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Supporting malaria elimination with 21st century antimalarial agent drug discovery

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The burden of malaria has been considerably reduced over recent years. However, to achieve disease elimination, drug discovery for the next generation needs to focus on blocking disease transmission and on targeting the liver-stage forms of the parasite. Properties of the 'ideal' new antimalarial drug and the key scientific and technological advances that have led to recent progress in antimalarial drug discovery are reviewed. Using these advances, Novartis has built a robust pipeline of next-generation antimalarials. The preclinical and clinical development of two candidate drugs: KAE609 and KAF156, provide a framework for the path to breakthrough treatments that could be taking us a step closer to the vision of malaria elimination.

Introduction

Malaria is a devastating and often fatal disease caused by protozoan parasites from the *Plasmodium* genus. Owing to increased prevention and control measures, such as the introduction of insecticide-treated bed nets and use of artemisinin-based combination therapy, the burden of malaria has been reduced such that between 2000 and 2013 global malaria mortality rates decreased by 47% [1]. Nevertheless, in 2013 an estimated 584 000 individuals (90% from sub-Saharan Africa and 78% children <5 years of age) died from malaria [1]. The current challenge is whether malaria can be completely eliminated on a global scale. Several countries have now developed and started to implement malaria elimination strategies [2], but it is widely accepted that currently available drugs are not ideally suited for disease elimination campaigns [3]. Specifically, there is a need for safe single-dose therapies that are also suitable for mass drug administration to asymptomatic carriers and capable of blocking malaria transmission through the anopheles vector. In addition, chemoprophylaxis prevention requires drugs that are able to eliminate the liver-stage forms of the parasite (especially for *Plasmodium vivax*).

The looming threat of artemisinin drug resistance [4–6], coupled with intense lobbying and the financial support of philanthropic organisations such as the Wellcome Trust and the Bill &

Melinda Gates Foundation, as well as the creation of public–private partnerships such as the Medicines for Malaria Venture [7], triggered a recent surge in antimalarial drug discovery and development activities [8,9]. These research efforts are focused on identifying drugs with novel mechanisms of action. Ultimately, this should mitigate the risk of cross-resistance with existing antimalarials and, by targeting multiple stages of the malaria parasite lifecycle, facilitate prevention, radical cure and transmission blocking [10].

Over the past 10 years, recognition of the need for antimalarial agents with properties that differ from existing treatments has led to dramatic changes in the way in which new targets are identified and new drugs developed. Until very recently, no antimalarial drug with a novel mechanism of action had entered Phase II clinical trials since the hydroxy-1,4-naphthoquinone atovaquone more than 20 years ago [11]. An important milestone in malaria research, however, was achieved in early 2014 with the publication of the results of a Phase II trial of KAE609 [12], the first member of a novel class of antimalarials: the spiroindolones. KAE609 emerged from a malaria drug discovery effort led by the Novartis Institute for Tropical Diseases in partnership with the Genomics Institute of the Novartis Research Foundation, the Swiss Tropical and Public Health Institute, the Biomedical Primate Research Centre and the Medicines for Malaria Venture, with financial support from the Wellcome Trust. KAE609 is being further developed by Novartis as

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part of the company's efforts to contribute to global malaria elimination. This compound is the front-runner in a growing pipeline of antimalarials emerging from Novartis that also includes a second novel antimalarial, the imidazolopiperazine KAF156 [13,14] currently in clinical trials [15], and a preclinical programme aiming to develop inhibitors of phosphatidylinositol-4-OH kinase [PI(4)K], a novel drug target that operates across all the major lifecycle stages of the parasite in its host [16].

Here, we review and discuss the latest scientific and technological advances in malaria drug discovery that have enabled the development of a robust Novartis drug portfolio, and offer some personal perspectives on the discovery and development of these novel antimalarial agents. A comprehensive overview of the global antimalarial pipeline has been the subject of another recent publication [8].

Scientific and technological advances in antimalarial drug discovery

Beyond already well-exploited drug targets, such as the *Plasmodium* dihydrofolate reductase, dihydropteroate synthetase, cytochrome bc1 complex and the haemoglobin degradation pathway, there remains a dearth of novel validated targets. The sequencing of the *Plasmodium falciparum* genome [17] and use of modern genetics tools were expected to yield many attractive drug targets, reviewed by Winzeler 2008 [18]. However, few proposed targets have subsequently been chemically validated; most targets validated by genetic knockouts have not been tractable. A notable exception is the dihydroorotate dehydrogenase enzyme, which led to the identification of the clinical candidate DSM265 [19]; DSM265 clinical studies represent the first attempt clinically to validate a genetically identified target.

