Plasmodium vivax* and *Plasmodium ovale*)

Like primaquine, tafenoquine is an 8-aminoquinoline active against asexual stages of *P. vivax*, but more importantly against hypnozoites which cause relapse. This long-acting drug (elimination half-life 14–19 days) is being developed for single-dose relapse prevention for vivax malaria and a new drug application was submitted to US FDA and the Australian Therapeutic Goods Administration in late 2017 [78]. The development process has been slow and painstaking, necessitated by the known potential for haematological toxicity (acute haemolytic anaemia) in persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked enzymopathy found in malaria-endemic areas. No clinically significant PK-PD interactions or safety concerns were observed when chloroquine and tafenoquine were co-administered in healthy adult volunteers [79]. Tafenoquine has the potential to revolutionise radical therapy of the relapsing malarial by enhancing patient adherence compared to the standard 14 days of primaquine and may be a potent tool to accelerate vivax malaria elimination; however, it will need to be deployed with concurrent reliable point-of-care G6PD activity testing to screen out individuals at risk. Differences in metabolism of primaquine with a resulting impact on efficacy for relapse prevention have been observed in association with allelic variation in the cytochrome 2D6 gene [80]. Preliminary investigation has indicated the same may not be true for tafenoquine, but more evidence is needed [81].

### 3.4 New Drugs in Clinical Development for Transmission-Blocking

Reducing malaria transmission substantially will accelerate malaria elimination. Drugs preventing malaria transmission target gametocytes, sporogony or the mosquito vector by reducing its survival (endectocides). Single low-dose (0.25 mg/kg) primaquine is now recommended alongside antimalarial treatment in endemic countries targeting elimination for its activity against mature gametocytes; thus, the potential for drug-drug interactions with new blood schizonticides needs to be evaluated. Very few new agents are in development specifically for transmission-blocking, although some have multistage activity, e.g. KAF 156. *Methylene blue* is an old parasitocidal agent with blood stage activity and the added benefit of activity against mature male and female *P. falciparum* gametocytes. It is being developed by the University of Heidelberg in combination with ACTs as a strategy to protect against artemisinin resistance emergence and reduce transmission [82].

### 3.5 Re-purposing of Antimicrobial Drugs for Malaria Prevention or Treatment

Several antibiotics have weak-to-moderate antimalarial activity, e.g. doxycycline, clindamycin, azithromycin, and cotrimoxazole. Cotrimoxazole is given to patients with HIV to protect against *Pneumocystis jirovecii* pneumonia and has been shown to reduce malaria infections, and is a possible candidate for use as prevention in HIV-uninfected pregnant women or children [83]. Azithromycin is amenable to once-daily dosing and has a good safety profile; therefore it could theoretically be combined as part of a short-course once-daily combination therapy or be used for prevention. Drawbacks include selection for resistance in other common important pathogens, e.g. *Salmonella enterica* serovar Typhi, *Streptococcus pneumoniae*. In the 1970s, the discovery of ivermectin, a common anti-parasitic agent used to treat animals, led to the Nobel Prize in Medicine being awarded to William C. Campbell and Satoshi Ōmura in 2015. The prize was shared with Tu Youyou in recognition of the discovery of qinghaosu (artemisinin) to treat malaria. Ivermectin has also been shown to reduce malaria transmission by reducing mosquito survival [84]. It has other uses for human disease, e.g. mass treatment for lymphatic filariasis, thus has the potential to be used as a tool in integrated disease-control programmes [85].

### 3.6 New Adjunctive Therapies in Development for the Treatment of Severe Malaria

The search for adjunctive therapies capable of improving the outcome from severe malaria continues after serial alternatives have failed to show any benefit, or in some cases, caused harm [16]. Sevuparin is a polysaccharide heparin analogue, which retains the anti-adhesive effects of heparin without the antithrombin properties and has been shown to block merozoite invasion, cytoadherence and rosetting [86, 87]. Studies in patients with severe malaria are planned.

