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The early preclinical and clinical development of cipargamin (KAE609), a novel antimalarial compound

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ABSTRACT

Background: Cipargamin (KAE609) is a novel spiroindolone class drug for the treatment of malaria, currently undergoing phase 2 clinical development. This review provides an overview and interpretation of the pre-clinical and clinical data of this possible next-generation antimalarial drug published to date.
Methods: We systematically searched the literature for studies on the preclinical and clinical development of cipargamin. PubMed and Google Scholar databases were searched using the terms 'cipargamin', 'KAE609' or 'NITD609' in the English language; one additional article was identified during revision. Nineteen of these in total 43 papers identified reported original studies; 13 of those articles were on pre-clinical studies and 6 reported clinical trials.
Results: A total of 20 studies addressing its preclinical and clinical development have been published on this compound at the time of writing. Cipargamin acts on the PfATP4, which is a P-type Na + ATPase disrupting the Na + homeostasis in the parasite. Cipargamin is a very fast-acting antimalarial, it is active against all intra-erythrocytic stages of the malaria parasite and exerts gametocytocidal activity, with transmission-blocking potential. It is currently undergoing phase 2 clinical trial to assess safety and efficacy, with a special focus on hepatic safety.
Conclusion: In the search for novel antimalarial drugs, cipargamin exhibits promising properties, exerting activity against multiple intra-erythrocytic stages of plasmodia, including gametocytes. It exhibits a favourable pharmacokinetic profile, possibly allowing for single-dose treatment with a suitable combination partner. According to the clinical results of the first studies in Asian malaria patients, a possible safety concern is hepatotoxicity.

1. Introduction

Despite global efforts, malaria continues to be an important global health problem, responsible for an estimated 228 million cases in 2018, and an estimated number of deaths of 405.000. Although numbers have decreased since 2010, this trend has stagnated over the past years [1]. Most cases (more than 90%) occurred in sub-Saharan Africa, followed by Southeast Asia. Children younger than five years and pregnant women are the most vulnerable group affected by malaria, with anaemia as the main cause for poor outcomes. Uncomplicated malaria can develop into severe malaria and death due to a number of influencing factors like age, immunity, treatment availability, *Plasmodium* species and *Plasmodium* isolate-specific genetic diversity [2]. Overall, *Plasmodium falciparum* continues to be the most prevalent species responsible for human malaria cases globally, associated with severe outcomes [1].

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The current first-line treatment regimens in use for uncomplicated (falciparum) malaria (but being effective against blood stages of all *Plasmodium* spp. afflicting man [3]) are all artemisinin-based combination therapies (ACTs), which currently retain an overall efficacy of 98% [1]. That notwithstanding; the control, elimination and eradication agenda promoted by the World Health Organization (WHO) expresses the need for new and better drugs that will not only treat acute malaria illness and prevent complications in vulnerable groups but also be used for elimination-specific indications [4].

The ultimate goal is to develop novel compounds that can be used for case management and chemoprotection, as defined in TPP-1 and TPP-2 (Target Product Profiles) [5]. TPP-1 relates to case management, with a candidate compound being expected to be suitable for treating acute complicated and uncomplicated malaria in children and in adults. It needs to be able to clear asexual blood-stage parasitaemia, block transmission and prevent the formation of hypnozoites in *P. vivax* infection. TPP-2 stands for chemoprotection, used e.g. for migrants to highly-endemic areas or during epidemics.

An ideal new antimalarial would exhibit cidal activity against sexual and asexual blood stages, and target all stages of the parasite lifecycle [4,5]. Favourably, it would also block transmission from human to mosquito, and that way decrease the number of new infections. It should be active against multi-drug resistant parasites and be safe in vulnerable populations, such as children, pregnant women, and immune-compromised patients. Finally, it is desirable that its pharmacokinetic properties might allow for single oral-dose administration. Nowadays, all dosing regimens are three-day courses, which are not ideal regarding patient compliance and the associated potential to foster development of resistance [6].

Amongst a range of novel antimalarial compounds under development in the quest for next-generation antimalarials [7], cipargamin (formerly known as KAE609 and previously referred to as NITD609) is a new antimalarial compound currently undergoing phase 2 clinical development for the treatment of uncomplicated malaria, with promising properties indicating action against all parasitic blood stages. Cipargamin (Fig. 1) belongs to the spiroindolones, emerging from a lead-optimisation program [8]. Spiroindolones are a distinct class of novel antimalarial compounds, acting through a mechanism of action not exploited before that disrupts intracellular Na^+ homeostasis in the parasite. The molecule contains an indolone ring, substituted with another ring in a spiro-configuration. The early preclinical development of cipargamin has been mentioned and briefly discussed in recent reviews of malaria drug development [9,10].

2. Areas covered

This review provides an overview on the pre-clinical and clinical data available on cipargamin at the time of writing, and assesses its potential to become registered as next-generation antimalarial. A total of 42 articles was identified through searches of the PubMed and Google Scholar databases (last update Cion 30-01-2020) using the search terms ‘cipargamin’, ‘KAE609′ or ‘NITD609’ in the English language. During revision, another yet unpublished article in press was identified and added. Eighteen of these in total 43 papers identified were original studies; 13 of those articles were on pre-clinical studies (Table 1) and 6 reported clinical trials (Table 2). Two additional documents published online by the manufacturer of the investigational drug pertaining to yet unpublished clinical trials were identified and the data provided included into this review. Fig. 2 provides a synopsis on the cipargamin past and anticipated future development path (see Ref. [11] for additional regularly updated information).