A number of scientific and technological advances have recently enabled the rapid identification of clinical candidates and also enabled the discovery and chemical validation of novel malaria drug targets. Undoubtedly, the first important technological advance was the miniaturisation of *Plasmodium* growth assays [20,21]. This development made automated HTS possible and enabled Novartis and many other companies and academic groups to screen large compound libraries for chemical 'starting points' with promising antimalarial activity [21–23]. An open-access repository of screening data is available online at the ChEMBL – Neglected Tropical Disease archive (https://www.ebi.ac.uk/chembl ntd) [24]. The second advance has been the development of a wide array of genetic and biochemical techniques that have enabled rapid identification of the molecular targets of different screening hits [10,16,25–31].

An important barrier with respect to the elimination of malaria is the liver stage of the parasite lifecycle, because *P. vivax* can remain dormant in the liver as hypnozoites for many months or years [32]. Currently, the only treatment targeting the liver stage of vivax malaria is primaquine, which cannot be used in individuals with glucose-6-phosphate dehydrogenase deficiency, a common genetic abnormality in malaria endemic areas. However, there is also a new hypnozoiticidal compound in Phase III development: tafenoquine, which might offer another liver-stage treatment in the future [33,34]. The discovery of new liver-stage drugs has been hampered by the dearth of models to screen for activity against hypnozoites; until recently, only the *in vivo Plasmodium-cynomolgi*–simian malaria

model was available. An important step towards the screening of novel hypnozoiticidal compounds was recently made with establishment of an *in vitro* assay that discriminates the activity of primaquine and atovaquone against the growing schizont and dormant hypnozoite [35–37]. It was this assay that eventually suggested the *P. vivax* radical cure potential of a novel class of blood-stage antimalarial drugs: the imidazolopyrazines, which target *Plasmodium* PI(4)K [16].

The availability of new drugs that prevent the transmission of sexual-stage parasites (gametocytes) to mosquito vectors has also been recognised as a crucial component of future malaria elimination strategies [38]. This task has been aided by the recent development of a novel assay that utilises *P. falciparum* cell lines to measure the effects of tested compounds on gametocyte maturation and transmission [38]. A variety of *in vitro* assays have also been employed to show that early- and late-stage gametocytes are susceptible to KAE609 in a dose-dependent manner and that this agent reduces transmission to the vector, providing further evidence that blockage of transmission might be possible with new-generation antimalarials [39].

Another important advance in malaria research was the development and refinement of small animal models, reviewed by Vaughan et al. [40] and Kaushansky et al. [41]. Humanised mouse models, such as the severe combined immunodeficiency model, enable the study of blood- and liver-stage *P. falciparum* and have been used to establish the antimalarial potential of new drugs.

As a result of these advances, the emerging global pipeline for new antimalarials [8,9] was identified using different discovery strategies (e.g. target- vs cell-based hits). To have maximum public health impact, novel antimalarial drugs must ideally fulfil a number of pharmacological and safety requirements throughout development. We describe the various approaches employed by Novartis and collaborators to ensure that drug candidates meet these key pharmacological requirements.

Preclinical development strategy

In the preclinical profiling stages of drug development, the overall goal is to ensure that the profile of a new drug candidate is compatible with the probable use of the compound in the clinic. To mitigate the risk of failure owing to a possible difference in activity between laboratory-adapted strains and clinical isolates, KAE609 was shown early to be as effective as artesunate in an *ex vivo* assay against field isolates of *P. falciparum* and *P. vivax* collected from areas of drug resistance [42,43]. As noted, there are several *in vivo* malaria models available for drug testing. The lethal *Plasmodium berghei* malaria mouse is inexpensive, rapid and enables the evaluation of cure (i.e. prevention of recrudescence) but species differences in drug sensitivity might warrant the use of the more expensive *P. falciparum* SCID model [19]. Potent antimalarial activity across *Plasmodium* species is generally desirable because it is suggestive of strong evolutionary conservation of the compound-binding site on its molecular target, which is a harbinger for a reduced risk of drug resistance development.

In preclinical species, KAE609 displayed pharmacokinetic profiles upon oral dosing consistent with once-daily oral dosing in humans. In the *P. berghei* malaria mouse model, KAE609 demonstrated a fast onset of action and potently reduced parasitaemia. Importantly, KAE609 was the only one of the tested drugs (which

included artesunate, artemether, chloroquine and mefloquine) to display single-dose cure efficacy [42]. *P. berghei* appears *in vitro* to be less sensitive to spiroindolones than *P. falciparum* and close analogues of KAE609 display faster clearance antimalarial properties in the *P. falciparum* SCID mouse (unpublished data).

