Trends in Parasitology

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Opinion

Triple Artemisinin-Based Combination Therapies for Malaria – A New Paradigm?

Rob W. van der Pluijm,[1,2] Chanaki Amaratunga,[1,2] Mehul Dhorda,[1,2,3] and Arjen M. Dondorp[1,2,*]

Recent gains in the fight against malaria are threatened by the emergence and spread of artemisinin and partner drug resistance in *Plasmodium falciparum* in the Greater Mekong Subregion (GMS). When artemisinins are combined with a single partner drug, all recommended artemisinin-based combination therapies have shown reduced efficacy in some countries in the GMS at some point. Novel drugs are not available for the near future. Triple artemisinin-based combination therapies, combining artemisinins with two currently available partner drugs, will provide one of the last remaining safe and effective treatments for falciparum malaria that can be deployed rapidly in the GMS, whereas their deployment beyond the GMS could delay or prevent the global emergence and spread of resistance to currently available drugs.

Highlights

Artemisinin and partner drug resistance have resulted in high failure rates of artemisinin-based combination therapies (ACTs) in the GMS. Spread or emergence of resistance beyond the GMS are threats to malaria control.

Triple ACTs (TACTs), combining an artemisinin and two existing partner drugs, could be a stop-gap therapy for treating multidrug-resistant malaria until new antimalarials are available. Where resistance is not established, deployment of TACTs could delay or prevent emergence of resistance and could prolong the longevity of antimalarial compounds used in any triple-drug combination.

TACTs must be safe, well-tolerated, effective, and affordable. Fixed-dose combinations of three drugs in the same tablet will likely improve adherence. Barriers that hinder deployment and adherence must be identified and addressed early in the development of TACTs.

Artemisinin-Based Combination Therapies Are First-line Treatments for Malaria

Artemisinins are the most potent antimalarial drugs available to date, with a 10 000-fold reduction in *Plasmodium falciparum* parasite burden per 48 h asexual parasite life cycle period in infections caused by artemisinin-sensitive parasites [1]. Artemisinins have a short **elimination half-life** (see Glossary) of ~1 h, which then requires a longer regimen for complete elimination of the infection, but also shortens the window of selection in which drug-resistant parasites outgrow drug-sensitive parasites [2,3]. As early as 1984, Li *et al.* recognised that, even with 3 days of high-dose artemisinin treatment, ~40% of patients will come back with **recrudescent infections** [4]. Due to the short half-life of artemisinin, and probably also due to artemisinin-induced **dormancy** in asexual-stage parasites, even 7 days of monotherapy is not completely effective [5–7]. Early in its development, Chinese investigators already suggested that artemisinin should be used as combination therapy with one or more partner drugs [4]. Artesunate combined with mefloquine was one of the first artemisinin-based combination therapies (ACTs) evaluated, showing that a 3-day regimen was safe, well tolerated, and highly effective in treating uncomplicated multidrug-resistant *P. falciparum* malaria in Western Thailand [8]. Combining two existing drugs led to high efficacy rates despite the drugs not being fully effective on their own because of their pharmacokinetic properties (artemisinins) or parasite resistance (mefloquine). In the first 3 days of treatment the total parasite load is rapidly reduced by the highly potent yet quickly eliminated artemisinin component, and the remaining parasites are subsequently killed by the less potent yet long-lasting partner drug [9,10]. In 2006 the World Health Organization (WHO) recommended ACTs as global first-line treatments for *P. falciparum* malaria [11]. Five ACTs are recommended by the WHO: artemether–lumefantrine, artesunate–amodiaquine, artesunate–mefloquine, artesunate–sulfadoxine–pyrimethamine, and dihydroartemisinin–piperaquine [11]. A sixth ACT, artesunate–pyronaridine, has recently been added[i] [12]. Along with large-scale distribution of insecticide-treated bed nets and chemoprevention in pregnant women and children, the rollout of ACTs has been key in reducing the incidence of malaria and related deaths over the past decade, particularly in sub-Saharan Africa, which bears the brunt of the global malaria burden [13–15].