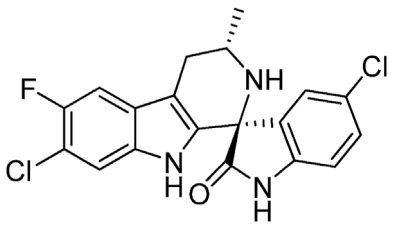

Fig. 1. Chemical structure of cipargamin. Source: Zou et al. [23]. Figure available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6268731/figure/molecules-17-10131-f001/(open access) (last accessed on 30 January 2020).

3. KAE609 – Preclinical development

3.1. Discovery and early plasmodicidal testing

The first reports on cipargamin were published in 2010 in two papers by Yeung et al. and Rottmann et al. describing the identification of cipargamin through screening and lead optimisation approaches [8,12]. Twelve-thousand natural compounds and synthetics with similar structures were screened for antimalarial activity, using *Plasmodium* whole-cell proliferation assays from cultured intra-erythrocytic parasites [8]. Antimalarial blood-stage activity was evaluated *in vitro* against a panel of culture-adapted *P. falciparum* strains. Cipargamin displayed low nanomolar 50% inhibitory concentration (IC_{50}) values (range 0.5–1.4 nM), with no evidence of diminished potency against drug-resistant strains [12].

3.2. Cytotoxicity and in vivo toxicity

Cytotoxic activity was measured *in vitro* with cell lines of neural, renal, hepatic or monocytic origins. No significant cytotoxity was observed when the cells were exposed to high concentrations of the drug. The *in vivo* safety profile was determined by exposing male rats during 2 weeks with daily doses corresponding to 10–20 times the concentration that causes 99% parasitaemia reduction. Under those conditions, no adverse events or histopathological findings were observed [12].

3.3. In vitro resistance

A multi-drug resistant strain of *P. falciparum* was exposed to increasing, sub-lethal concentrations of cipargamin for several months to evoke drug resistance. A low level of resistance was obtained, with IC_{50} values increasing 7-24-fold. None of the mutants showed cross-resistance to a panel of antimalarial agents that exhibited different modes of action [12]. The molecular basis of resistance was determined by a genome-wide hybridization analysis of the cipargamin-resistant clones. This analysis identified various mutations in the gene *pfatp4*, encoding a P-type Na^+ ATPase. The *pfatp4* gene was cloned and expressed in parasites. Cipargamin had less potency against these parasites, whereas IC_{50}s for artemisinin and mefloquine remained unchanged, confirming that resistance was specific to cipargamin [12]. *Saccharomyces cerevisiae* was exposed to cipargamin and developed mutations in a P-type ATPase (ScPMA1). These mutations caused resistance against cipargamin, but not against established antimalarials [13].

3.4. Antimalarial activity

Spillman and colleagues explored the mechanisms of Na + regulation in *P. falciparum* and found that intra-erythrocytic parasites actively extrude Na + against an inward gradient via PfATP4 [14]. Cipargamin perturbs ion homeostasis and increases host cell membrane rigidity. On *S. cerevisiae*, a more genetically tractable model, cipargamin has a similar function and inhibits ATPase (ScPma1p) activity [13]. In fact, another study on *Toxoplasma gondii* showed that by knocking down ATP4, the cytosolic Na^+ regulation was disrupted, causing growth defect, reduced viability and reduced virulence in mice [15]. Rosling proved that cipargamin targets the PfATP4 by

Table 1
Overview of preclinical studies.