The imidazolopiperazine class of compounds, which includes KAF156, was also discovered and initially optimised primarily for its asexual blood-stage activity, but was later found to show liver-stage activity *in vitro* (Fig. 1) [44]. KAF156 displayed potent parasitaemia reduction at doses lower than those of standard antimalarial drugs in blood-stage models, and also showed potent prophylactic activity in the *P. berghei* mouse model. Thus, preclinical data suggest that KAF156 could have clinical utility as a therapeutic and prophylactic antimalarial agent [45]. Similar to KAE609, the pharmacokinetic profile of KAF156 upon oral dosing in preclinical species is compatible with once-daily dosing. In terms of preclinical safety, oral doses of KAF156 were shown to be well-tolerated in studies of rodents and dogs, with no toxicities identified that would preclude use in humans.

New antimalarial drugs must also be fully tested *in vitro* with regard to the potential for drug–drug interactions to minimise the risk of adverse interactions with other anti-infective therapies frequently used in malaria-endemic countries, such as human immunodeficiency virus and tuberculosis drugs. Many existing antimalarial drugs carry some risk of cardiotoxicity through inhibition of hERG-encoded potassium channels [46,47]. Given that any novel antimalarial might be combined with some of these drugs, it is important to minimise the risk for cardiotoxicity through a thorough early evaluation of this risk in hERG *in vitro* assays and animal toxicology studies. In addition, because the populations most vulnerable to malaria are young children and pregnant women, the drugs need to be tested as early as possible with regard to their potential for reproductive toxicity, mutagenicity and genotoxicity. Because malaria is a tropical disease, it is also important to assess the risk of phototoxicity in *in vitro* and *in vivo* models [48]. KAF156 and KAE609 have been evaluated in these safety pharmacology assays and no significant risks have been identified [42,43,45]. Finally, the risk of drug resistance, together with suitability for combination with existing agents to minimise the risk of future resistance development, should also be assessed at an early stage [49]. Drug resistance to KAE609 and KAF156 can be generated in laboratory *P. falciparum* strains and, taking into
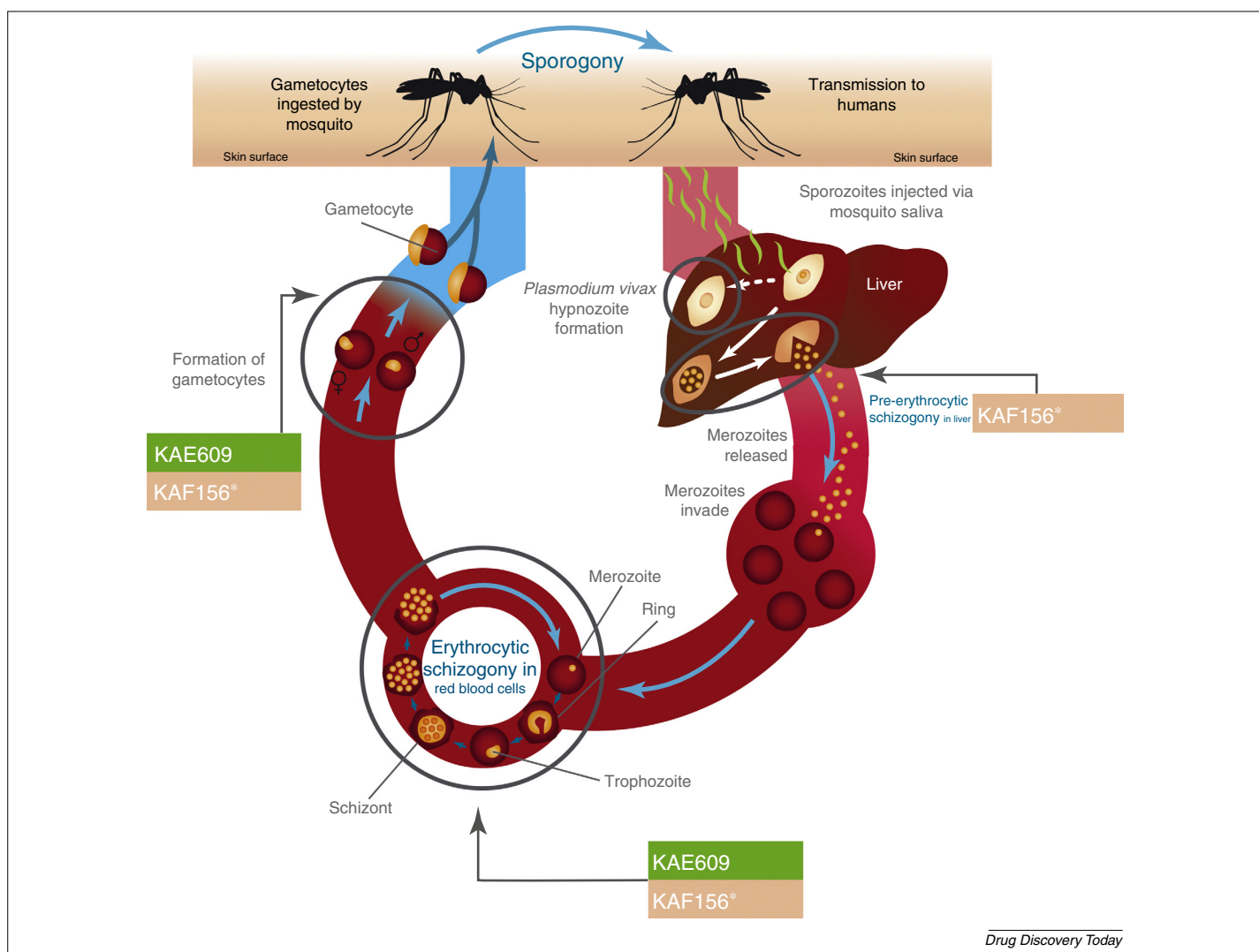