[1]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[2]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[3]WorldWide Antimalarial Resistance Network – Asia-Pacific Regional Centre, Bangkok, Thailand

*Correspondence:
arjen@tropmedres.ac (A.M. Dondorp).

Artemisinin and Partner Drug Resistance Cause ACT Failure

Fast clearance of ring-stage parasites distinguishes artemisinins from other antimalarials. Artemisinin resistance in *P. falciparum* is characterised by much slower ring-stage parasite clearance following treatment with an ACT [16], and was first described in 2009 in western Cambodia [1]. Since then, artemisinin resistance, defined clinically in the GMS as a **parasite clearance half-life** of >5.5 h, has spread through the GMS and is now present in >80% of infections in Northeast Thailand, Cambodia, and Vietnam [17]. The *in vivo* artemisinin-resistant phenotype associates with *in vitro* higher survival in a purposely designed **ring-stage survival assay**. It also has a strong association with point mutations in the propeller domain of the *P. falciparum Kelch* gene (*K13)* [18–20], which indicated the presence of artemisinin resistance at a lower frequency in Southern Laos [21]. Reduced sensitivity to artemisinin leads to a larger proportion of parasites surviving the initial 3 days of treatment, which results in an increase in parasite biomass from ~10^5–10^6 to 10^8–10^9 parasites exposed to only the ACT partner drug [22]. The residual partner drug levels will suppress sensitive parasites but allow selection of resistant parasites. Artemisinin resistance followed by selection for partner drug resistance has been observed recently for mefloquine (Thai–Myanmar border) and for piperaquine (northeast Thailand, Cambodia, and Vietnam), and maybe also amodiaquine (Cambodia), and has resulted in unacceptably high failure rates of these ACTs [17,23–26]. **Therapeutic efficacy studies** (**TESs**), following the WHO protocol, show that artesunate–mefloquine, artesunate–pyronaridine, and artemether–lumefantrine are currently efficacious in Cambodia [12], although all of these have shown compromised efficacy in past studies [27–29]. The three ACTs in Cambodia that now show treatment efficacies ≤90%, the WHO-defined threshold for minimal efficacy acceptable for a first-line antimalarial drug, are dihydroartemisinin–piperaquine, artesunate–sulfadoxine-pyrimethamine, and artesunate–amodiaquine [12]. A major concern is the potential for artemisinin and partner drug resistance to spread to Myanmar, Bangladesh, India, and sub-Saharan Africa, similar to the previous spread of resistance to chloroquine and sulfadoxine–pyrimethamine that contributed to millions of malaria-related deaths in African children [30–32] (Box 1). Recent reports

Box 1. Antimalarial Drug Resistance: Past and Present

Chloroquine-resistant *P. falciparum* parasites appear to have evolved independently in the 1950s and 1960s on at least four separate occasions, but the lineage originating from Cambodia in Southeast Asia migrated westward, and by the late 1980s chloroquine-resistant parasites had spread throughout the African continent [32] with a direct and severe impact. A two- to three-fold increase in malaria-related deaths was observed in multiple countries and different epidemiological contexts across the African continent, with up to a sixfold increase in malaria-attributable mortality reported in some locations [31]. Sulfadoxine- and pyrimethamine-resistant parasites again emerged and spread from Southeast Asia to Africa very soon after the combination was introduced to replace chloroquine, rendering these safe, cheap, and widely available drugs ineffective for the treatment of *P. falciparum* malaria [87]. Artemisinin-resistant *P. falciparum* emerged in Western Cambodia probably already in the early 2000s, even before artemisinin-based combination therapies (ACTs) were recommended by the World Health Organization as first-line treatments. It is again likely to have resulted from their widespread inappropriate use as monotherapies at suboptimal dosing in nonimmune populations with higher parasite densities, exacerbated by substandard drugs [88].