Study	Methods	Assessments	Results/outcomes
Rottmann (2010) [12]	*In vitro* culture-adapted *P. falciparum* strains efficacy assay *Ex vivo* fresh isolates of *P. falciparum* and *P. vivax* efficacy assay *In vitro* drug sensitivity assays with synchronized parasites in different stages *In vitro* measuring of the concentration leading to 50% cell death (CC50) *In vivo* pharmacokinetic tests in mice and rats *In vivo* antimalarial activity in *P. berghei* rodent malaria model *In vitro* development of resistance following 3–4 months of constant drug pressure in multidrug-resistant parasite strains	Antimalarial activity Safety profile/cytotoxity Pharmacokinetics Drug resistance	Cipargamin is potent against intra-erythrocytic stages of *P. falciparum* and *P. vivax*, including drug resistant strains Cipargamin targets all asexual blood stages Cipargamin blocks protein synthesis in *P. falciparum* parasites within 1 h No significant cytotoxicity was observed for cipargamin *in vitro* cell lines Cipargamin has efficacious doses (ED50/90/99) of 1.2, 2.7, and 5.3 mg/kg, respectively Mutations in the P-type cation-transporter ATPase4 (PfATP4) confer low level drug-resistance to spiroindolones
Van Pelt-Koops (2012) [20]	*In vitro* activity on asexual parasites *In vitro* effect on early and late gametocyte development of laboratory adapted *P. falciparum* *In vivo* effect on development of oocyst in mosquitoes	Antimalarial activity	Cipargamin inhibits the early and late development of *P. falciparum* gametocytes Cipargamin reduces transmission to *Anopheles stephensi*
Spillman (2013) [14]	*In vitro* assay to investigate the mechanisms of Na + regulation in *P. falciparum*	Mechanism of action	The intraerythrocytic parasite actively extrudes Na^+ against an inward gradient via PfATP4 mutations in PfATP4 confer resistance to cipargamin
Upton (2015) [21]	*In vivo* evaluation of impact on malarial infections over an entire transmission cycle (mouse-mosquito-mouse)	Transmission reducing potential	An effect size of 100% was estimated when examining the ability of cipargamin to block malarial transmission
Lakshminarayana (2015) [29]	*In vivo* dose-response efficacy of Cipargamin in the *Plasmodium berghei* murine malaria model	Antimalarial activity Pharmacokinetics	Cipargamin exhibits time-dependent killing in the *P. berghei* malaria mouse model
Zhang (2015) [17]	*In vitro* examination of the effect of cipargamin on the rheological properties of infected RBCs	Mechanism of action	Ring-stage parasite–infected red blood cells exposed to cipargamin become spherical and rigid, causing splenic clearance
Huskey (2016) [30]	*In vivo* assessment of absorption, metabolisation and metabolites in uninfected rats and dogs	Pharmacokinetics	Cipargamin was well absorbed and extensively metabolised in rats and dogs Elimination of cipargamin and metabolites was primarily mediated via biliary pathways in faeces
Goldgof (2016) [13]	*In vitro* examination of the effect of cipargamin on ScPma1p ATPase activity in *S. cerevisiae*	Mechanism of action	Cipargamin inhibits *S. cerevisiae* growth Cipargamin resistance is conferred by mutations in ScPMA1, an ortholog of PfATP4
Chavchich (2016) [19]	*In vitro* evaluation of potential of cipargamin to induce dormancy in *P. falciparum*	Antimalarial activity	Cipargamin does not induce dormant *P. falciparum* ring stage parasites
Dechering (2017) [22]	Computational strategy to assess compound effects on the infection prevalence at naturally occurring infection intensities	Transmission reducing potential	Infection prevalence can be derived from modelling the infection intensity on basis of oocyst numbers Cipargamin exhibits potential for achieving blockage of transmission at curative doses
Dennis (2018) [18]	*In vitro* characterization of physical changes in trophozoite-stage parasites and trophozoite-infected erytrhocytes	Mechanism of action	PfATP4-associated compounds cause Na^+-dependent swelling of isolated trophozoites and swelling of infected erythrocytes
Rosling (2018) [16]	*In vitro* characterization of the PfATP4-associated ATPase activity in membranes of blood-stage *P. falciparum* parasites	Mechanism of action	Cipargamin inhibits the Na^+-dependent ATPase activity present in *P. falciparum* membranes Potency reduced in cipargamin-resistant PfATP4-mutant parasites
Lehane (2019) [15]	*In vitro* characterization of ATP4 in *Toxoplasma gondii*	Mechanism of action	ATP4 in *Toxoplasma gondii* is a plasma membrane Na^+ pump, important for Na^+ homeostasis

characterisation of the PfATP4-associated ATPase activity in membranes of blood-stage *P. falciparum* parasites [16]. Zhang et al. examined the effect on the morphological properties of infected red blood cells (RBCs) [17]. Ring stage parasites exposed to cipargamin become spherical and rigid; as well that they are more likely to result in their retention in the spleen. RBC swelling is dependent on the presence of Na^+ in the environment and causes an increase in the osmotic fragility in the infected RBCs [18]. In another study, the ability to induce dormancy at ring stage parasites was determined. It was concluded that cipargamin does not induce dormant ring stages and thereby does not foster recrudescence [19].

3.5. Transmission-reducing potential

The effect of cipargamin on gametocytes and the transmission-reducing potential was investigated *in vitro* and compared to other antimalarials by Van Pelt-Koops et al. [20]. Firstly, the *in vitro* activity on asexual *P. falciparum* parasites from the drug-sensitive NF54 and K1 strains was measured, which showed favourable results for cipargamin compared to lumefantrine, artemether and primaquine. Secondly, gametocytocidal activity was measured, with cipargamin being the most effective inhibitor of early and late gametocyte development. Thirdly, these four compounds were added to blood meals of blood-feeding *Anopheles* mosquitoes, where cipargamin and lumefantrine both independent yielded reduced oocyst counts [20]. Upton and colleagues evaluated the effect of different antimalarials over an entire transmission cycle [21]. The drug was administered to five mice, which were infected with *P. berghei* clone 507cl1. The mice were exposed to mosquito bites, and subsequently those mosquitoes fed on naïve mice. For cipargamin, a 100% effect size was identified when examining the ability to block transmission [21]. In addition, an experimental computational method was used to describe the drug effects of 15

Table 2
Overview of clinical trials.