## 4 Drugs in Pre-clinical Development

An up-to-date analysis has estimated that molecules in pre-clinical development have an 8% chance of becoming a registered product [30]. Agents in active development are shown in Table 3. *MMV 253* (previously AZ13721412) is a very long-acting *Plasmodium* ATPase inhibitor in early preclinical development, with the goal of being part of a single-dose radical cure [88, 89]. *JPC-3210* is another promising long-acting drug shown to be active against multidrug resistant *P. falciparum* in vitro [90, 91].

*SC83288* is the only drug in preclinical development for severe malaria. This amicarbalide derivative is fast acting and adapted for parenteral administration. Preliminary findings in a murine model have demonstrated a three-log parasite reduction per asexual life cycle, which is approximately 10-fold lower than that observed with parenteral artesunate treatment in humans [92].

In late preclinical development for relapse prevention is another 8-aminoquinoline, *NPC1161B*, developed by the University of Mississippi [93]. The development plan is to see whether this single enantiomer drug has a more favourable haematological toxicity profile than tafenoquine in Phase 1.

## 5 Antimalarial Drug Discovery

Drug discovery for malaria is in a healthy state, with numerous compounds under investigation following successful screening programmes. As evidenced by the development of the artemisinin derivatives, it is possible to register highly successful antimalarials without fully understanding the drug target or mechanism of action. Phytochemical screening is still being pursued to identify new antimalarials. From the target-based approaches, a number of *Plasmodium* proteases have been singled out; these perform critical functions in the parasite life cycle [94]. There are different families such as the falcipain cysteine proteases, aminopeptidases, and plasmepsins (aspartic proteases). Plasmepsin V inhibitors have been proposed as powerful new agents, and recent elucidation of the function of plasmepsins IX and X has revealed them to be two new promising additional targets for potential multi-stage antimalarials [95–97]. Another target that has garnered a lot of interest is  $\text{Na}^+$ -ATPase 4, the site of action of the potent cipargamin and SJ557733, both in clinical development, and of 21A092, a pyrazoleamide in the discovery phase [98]. Pathways involved in haem detoxification are also under study [99]. Work continues on developing parasite-selective inhibitors of phenylalanyl-tRNA synthetase, histone deacetylase, hypoxanthine-guanine-(xanthine) phosphoribosyltransferase and N-myristoyltransferase [100–103]. Another avenue of research and development is the creation of hybrid molecules, i.e. combining two synergistic agents with different targets as a way of increasing the barrier to resistance [104, 105].

As a general rule, available libraries are biased in favour of compounds with blood-stage activity but there has been progress in the discovery of agents capable of preventing transmission through dedicated screening programmes such as that of the GSK Tres Cantos Antimalarial Set (TCAMS), which identified 98 out of 13,533 molecules with activity against trophozoites and stage V gametocytes

**Table 3** Agents in preclinical development

Name/ development partners	Type/target	Activity spectrum	Other characteristics	Vision for development
MMV 253 (previously AZ13721412)/MMV and Zydus Cadila [142]	Triaminopyrimidine, <i>Plasmodium</i> ATPase inhibitor	Blood schizonticide	Long acting	Part of a single-dose radical cure
AN 13762/MMV, University of California, San Francisco [143]	Benzoxaborole, mechanism of action unknown	Blood schizonticide	Reassuring pre-clinical toxicology	Part of a single-dose radical cure, prevention
JPC-3210/MMV and Zydus Cadila [90]	Aminomethylphenol	Blood schizonticide	Long acting	Treatment and prevention
UCT 943 (previously MMV 642943)/MMV, H3D, University of Cape Town	PfPI(4)K inhibitor [144]	Multi-stage, falciparum and vivax	–	Back-up for MMV048 [71] (part of a single-dose radical cure, transmission blocking, prevention)
NPC1161B/University of Mississippi [93, 145]	8-Aminoquinoline (single enantiomer)	Multi-stage	Superior radical curative activity to primaquine in animal models [146]	Vivax malaria relapse prevention
SC83288/University of Heidelberg [92]	Amicarbalide derivative	Blood schizonticide	Rapid action (log parasite reduction ratio ~ 3 in a mouse model)	Severe malaria treatment
SAR121 (previously MMV688533)/Sanofi, MMV	Unknown mechanism of action	Blood schizonticide	Long acting	Part of a single-dose radical cure
DM1157(PL 69)/DesignMedix	“Reversed chloroquine”, similar mechanism of action	Blood schizonticide	No published data (similar to chloroquine)	Part of a single-dose radical cure [147]