Molecular epidemiology has facilitated the tracking of artemisinin-resistant *P. falciparum* across Southeast Asia [89,90]. Its spread through the GMS triggered an emergency response to eliminate malaria from the region to prevent further spread to South Asia and onwards to Africa. There have nonetheless been observations of artemisinin-resistant *P. falciparum* in India, and more recently independent emergence of artemisinin resistance has been confirmed in Rwanda [33,34,39]. Both settings provide a context for the emergence of artemisinin resistance. In India, artesunate–sulfadoxine–pyrimethamine was until recently the first-line ACT in the presence of widespread sulfadoxine–pyrimethamine resistance, providing an environment for the emergence and spread of artemisinin resistance. In Rwanda, ACT coverage has risen to very high levels, which has contributed to an impressive reduction in *P. falciparum* transmission. However, this has left an intense selective drug pressure on the remaining parasites, infecting a population with low immunity. This implies a worrying scenario of artemisinin-resistant *P. falciparum* spreading across South Asia simultaneously with its *de novo* emergence in Africa. They underline the immediacy and importance of the need for globally deployable novel treatments for multidrug-resistant *P. falciparum*.

Glossary

Dormancy: a state in which the metabolism of *P. falciparum* parasites, hence progression through their life cycle, is slowed or arrested. It has been proposed as a characteristic of artemisinin-resistant parasites which allows escape from killing by artemisinins.

Elimination half-life ($t_{½}$): an estimate of the time it takes for the *in vivo* concentration of a drug to be reduced by 50% from its maximum. The $t_{½}$ of antimalarial drugs is variable, ranging from approximately 45 min (artemisinins) to weeks (e.g., 3–5 weeks for piperaquine). It is easier for parasites to develop resistance to drugs with a longer $t_{½}$ since they remain at subinhibitory levels in the blood over multiple parasite life cycles.

Parasite clearance half-life ($PC_{½}$): a measure of the rate at which parasites are cleared from the peripheral circulation following treatment with an antimalarial drug. It is defined as the slope of the log-linear parasite density curve when plotted against time in hours. $PC_{½}$ estimation is the only way in which an artemisinin-resistance phenotype can be detected *in vivo*. A $PC_{½}$ of >5.5 h is considered to be indicative of artemisinin resistance in southeast Asia.

QT- or QTc-interval: a measurement of the time elapsed between certain electrical impulses in heart muscles. In an electrocardiogram, it is calculated as the time from the start of the 'Q' wave to the end of the 'T' wave. A prolongation of QT-intervals is considered to be an indicator of potential cardiotoxicity of drugs. QT-intervals increase naturally as fever subsides and heart rates decrease, and hence corrected QT ('QTc') intervals are often used in cardiac safety assessments of antimalarial drugs.

Recrudescent infections: recurrence of microscopically detectable parasitaemia with progeny of parasites from the primary infection. They are distinct from reinfections which occur following a new infective mosquito bite. Recrudescences must be confirmed by verifying that the genotype of parasites from the primary and recurrent infections are the same.

Ring-stage survival assay (RSA): an *in vitro* drug susceptibility assay used to detect artemisinin resistance in *P. falciparum*. It is different from classical drug susceptibility assays in that

from India of decreased *in vitro* and *in vivo* sensitivity of *P. falciparum* to artemisinins, although in only a few cases or isolates, will have to be monitored closely [33,34]. Mutations in the *K13* gene have been reported from Africa, but in low frequencies consistent with background mutation rates rather than gene selection, or in *K13* positions that are not associated with artemisinin resistance, such as the *K13* A578S mutation [15,35–37]. However, recent reports from South America and Africa of what appears to be independent emergence of artemisinin-resistant parasites with *K13* mutations are a cause for alarm. Parasites carrying a *K13* C580Y mutation of independent origin and showing an artemisinin-resistant phenotype *in vitro* have been isolated from patients in French Guyana [38]. Studies conducted at three sites in Rwanda showed a 1–20% prevalence of parasites with a single origin carrying a validated artemisinin-resistant marker, *K13* R561H. Infections with these parasites were associated with delayed parasite clearance and constitute strong first evidence of local emergence and spread of artemisinin resistance in Africa [39].