Author	Population (years)	Healthy/infected	Location/ethnicity	Follow-up	Dosing	Assessments
White (2014) [33] Phase 2	21 adults	Uncomplicated *P. vivax* malaria (10) or *P. falciparum* malaria (11)	Thailand	30 days	30 mg per day for 3 days	Pharmacokinetics, safety and tolerability, antimalarial activity, efficacy against drug resistant parasites
Leong (2014) [31] Phase 1	95 male adults	Healthy	Australia, majority white (94.68%)	6–8 days post-dose	Multiple-dose cohorts (10–150 mg once daily for 3 days)	Pharmacokinetics, safety and tolerability
Stein (2015) [43] Phase 1	110 male adults (18–45)	Healthy	Mostly Caucasian (81.8%)	89 days	75 mg cipargamin plus 320 mg PPQ, 25 mg cipargamin plus 1,280 mg PPQ, 25 mg cipargamin alone, 320 mg PPQ alone, or 1,280 mg PPQ alone	Pharmacokinetics, safety and tolerability
Huskey (2016) [30] Phase 1	6 adults (35–55)	Healthy	White	10–14 days	Single oral dose of 300 mg	Pharmacokinetics, safety and tolerability
Hien (2015) [36] Phase 2a	25 adults (20–60)	*P. falciparum* malaria	Vietnam	42 days	Single oral dose of 10, 15, 20, 30 mg	Pharmacokinetics, safety and tolerability, antimalarial activity
Study CKAE609X2202 [37]	11 adults (20–60)	*P. falciparum*	Thailand, Vietnam	28 days	Single oral dose of 75 mg	Safety and tolerability, antimalarial activity
Study CKAE609A2109 [38]	8 adults (18–55)	Human challenge model -induced *P. falciparum* malaria in healthy adults	Australia	36 days	Single oral dose of 10 mg	Pharmacokinetics, safety and tolerability, antimalarial activity

Additional study in press: Chuglay et al. [41] report (in the context of a synopsis on liver enzyme elevations in participants of *P. falciparum human* challenge studies on a yet otherwise unpublished trial (QP15C01; clinical trial ID: NCT02543086) where at least 1/8 subjects receiving a combination of oral primaquine + cipargamin developed a reversible grade 4 transaminase rise.

antimalarials on the prevalence of plasmodial mosquito infestation. Amongst those put to test, cipargamin seemed to be the most advanced candidate in blocking human-to-mosquito transmission [22]. This property of the drug could potentially reduce the number of new malaria infections and thereby the overall prevalence.

3.6. Synthesis of cipargamin

At first, a high degree of diastereoselectivity was observed during the synthesis of cipargamin, with only one diastereoisomer yielding the desired level of antimalarial activity [8]. A mechanistic study was performed to gain insights into the source of the diastereoselectivity [23]. Different reaction methods were developed to obtain an appropriate enantioselectivity [24–26]. The reaction process was simplified by performing region-selective indole alkylation, which showed a good yield and high region selectivity, a method that renders the synthesis of cipargamin more efficient [27]. Another method was developed by rhodium-catalysed addition of arylboroxines to N-unprotected ketimines, forming directly α-tertiary amines in excellent yield. The method could be an efficient enantioselective synthesis of cipargamin [28].

3.7. Pharmacokinetic profile – Preclinical studies

Rottmann et al. obtained first data on the pharmacokinetic profile of cipargamin from an *in vivo* mouse model. They found effective doses of 50%, 90% and 99% decrease in parasitaemias after single doses of 1.2 mg/kg, 2.7 mg/kg and 5.3 mg/kg, respectively [12]. Lakshminarayana and co-workers conducted a pharmacokinetic-pharmacodynamic analysis of cipargamin in an in *vivo* mouse model. The effective dose causing 90% reduction in parasitaemia was 5.6 mg/kg [29]. In another study, absorption, metabolisation and elimination were characterised in rats and dogs. Cipargamin was well absorbed; only 11% of the original cipargamin was found in rat faeces, and cipargamin was not detected in dog faeces. The elimination of cipargamin and metabolites was primarily mediated via biliary pathways (93% of the dose) in faeces [30].

4. Cipargamin - Clinical development

4.1. Pharmacokinetic profile – Phase 1 studies in humans

The pharmacokinetic profile in healthy volunteers was evaluated by both Leong et al. and Huskey et al. [31,32]. Findings of these two studies are compared in Table 3.

Leong et al. assessed the pharmacokinetics of cipargamin in a group of 95 healthy male adults [31]. Oral administration was evaluated in single-dose cohorts (1 mg, 3 mg, 10 mg, 30 mg, 100 mg, 200 mg, 300 mg) and in multiple-dose cohorts (10 mg, 30 mg, 60 mg, 100 mg, and 150 mg once daily) for 3 days [31]. In the single-dose cohorts, the maximal concentration (C_{max}) ranged from 14 ng/mL (1 mg dose) to 2,090 ng/mL (300 mg dose). The time until the maximum concentration was reached (T_{max}) was in the range from 1 to 5 h, rising with increasing doses. Systemic exposure increased in approximately dose-proportionality. For the single-dose cohorts, the elimination half-life ($T_{1/2}$) was in the range of 19–26 h. Administration of multiple doses showed an accumulation ratio ($AUC_{0\text{-}24}$, day3/$AUC_{0\text{-}24}$, day 1) in the range of 1.5–2. For the multiple-dose cohorts, $T_{1/2}$ was 21–28 h [31].