FIGURE 1

Breaking the malaria life-cycle with novel antimalarials. * Potential for prophylactic activity and stage of action is based on in vitro/in vivo preclinical data.

account the frequency of mutations, the fitness of the mutants and the fold-shift drug resistance associated with single mutations, the overall risk of resistance is viewed as moderate.

Clinical development strategy for new antimalarial drugs

After Phase I healthy volunteer studies establish preliminary safety and the maximum tolerated dose of a novel agent, demonstration of the efficacy of a well-tolerated regimen predicted to deliver therapeutic and efficacious drug levels is required in a small proof-of-concept (PoC) Phase II study. This is usually performed in patients with uncomplicated malarial infection and low-to-moderate parasitaemia (<50 000 parasites/µl). Such PoC studies also offer the opportunity to investigate the pharmacokinetics, pharmacodynamics and tolerability of the drug in the target population. Assuming the success of the PoC study, additional Phase II studies might then be initiated to test whether single doses of the drug are sufficient to cure acute infections and to define the minimum inhibitory concentration (MIC) [50]. The final step before large-scale Phase III trials can be initiated is usually dose-range-finding studies evaluating various combination treatments with other antimalarial drugs.

In a Phase I study (single and multiple ascending doses) in healthy adult volunteers [51], KAE609 showed dose-proportional pharmacokinetics (from 1 to 300 mg) with no significant food effects. It has been proposed that for fast-acting antimalarial drugs with a parasite reduction ratio of $\geq 10^4$ cure can theoretically be achieved in uncomplicated malaria patients if drug levels are maintained above the MIC for three-to-four *Plasmodium* lifecycles (144–192 h) [50]. In the KAE609 Phase I study, a single 200 mg dose yielded drug levels above the *in vitro* IC_{99} for more than 144 h, suggesting that a single-dose cure might be feasible. Although this is an enthralling possibility, the *in vitro* IC_{99} might not accurately predict the actual patient MIC and further patient trials are underway to determine whether single-dose cure can be achieved with KAE609 (see below).

Subsequently, KAE609 underwent an open-label PoC trial involving 21 patients with *P. falciparum* or *P. vivax* mono-infection [12]. After treatment with 30 mg KAE609 daily for three days, parasites were cleared in a median of 12 h, an effect that is more rapid than that observed with artemisinin-based combination therapies. In addition, in the five patients for whom gametocytaemia was detected at baseline (all with vivax malaria), gametocytaemia was cleared by 8 h post-dose, confirming the potent transmission-blocking potential of this novel class of compounds. All patients recovered uneventfully, with no patients discontinuing treatment as a result of adverse events. Absorption of KAE609 was reliable and the terminal elimination half-life (~21 h) was fully consistent with once-daily dosing. KAE609 is currently under evaluation in another single-dose-range-finding Phase II trial that will assess efficacy, safety and pharmacokinetics in uncomplicated *P. falciparum* mono-infection and should provide information on the feasibility of a 28-day single-dose cure [52]. Another Phase II study will aim to identify the MIC of KAE609 in *P. falciparum* mono-infection [53]. In terms of resistance, *in vitro* selection resulted in strains with stable mutations, all mapping to the *PfATP4* gene [42]. It is therefore important that KAE609 is combined with a second drug to protect it from the emergence of