New treatments are urgently needed in areas where artemisinin and partner drug resistance are established. They must be effective, well tolerated, safe, affordable, and less likely to fall to resistance. Although antimalarial drugs in the development pipeline look promising, it is difficult to predict if and when these will reach the market. Most estimates are that new compounds will not be available for wide-scale deployment within the next 4 years [40–42]. Meanwhile, the window of opportunity to prevent widespread resurgence of multidrug-resistant malaria followed by a likely increase of difficult-to-treat multidrug-resistant falciparum malaria infections is narrowing. Occasional case reports of patients with severe falciparum malaria not responding to intravenous artesunate therapy is particularly concerning [43]. In the recent past, high treatment failure with the then first-line antimalarial dihydroartemisinin-piperaquine in Eastern Cambodia and Southwestern Vietnam was anecdotally associated with an increase in falciparum malaria cases.

Strategies to Overcome ACT Failure Using Existing Antimalarials

Using existing antimalarials in novel ways could sustain their efficacy [22,44]. One option is to prolong the duration of therapy. A 3-day course of oral artesunate followed by a 3-day course of ACTs was fully effective, even in areas with clearly established artemisinin resistance [45]. However, this could likely result in lower adherence to the therapy since patients generally feel better quickly, which makes them less likely to complete a 6-day treatment course. Alternatively, different ACTs could be used as first-line treatment, either sequentially or at the same time [46]. If deployed sequentially, the first-line treatment policy would be changed at fixed intervals or when resistance to one of the partner drugs reaches a predefined threshold. In theory, using multiple ACTs at the same time could be the better approach as it would reduce the selective pressure on individual partner drugs – the chance for a parasite to develop resistance to the partner drugs in circulation approaches the product of the probabilities of developing resistance to each partner drug, provided there is no cross-resistance between the drugs in use [47,48]. Comprehensive and consistent implementation of such strategies, however, is complex, costly, and challenging in under-resourced countries. In the GMS, rapid change to a new ACT has proven to be difficult – in two countries, it could not be achieved before multidrug-resistant parasites had already spread widely [17].

Triple Artemisinin-Based Combination Therapies as First-Line Treatment for Malaria

Another strategy to prolong the utility of existing antimalarials is to combine two partner drugs with artemisinins in triple artemisinin-based combination therapies (TACTs). Such triple-drug combinations are now standard for treatment of tuberculosis and HIV infections [49,50]. Compared to ACTs, this could sustain efficacy of the drugs over longer periods, even in the context of artemisinin resistance, wherein partner drugs are exposed to a higher parasite biomass,

cultured parasites are tightly synchronised and exposed to artemisinins over a brief segment of their life cycle in order to approximate *in vivo* exposure.

Therapeutic efficacy study (TES): clinical trials conducted per a standardised methodology to assess the efficacy of antimalarial drugs. TESs are performed periodically in malaria-endemic countries to verify the efficacy of drugs being used as first-line treatments or their potential replacements. Recrudescences in ≥10% of adequately treated patients in a TES constitutes a sign of emerging resistance and should trigger a change in the first-line treatment policy.

are unprotected, and are thus more prone to the development of resistance. Partner drugs for TACTs would need to be carefully chosen to provide mutual protection. Their elimination half-lives, or more precisely the time periods of blood concentrations above the minimal parasiticidal concentrations, must be similar to avoid in effect exposing parasites to a single drug (Figure 1, Key Figure). Further, the mechanisms of resistance to either drug must at least be distinct if not mutually counteractive. As with multiple first-line treatments, this approach would reduce the chance of resistance emerging to either of the partner drugs while delivering highly effective treatment to the individual patient.

The recent Tracking Resistance to Artemisinin Collaboration II (TRACII) trial assessed the safety, tolerability, and efficacy of two such TACTs [51]. Based on their pharmacokinetic profiles, mefloquine was added to the ACT dihydroartemisinin–piperaquine (DHA–PPQ) and amodiaquine was added to artemether–lumefantrine (AL) respectively to constitute the TACTs. These combinations also took advantage of counterbalancing resistance mechanisms observed in field and laboratory studies between piperaquine versus mefloquine and lumefantrine versus amodiaquine [24,52–57]. Both TACTs were highly efficacious throughout Asia. In Thailand, Cambodia, and Vietnam, where DHA–PPQ failed in ~50% of patients, DHA–PPQ+MQ was 98% effective, whereas efficacy data of AL+AQ in the same areas will be published shortly. TACTs were safe and generally well tolerated, although loss of appetite, nausea and vomiting occurred with slightly higher frequency. This indicates that TACTs could present an effective option to treat drug-resistant malaria and could be made available quickly. Atovaquone and pyronaridine have very different mechanisms of action, which makes this also a potentially attractive combination [58,59]. A trial comparing the TACT artesunate–pyronaridine with atovaquone–proguanil and the TACT artesunate–mefloquine with atovaquone–proguanil versus the ACT artesunate–pyronaridine is currently underway in Cambodia (ClinicalTrials.gov identifier: NCT03726593).