Huskey et al. evaluated the pharmacokinetics in a group of six healthy male adults after oral administration of a single dose of 300 mg. The mean C_{max} was 1,780 ng/mL, with a median T_{max} of 3.5 h (range 3–8 h). The apparent systemic clearance from plasma following extravascular administration (CL/F) of cipargamin was 4.99 L/h; the $T_{1/2}$ was 33.4 h. The mean apparent volume of distribution during terminal elimination following extravascular administration (Vz/F) for cipargamin was 238 L, indicating that cipargamin is probably not extensively distributed into tissues [32].

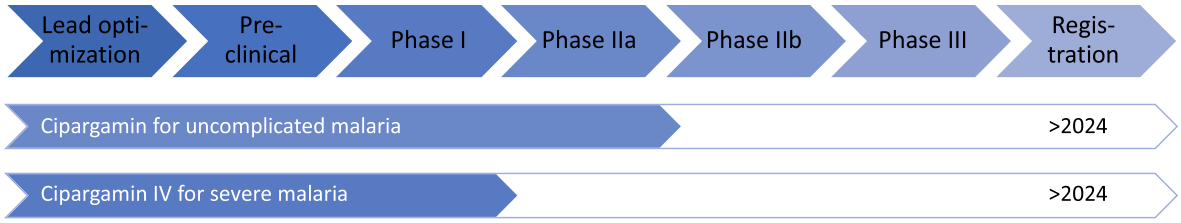

Fig. 2. Development of cipargamin (status May 2020).

Table 3
Summary of pharmacokinetic parameters of cipargamin following 300 mg oral administration in healthy volunteers, assessed in two different studies.

PK parameter	Unit	Leong [31] (2014) N = 95	Huskey [32] (2016) N = 6
C_{max}	ng/mL	2090 ± 482	1780 ± 676
AUC_{last}	h·μg/mL	79.7 ± 15.8	66.7 ± 24.1
AUC_{inf}	h·μg/mL	81.3 ± 17.7	67.0 ± 24.2
AUC_{0-24}	h·μg/mL	36.0 ± 2.40	25.7 ± 8.95
T_{max}	h	5.43 (3.0–8.15)	3.50 (3.00–8.00)
$T_{1/2}$	h	24.0 ± 7.59	33.4 ± 4.23
CL/F	L/h	3.85 ± 1.0	4.99 ± 1.74
Vz/F	L	124 ± 17.7	238 ± 89.8

All values are mean (± SD) except for T_{max}, which is median (range).
C_{max}: observed maximum plasma concentration following drug administration. AUC_{last}: area under the plasma concentration–time curve from time zero to the time of last measurable concentration. AUC_{inf}: area under the plasma concentration–time curve from time zero to infinity. AUC_{0-t}: area under the plasma concentration–time curve from time zero to time 't' where t is a defined time point after administration. T_{max}: time to reach the maximum concentration after drug administration. $T_{1/2}$: terminal elimination half-life. CL/F: apparent systemic (or total body) clearance from plasma following extravascular administration. Vz/F: apparent volume of distribution during terminal elimination phase following extravascular administration.

The effective dose causing a 99% reduction in parasitaemia in the *P. berghei* mouse model is 5.3 mg/kg, with an estimated exposure AUC_{0-24} of 3.260 h·μg/mL. Allometric scaling predicts an equivalent human efficacious exposure of 3.570 h·μg/mL. This suggests that a daily dose of 30 mg for a 70 kg human is sufficient to maintain a total plasma concentration greater than the minimal inhibitory concentration [31].

Food effect on the pharmacokinetics of cipargamin was evaluated with a single dose of 30 mg after a high fat standard breakfast. There were no statistically significant differences between fasting and fed groups with respect to AUC. The geometric mean ratio of fed/fasting for AUC_{0-last} and AUC_{0-inf} were 1.063 (90% CI 0.979–1.153) h · μg/mL and 1.016 (90% CI 0.947–1.091) h μg/mL, respectively. However, after a meal, the maximum concentration was reduced, and the absorption was delayed. C_{max} decreased from 356 ng/mL to 254 ng/mL, and T_{max} was prolonged from 2.5 h under fasting conditions to 4 h when participants were fed. Fatty food did not alter the CL/F, which was 4.00 L/h under fed conditions and 4.03 L/h under fasting conditions. The apparent volume of distribution (Vz/F) ranged from 129.36 L under fed conditions to 123.46 L when participants were fasting [31].

The pharmacokinetic properties were assessed in a group of 21 *P. falciparum* and *P. vivax* patients after a dose of 30 mg for three days [33]. C_{max} was reached after a median of 3 h. $T_{1/2}$ was 20.8 h (range, 11.3–37.6 h). Accumulation ratios were 1.56 in *P. vivax* patients and 1.60 in *P. falciparum.* No substantial differences were seen between the two patient cohorts and between the first and last doses. Compared to healthy volunteers, exposure values (C_{max} and AUC) were 2–3 times as high [33]. A plausible explanation could be a higher exposure due to higher protein binding in malaria patients, due to raised alpha-1 acid glycoprotein (AAG) levels. AAG is an acute-phase protein, which increases 2- to 5-fold during injury, infections including malaria, and inflammation [34,35]; observations initially made with quinine [35]. In a study of 25 *P. falciparum* patients, receiving a single dose of 10–30 mg, pharmacokinetic modelling showed that dosing did not significantly affect the PK properties, suggesting dose-linear kinetics [36].