and prioritized 56 compounds [106]. Torin 2, an agent with multistage activity, including gametocytes, identified in a different screening programme, has been selected for lead optimisation by the National Center for Advancing Translational Sciences (NCATS) programme of development of malaria transmission-blocking drugs [107, 108]. Detailed studies of mature *P. falciparum* gametocytes in human infections have discovered that females tend to outnumber males by a ratio of ~four to one and that they differ in their susceptibility to antimalarial drugs, with males tending to be more sensitive [109, 110]. This knowledge may be exploited in the development of new gametocytocidal drugs but requires new methods of assessment to distinguish males from females [111, 112]. To date, the assessment of transmission blocking in clinical trials of new drugs has relied on gametocyte quantitation in peripheral blood by microscopic examination, which is inadequate.

Discovery of new drugs for relapse prevention is hampered by the challenge of finding suitable pre-clinical models to screen for activity against hypnozoites but there has been progress, for example, with the development of an in vitro liver stage drug assay [113, 114]. A novel class of non-8-aminoquinoline compounds, the PI4K inhibitors e.g.

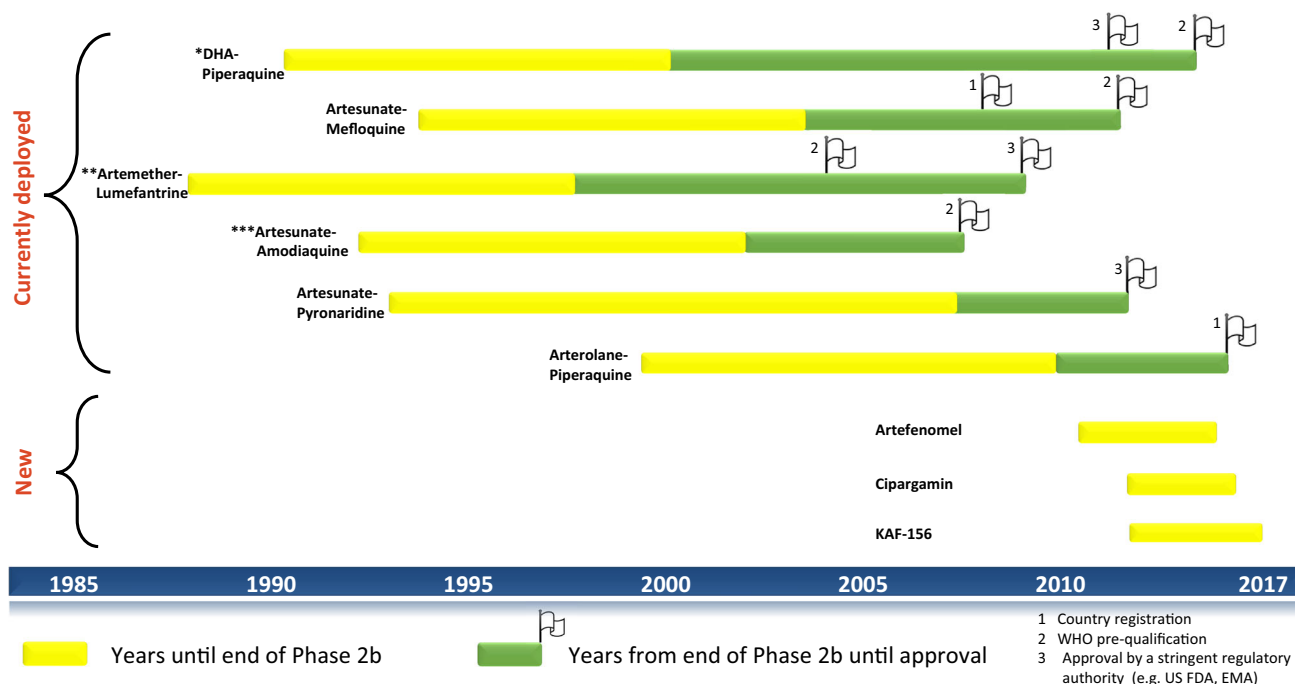
*KAI407* and *KDU691*, has been shown to kill early *P. cynomolgi* dormant liver stages in vitro [113, 114].