Global Deployment of TACTs: Potential Strategies and Barriers

Multiple reasons prevented rapid uptake of ACTs throughout the world, including costs and availability, delays in decision making and implementation of policy, and concerns from policymakers, healthcare providers, and patients. Similar and additional issues will likely surface with the potential future roll-out of TACTs.

Dosing, Formulation, and Production of TACTs

Optimizing the composition of TACTs and dosing regimens has to consider age-stratified pharmacokinetic drug profiles, dose–effect relationships, and dose-related toxicity and tolerability [60–62]. Suboptimal dosing of any of the components of the TACTs facilitates incomplete parasite clearance and subsequent recrudescences, and thus selection of drug-resistant parasites [62–65]. Coformulation enables accurate dosing, but pharmaceutical issues sometimes affect drug stability. Additionally, *in vivo* interactions between the drugs in the combination need to be assessed [66]. A multitude of factors will determine the additional costs associated with the development of the TACTs. However, most antimalarials currently in use are off-patent, thereby limiting the costs of individual drugs. Although coformulation of TACTs would be preferable regarding patient adherence, coblistering of an ACT and a second partner drug should be considered as a more rapidly available option in case TACTs are needed urgently in the coming years.

Efficacy, Safety, and Tolerability of TACTs in Adults and Children in Asia and Africa

The safety and tolerability of all currently available ACTs and individual antimalarial components are well established. Common side effects of antimalarial drugs are fatigue, headache, dizziness, nausea, vomiting, and abdominal pain. Most of these symptoms are also common in malaria infections and therefore often result in an overestimation of the side effects of antimalarials.

Key Figure

Parasite Clearance Dynamics and Partner Drug Elimination Dynamics in Artemisinin-Based Combination Therapies (ACTs) and Triple Artemisinin-Based Combination Therapies (TACTs)