4.2. Antimalarial activity in P. vivax and P. falciparum patients

The first phase 2 study in malaria patients was conducted by White et al. at three centres in Thailand [33]. Twenty-one patients aged 20–60 years, with uncomplicated *P. vivax* malaria (10) and falciparum malaria (11) were treated at a dose of 30 mg per day for 3 days. Baseline parasitaemia ranged from 6,816/μL to 53,082/μL for *P. vivax* malaria and from 4,139/μL to 63,745/μL for *P. falciparum* malaria, respectively. The median parasite clearance time was 12 h in each cohort, with a parasite clearance half-life ($PC_{1/2}$) of 0.95 h for *P. falciparum* and 0.90 h for *P. vivax*, respectively. Gametocyte clearance time was 8 h in the *P. vivax* patients; no gametocytes were detected in the *P. falciparum* patients. Resolution of fever was reached after 8 h and 12 h for *P. vivax* and *P. falciparum*, respectively [33]. A second phase 2 study in Asian patients aged 20–60 years with *P. falciparum* infections was designed to assess efficacy and safety of single-dose treatment of cipargamin and to correlate the outcome with pharmacokinetics. In four sequential ascending single-dose cohorts, 75 mg, 150 mg, 225 mg and 300 mg were to be tested. However, the study was terminated following recruitment of the first cohort of 11 patients treated with 75 mg. Efficacy was evaluated as clinical cure at day 29. All 11 patients achieved parasite clearance at day 6, but 4/11 patients experienced recrudescence during the study [37]. A human challenge model phase 1 study assessed efficacy and safety of single dose cipargamin treatment of *P. falciparum* malaria induced in healthy Australian adults, aged 18–55 years. This study was planned as a multiple-cohort study, with the first cohort assessing a single-dose oral treatment of 10 mg cipargamin and in the second cohort a combination of cipargamin with pre-administration of 480 mg piperaquine. Eight healthy adults were inoculated with blood stage malaria and subsequently treated. The study was terminated after liver function test abnormalities occurred (see section 4.3); however, rapid parasitaemia declines were observed. Six out of eight subjects showed a decline in parasitaemia by 40 h post-dose and the remaining two subjects showed a steady decline until 80 h post-dose and beyond [38].

Hien et al. [36] determined the minimal inhibitory concentration (MIC), which is defined as the drug level with a net parasite multiplication rate of one per asexual cycle. In this phase 2 study, 25 Vietnamese adults, aged 20–60 years, with falciparum malaria were treated with single doses either of 10 mg (7), 15 mg (7), 20 mg (5) or 30 mg (6), $PC_{1/2}$ estimates were 4.35 ± 2.21, 3.79 ± 1.22, 1.91 ± 1.64, and 1.47 ± 0.83 h for doses of 10 mg, 15 mg, 20 mg, and 30 mg, respectively. The estimated MIC values ranged from 0.0375 to 0.803 ng/mL (median, 0.126 ng/mL) [36].

4.3. Safety and tolerability in humans – Phase 1 and 2 studies

Several studies assessed safety and tolerability (Table 2). No deaths were reported in any of the studies altogether. Seven serious adverse events (SAEs) were reported in six patients, mainly based on liver function abnormalities based on protocol defined thresholds for

reporting [37,38]. Leong et al. assessed the safety and tolerability of cipargamin in a phase 1 study. Ninety-three healthy adult male volunteers, aged 18–55 years, received single (1 mg, 3 mg, 10 mg, 30 mg, 100 mg, 200 mg and 300 mg) or multiple (10 mg, 30 mg, 60 mg, 100 mg and 150 mg once daily for three days) dosing. Using the Criteria for Adverse Events (CTCAE) scale, adverse events grade 1 to 2 (i.e. AEs of mild or moderate severity) were reported. After single doses, 50% of the study subjects reported adverse events, with 73% reporting them after multiple dose intake. Most of them were self-limiting. Adverse events mentioned in the single-dose group were semen discolouration (16.7%), diarrhoea (7.1%), nausea (7.1%), and fatigue (4.8%). Headache was experienced both in the cipargamin and placebo groups. In the multiple-dose group, dizziness (10.8%), catheter site haematoma (8.1%), back pain (5.4%), and nausea (5.4%) were the adverse events reported. Three types of adverse events were reported in multiple-dose groups, namely headache (45.9% versus 33.3%, respectively), semen discolouration (59.5% versus 8.3%), and confusion (2.7% versus 16.7%). One subject who received multiple doses of cipargamin developed elevated blood alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK), which returned to within normal limits within four days after dosing and was not attributed to the study drug [31]. The adverse event of semen discolouration was caused by yellow-coloured metabolites of KAE609 with M23 being the main metabolite. M23 has no cytotoxic potential against a human hepatic cell line and no azoospermia, diminished spermatozoon motility or other clinically significant abnormalities were recorded in patient samples [31].