## 6 Conclusion

The malaria drug development pipeline, at present, is in a healthier state than for many years; however, there are no novel compounds ready to be deployed in areas where existing treatments are failing (Fig. 3). Of the newer agents, some classes possess multistage activity and are highly potent in terms of their ability to inhibit parasite multiplication (Fig. 1). While there has been encouraging progress towards malaria elimination in some countries, e.g. Sri Lanka was certified as malaria free in 2016, this is not universal, and recent reports suggest progress has stalled [1]. This makes it highly likely that there will be a continued need for new drugs for many years to come.

Looking to the future, a variety of target product profiles tailored to control and elimination goals in different populations have been defined, but the highest priority is still to ensure a continued supply of effective blood schizonticides and to maximise their useful therapeutic life. Single exposure treatments are desirable but not essential.

### Developmental timeline of currently deployed ACTs and leading new compounds



**Fig. 3** Developmental time-line of currently deployed ACTs versus leading compounds. Information sourced from [11, 31, 148, 149]. Single asterisk: Dihydroartemisinin–piperaquine (WHO pre-qualification refers to Eurartesim<sup>®</sup> by sigma tau in 2015); approved by EMA in 2011. Double asterisk: Artemether–lumefantrine (WHO pre-

qualification for Coartem<sup>®</sup> by Novartis in 2004, products by Ipca and Cipla in 2009; others have followed since then). Triple asterisk: Artesunate–amodiaquine (WHO pre-qualification for Sanofi’s ASAQ Winthrop<sup>®</sup> in 2008, Ipca and Guilin products in 2012, Ajanta in 2013 and Cipla in 2014)

Continued investment in drug discovery will help to sustain the current pipeline.

Prevention for groups at highest risk is likely to rely on the same classes of antimalarials as used for treatment, given the difficulties of developing drugs for use in pregnant women and children. There are calls to accelerate reproductive toxicology studies by bringing them further forward in the development process [31].

For relapse prevention there is good news, with tafenoquine closer to registration. However, the lack of options safe for use in populations where G6PD deficiency is prevalent is an impediment to the elimination of vivax malaria [34]. Recent progress in the development of methods to screen for hypnozoitocidal and transmission-blocking compounds may lead to more agents being identified in the coming years.

The failure of the ACTs in Southeast Asia has led to a re-evaluation of combination therapy for malaria. Rather than only using triple combinations of existing drugs as a salvage strategy to try to improve cure rates in areas where resistance has already taken hold, there is an argument for introducing them in countries with no evidence of resistance, sooner rather than later, as well as ensuring all new combinations developed are composed of three or more drugs. The drawbacks of this

approach include increased development time, increased costs and increased risks of toxicity; thus, any ambition to produce a novel triple combination that is efficacious against all strains of malaria in time to replace currently failing ACTs may have to be sacrificed in the interests of expediency. However, this could be the goal for future generations of novel antimalarial combination therapies.

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#### Compliance with Ethical Standards

**Conflict of interest** APP was an investigator on trials of artefenomel sponsored by Medicines for Malaria Venture and KAF156 and cipargamin sponsored by Novartis. EAA was a co-investigator on studies of DHA–piperaquine funded by Medicines for Malaria Venture and of artesunate–mefloquine funded by Drugs for Neglected Disease Initiative.

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