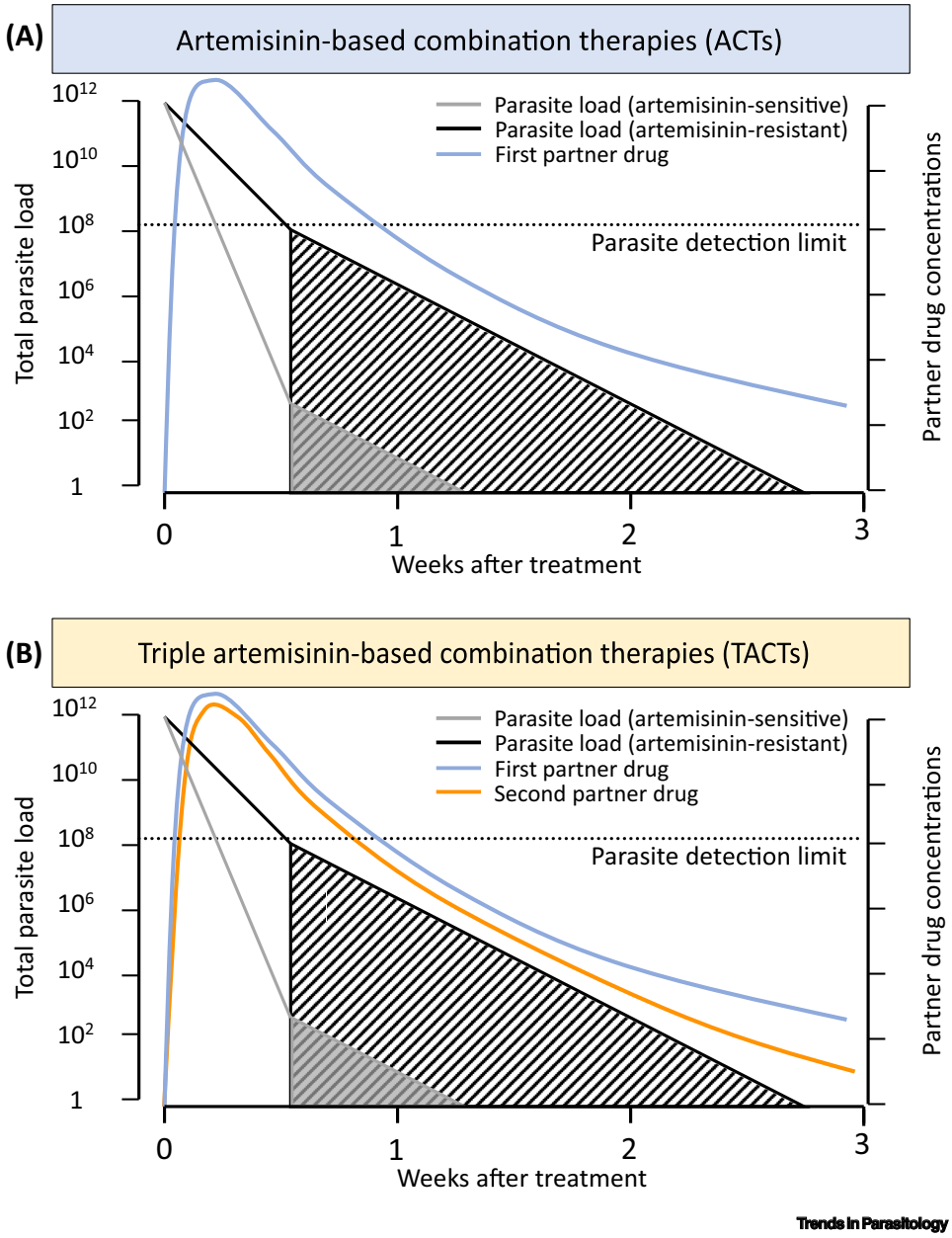

Figure 1. (A) Following 3 days of treatment, parasites are rapidly cleared predominantly by the highly potent yet rapidly eliminated artemisinins. With effective ACTs, any remaining parasites that have not been cleared by artemisinins will be cleared by the partner drug. In the case of artemisinin resistance, parasite reduction over one 48 h parasite life cycle is

(Figure legend continued at the bottom of the next page.)

Combining an ACT with another drug will likely result in additional side effects or increase the intensity of side effects attributable to the antimalarial treatment. It is important to quantify this increased risk of adverse events and to assess whether they are dose- or concentration-dependent. Many current antimalarials, including quinine, chloroquine, amodiaquine, mefloquine, and piperaquine have cardiovascular side effects such as exacerbation of malaria-related orthostatic hypotension, sinus bradycardia, and **QTc-interval** prolongation, but all well within acceptable safety limits when used in therapeutic doses [67,68]. The QTc-interval is often used as a surrogate marker for the arrhythmogenic risk of antimalarials. The TRACII trial (1100 patients randomised to receive DHA–PPQ (n = 183), DHA–PPQ+MQ (n = 269), artesunate–mefloquine (n = 73), AL (n = 289) and AL+AQ (n = 286)) showed that the addition of mefloquine to DHA–PPQ did not further increase the prolongation of the QTc-interval that is seen over time after administration of DHA–PPQ [51]. Addition of amodiaquine to AL slightly increased the prolongation of the QTc-interval, comparable to administration of amodiaquine alone, and did not raise safety concerns. More extensive data on the safety, tolerability, and efficacy of TACTs, in particular in children from sub-Saharan Africa, will become available in the near future (ClinicalTrials.gov Identifiers: NCT03923725 and NCT03939104).