Huskey et al. did not report any significant adverse events or safety concerns in a phase 1 study of six healthy males, aged 35–55 years, after administering a single dose of 300 mg. Semen discolouration caused by the yellow-coloured metabolite M23 was reported as adverse event by all male participants [32]. White et al. reported vomiting as the most common adverse event in Asian patients receiving a dose of 30 mg daily for 3 days. Additionally, in three patients, ALT and AST were elevated. The elevations were not considered related to study medication as the values were already slightly elevated prior to treatment [33]. Elevated liver function tests are regarded as an inherent effect of malaria with a certain degree of variability and individual differences in susceptibility [39]. In another study, which evaluated the treatment of 25 patients with *P. falciparum*, adverse events were reported in 76% of the patients. Nausea, myalgia and headache were considered as related to the study treatment. Laboratory abnormalities were mild to severe, but not further discussed [36].

Two studies published to date were prematurely terminated due to transient liver function test elevations. In a phase 2 dose-escalating multiple cohort study, cipargamin was given to patients with *P. falciparum* in Thailand and Vietnam. In the first cohort, eleven patients were treated with a single dose of 75 mg cipargamin. Three of these patients experienced 4 SAEs, including increased ALT and AST levels, and jaundice [40]. The study was terminated following recruitment of the first cohort of patients treated at 75 mg. The decision was prompted by the observation of asymptomatic and reversible liver function test abnormalities and was taken by the sponsor based on the evidence of a metabolite which required characterization by pre-clinical animal toxicology [37].

Another study was designed as a dose-escalating multiple cohort study in healthy volunteers, with induced blood stage malaria. Eight patients were treated with 10 mg of cipargamin. In this group of patients, three SAEs were reported, all related to significant and reversible (not further described) liver function test abnormalities. Due to these findings, the study was terminated by the sponsor after completion of the first cohort [38]. In another human challenge model study [40], reported in the context of a synopsis on liver enzyme elevations in *Plasmodium falciparum* human challenge studies, at least one of eight volunteers in total who received cipargamin (in this case in combination with primaquine) developed a reversible grade 4 transaminase rise. Indeed, data from human infection model studies support the hypothesis that liver injury in uncomplicated (falciparum) malaria might be in part rather disease/inflammation-related than drug-induced [41], multifactorial, and with regard to human challenge studies, even in part model-related [42].

Papers published to date [31–33,36,41,43] suggest that cipargamin seems to be well tolerated, both in healthy volunteers and in patients. However, in the terminated studies, patients experienced liver function test abnormalities, which were reported as SAEs based on the protocol. According to these studies, the main safety concern is potential hepatotoxicity. Nevertheless, the number of people that received cipargamin so far is small: 152 healthy subjects and 57 patients have been assessed after oral administration.

A phase 2 study was designed and set-up to address the potential hepatotoxicity of cipargamin in a systematic way. It is an open-label study with sequential dose-escalating cohorts and single-doses as well as multiple-dose arms. In the single-dose arm 10 mg, 25 mg, 50 mg, 75 mg and 150 mg and in multiple-dose arms 3 daily doses of 10 mg, 25 mg and 50 mg are being investigated. The study has accomplished recruiting patients with uncomplicated *P. falciparum* malaria in five countries in West- and East Africa (Mali, Gabon, Ghana, Uganda and Rwanda) and is currently (June 2020) prepared for publication. As part of this study, approximately 130 patients in total will be treated with increasing doses of cipargamin. In all patients, laboratory parameters will be monitored carefully throughout the entire study duration of 29 days [44]. The results of this study regarding safety of the drug in malaria patients will guide further clinical development plans.

4.4. Efficacy against drug-resistant parasites

Five of the patients with *P. falciparum infection* in the study conducted by White at al. carried kelch protein K13 polymorphisms, which are to some extent associated with artemisinin resistance [33]. Four of them had parasite clearance comparable to the patients without mutations. The fifth patient withdrew from the study [33]. To the best of our knowledge, no other indicating events pointing towards clinically relevant resistance in patients have been reported so far. However, the presence of a specific mutation in PfATP4 (G223R) was recently described in African Plasmodium falciparum isolates at moderate but significant levels [45], indicating the need to monitor presence of PFATP4 mutations in clinical trials with cipargamin.

4.5. Cipargamin as combination therapy partner

Stein and colleagues tested the drug-drug interaction of cipargamin and piperaquine (PPQ) in an open-label, parallel group, single-dose study with 110 healthy male adults aged 18–45 years. Subjects received 75 mg cipargamin plus 320 mg PPQ, 25 mg cipargamin plus 1,280 mg PPQ, 25 mg cipargamin alone, 320 mg PPQ alone, or 1,280 mg PPQ alone. Pharmacokinetics, safety and potential QT interval interactions were assessed. The QT interval was corrected by the Fridericia method (QTcF) [43]. Co-administration of PPQ had no overall effect on exposure to cipargamin. No significant differences were measured in PK parameters between the group that received 25 mg cipargamin plus 1,280 mg PPQ and the group that received 25 mg cipargamin alone, except for a 17% decrease in the cipargamin C_{max} in the presence of 1,280 mg PPQ. PK parameters for PPQ in plasma showed an increase in the $AUC_{0-\infty}$ when combined with 25 mg cipargamin but showed a decrease when combined with 75 mg cipargamin. The reason for these inconsistent results remains unclear [43]. No SAEs were reported, but 54.5% of the subjects experienced one or more adverse events grade 1 or 2, in all groups. The most common adverse event was respiratory tract infection, followed by cephalgias [43]. No increase in QTcF was seen except for the two treatment groups that received 1,280 mg PPQ. PPQ is associated with QTc prolongation in human studies [46]. Cipargamin did not show any QTc changes in humans so far [43].