resistance, particularly if the drug is ultimately used as a single-dose therapy. Several preclinical and clinical studies are underway or planned to evaluate potential drug partners to enable combination therapy in the clinic. A Phase IIb dose-ranging study and concurrent safety studies with the selected drug partner would follow, with a view to testing the optimal combination candidate in Phase III non-inferiority studies versus the appropriate comparator. The goal would then be to investigate the combination treatment in children and pregnant patients further. Following a strategy similar to KAE609, a Phase I study in healthy adults has been completed for KAF156 [54] and a Phase II PoC study in patients with uncomplicated *P. vivax* or *P. falciparum* infection is underway [15]. Further studies could also be conducted utilising a human sporozoite challenge model [55] to investigate the potential prophylactic activity of KAF156.

Challenges and opportunities

Thanks to significant investments over the past decade and technological advances in HTS, the current global pipeline of new antimalarial drug candidates is robust. Ironically, these successes are now challenged by the fact that we might have identified most – if not all – mechanisms accessible through HTS of conventional compound libraries representing the currently accessible chemical space [24]. Indeed, we suspect that most of our compounds active against *Plasmodium* asexual stages act through a limited number of known mechanisms and molecular targets [e.g. ATP4, PI(4)K, dihydroorotate dehydrogenase, cytochrome bc1 complex and dihydrofolate reductase]. Accessing alternative mechanisms of action will probably require the use of different screening procedures with different culture conditions and assay readouts.

Although most of the current clinical candidates have been identified for their asexual blood-stage activity, many have additional activity against the sexual stages and the developing liver stages, which could offer prevention and disease-transmission-blocking properties. Unfortunately, activity against the hypnozoite remains extremely rare and further investments to develop liver- and blood-stage culture systems for the *P. vivax* parasite are desperately needed.

A number of challenges can also be identified with respect to the clinical stages of drug development. Firstly, for new investigational drugs, access to non-immune patients in a good clinical practice (GCP)-compliant setting is restricted to few countries (mainly in South-East Asia) and, therefore, our knowledge of the efficacy and safety of new drugs in diverse populations is relatively limited. Secondly, current methods of dose finding for new antimalarial drugs are imprecise, such that some agents have been introduced in the field at suboptimal doses. This potentially exacerbates emergence of resistance and results in inefficient treatment of the patient [50]. Recent recognition of the importance of identifying the optimal dose has led to proposals for alternative dose-finding strategies in specific patient populations, discussed by White [50]. In this context, the recent and promising results obtained in a malaria challenge model using healthy volunteers could allow some of these limitations to be overcome [56,57].

A third key challenge for clinical development lies in the selection of the most appropriate partner drug for combination treatment and in the strategies employed to study these combinations once they are selected. Existing drugs, such as piperaquine or

lumefantrine, have well-characterised safety profiles that could enable an accelerated development path towards registration, whereas new chemical entities might require additional preclinical and clinical safety studies. By contrast, combinations that consist of entirely new chemical entities could enable an aggressive global roll-out once the combination has been approved because there are no concerns over pre-existing drug resistance. For each novel drug combination the decision tree could vary slightly depending on the constituent drug profiles because many pharmacological factors can influence the optimal drug combination strategy. Of utmost importance is ensuring all new antimalarial drugs are made available to the most vulnerable populations as early as possible, which necessitates rapid clinical evaluation in children and pregnant women for whom the tolerance to safety risk is lowest.

Concluding remarks

Over the past decade, significant progress has been made in the global fight against malaria. More recently, a series of scientific and technological advances have led to the development of a new generation of antimalarial candidate drug that holds much promise. In particular, KAE609 and KAF156 might possess some of the attributes of an 'ideal' antimalarial and, in combination with other drugs, have the potential to become the first of a new generation of malaria therapies to reach the clinic. Although large-scale trials are still required and several research and clinical development issues remain to be addressed, new drugs such as KAE609 and KAF156 will help to ensure there is sustained pressure on the parasite and, thus, take us a step closer to eliminating this devastating disease.

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