Combination therapy of antimalarial drugs with different modes of action is the most efficient way to treat malaria and is an effective manner to delay the development of resistance. Currently, ACTs are the recommended first-line therapies for *P. falciparum* malaria. Piperaquine, one of the partner drugs (in combination with dihydroartemisin), belongs to the group of 4-aminoquinolines, which also contains chloroquine. The mechanism of action is not fully understood; however, the drug seems to prevent the detoxification of toxic haeme, the end product of the plasmodial haemoglobin digestion pathway [47]. Piperaquine shows a rapid parasite-clearance, but monotherapy induces gametocytaemia [48]. This study shows that the combination of cipargamin and piperaquine is well tolerated in healthy adults. According to the results of the above study, piperaquine can possibly be a combination therapy partner of cipargamin, but further investigation on efficacy in patients would be required. Among the group of approved antimalarials, other possible combination partners for cipargamin would include lumefantrine. In addition, a range of other compounds currently progressing through early clinical studies would need to be evaluated for their suitability as combination partners for cipargamin.

5. Relevance for travel medicine

There is a continuous need for the further development of antimalarials suitable for malaria prophylaxis [49] and therapy [50] in order to stay abreast of drug resistance development and to move closer to compounds ideally fulfilling the criteria laid down in respective target product profiles.

With regard to its pharmacological properties and its promising mode of action and overall profile, cipargamin could evolve into a potentially very interesting compound with regard to travel medicine applications, given its prophylactic and treatment potential, and its anticipated favourable resistance profile. With a half-life of around 24 h - longer than artemisinins but shorter than aminoquinolines used in combination for malaria combination chemotherapy); and longer than doxycycline and proguanil but shorter than atovaquone and mefloquine, for example, as used for malaria chemoprophylaxis, cipargamin could turn out, pending further favourable clinical trial results, to become a combination partner of interest not only for the therapy of authochtonous and imported malaria cases, but also as a potential combination partner or single drug for malaria chemoprophylaxis, despite its lack of radical-prophylactic and -curative potential.

6. Summary

Cipargamin is part of the Medicines for Malaria Venture (MMV) drug pipeline [7]. A large multicentre phase 2a trial assessing safety and efficacy of cipargamin has been recently completed (publication in preparation) in Africa [42]. If safety and efficacy data from this trial are favourable, clinical development shall progress to a phase 2b combination study assessing different dose combinations of cipargamin and a to-be-determined partner compound. Confirmatory phase 3 clinical trials in uncomplicated malaria caused by *P. falciparum* and *P. vivax* would be required for registration of a cipargamin combination therapy for this indication. The fast plasmodicidal activity of cipargamin *in vitro* and in patients makes it also a good drug candidate for the treatment of severe malaria.

In the search for a new antimalarial compound with properties defined as TPP-1 and TPP-2 by MMV, cipargamin exhibits promising properties. The fact that it has a novel mechanism of action, which is also effective against drug-resistance parasites, makes it an interesting new compound. According to the clinical data, cipargamin shows to be highly effective against malaria, even in low doses. It is effective against multiple erythrocytic stages of the malaria parasite and seems to have the capacity to block transmission to mosquitoes. The pharmacokinetic profile supports once-daily dosing and possibly a single-dose treatment. The potential issue is the hepatic safety of the drug, as in studies with malaria patients, cases of elevated ALT and AST were reported at a single dose of 75 mg. In contrast, no significant elevation of liver function test was identified in healthy volunteers exposed to up to a single dose of 300 mg. Currently, a large phase 2a study is ongoing to assess the safety of cipargamin in African malaria patients. Phase 3 trials including young children will start if cipargamin with combination partner has a favourable efficacy and safety profile.

7. Conclusion

In preclinical and clinical studies, cipargamin proved to be effective against malaria in the asexual and sexual erythrocytic stages of the parasite. Thereby, it also blocks transmission from human to mosquito. The pharmacodynamic profile is favourable and seems to allow for a single-dose or once-daily regimen. The hepatic safety concern needs to be fully characterised to inform further clinical development of cipargamin as a next generation antimalarial. The currently ongoing phase 2 study in African patients with uncomplicated malaria was designed to provide clinical data supporting this evaluation.

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CRediT authorship contribution statement

Suzan AM. Bouwman: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Rella Zoleko-Manego:** Data curation, Formal analysis, Investigation, Resources, Validation, Writing - review & editing. **Katalin Csermak Renner:** Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing. **Esther K. Schmitt:** Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing - original draft, Writing - review & editing. **Ghyslain Mombo-Ngoma:** Data curation, Formal analysis, Methodology, Validation, Writing - review & editing. **Martin P. Grobusch:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

MP Grobusch is Principal Investigator on the cipargamin phase 2 trial [42]. G Mombo-Ngoma is co-PI, and R Zoleko Manego is CERMEL/Gabon Site Investigator. SAM Bouwman served as co-investigator on that trial. K Csermak Renner and EK Schmitt are Novartis employees. The authors have no other relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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