The (Cost-)effectiveness of TACTs to Prevent or Delay the Emergence of Resistance

Clinical trials on TACTs will not be able to address the potential long-term benefits of the deployment of TACTs to prevent or delay the development of antimalarial drug resistance, and modelling could provide valuable insights here. In the past, mathematical modelling identified the role of mismatched pharmacokinetic and pharmacodynamic (PK/PD) profiles in promoting the evolution of drug-resistant malaria [69]. In recent times there has been a rapid growth in the development and application of models to guide antimalarial drug development, including PK/PD modelling to identify appropriate candidates for development, incorporating laboratory measures of drug resistance and the epidemiological spread of resistance, and transmission models to guide drug deployment and use scenarios [70,71]. Mathematical modelling has been used to estimate economic losses that would follow ACT failure; direct medical costs for malaria treatment would be 28% higher (US$ 146 million) and the cost of policy change if switching between ACTs would be US$ 130 million [72]. Extensions of such models may be helpful in forecasting the future impact of deploying novel antimalarial therapies, although there are limitations and assumptions inherent to each model. Mathematical modelling could assess the likely epidemiological impact of TACTs as first-line antimalarial treatments in different malaria-endemic settings. This will quantify the potential of TACTs to delay the emergence and spread of artemisinin- and partner-drug-resistant falciparum malaria in the context of different transmission intensities, population mobility, and fitness costs of mutations conferring drug resistance. Pharmacokinetic modelling for partner drugs could determine the dose of the partner drugs at which toxicity is minimised while drug levels are sufficiently high to kill parasites that were not eliminated in the first 3 days of treatment, while shortening the window of selection, defined as the time that parasites are exposed to sub-optimal drug levels [73]. In addition, economic modelling could assess the cost-effectiveness of deploying TACTs in different endemic settings, incorporating the potential additional costs of

100-fold compared to 10 000-fold in sensitive strains, leaving a much higher parasite load after 3 days of ACT treatment for the partner drug to clear (lined area) compared to the case of artemisinin-sensitive parasites (grey area) [1]. Thereby artemisinin resistance leads to a larger parasite biomass exposed to a single partner drug. This higher parasite burden, facing declining partner-drug concentrations during an extended period, enables the selection of partner-drug-resistant parasites. Treatment failure occurs when parasites survive both the artemisinin and partner drug. (B) TACTs involve addition of a second partner drug, carefully chosen on the basis of having a matching pharmacokinetic profile, different mode of action, and no cross-resistance, compared to the other partner drug. This approach could increase the efficacy of TACTs compared to ACTs, when resistance already exists, and provide a stronger defence against resistance when resistance has not yet emerged, thereby prolonging the longevity of artemisinins and partner drugs.

TACTs versus conventional ACTs versus the savings made through reducing and preventing malaria morbidity and mortality resulting from antimalarial drug resistance.

Will TACTs Be Accepted in the Setting of ACTs That Are Still Efficacious?

Ethical aspects of malaria control and elimination have received limited attention but need to be addressed to minimise potential injustice to the autonomy of individuals while ensuring an advantageous risk–benefit balance [74]. Deploying TACTs as first-line treatment in Africa in the absence of widespread ACT failure raises an ethical question: why should a patient in Africa, often a child, take three drugs for malaria instead of two when two drugs work, and when the addition of a drug may result in increased side effects? This question shares some similarities with the ethical considerations of mass antimalarial drug administration, where the long-term benefit for the community will have to be taken partly on trust and cannot be guaranteed [75]. Social and cultural issues delayed the adoption of ACTs over inefficacious conventional antimalarials in Africa [76]. Past experience suggests that TACTs may not be accepted and prescribed without prior engagement with communities to devise culturally sensitive methods of increasing acceptance and adherence. Critical examination of the views of stakeholders, ranging from patients and parents to regulatory authorities and national malaria control programmes, will contribute to developing recommendations to guide deployment of TACTs in Africa. Without such engagement, acceptance of TACTs is likely to be poor which, in turn, would reduce the impact of deploying TACTs to prevent or delay emergent resistance. The recent emergence of artemisinin resistance in Rwanda, however, might change the risk–benefit and ethical analyses on the deployment of TACTs in Africa, making TACTs more urgently needed.

Market Positioning and Large-Scale Production and Deployment of TACTs

The transition to ACTs for the treatment of malaria has been described as one of the major challenges faced by malaria control in the recent past [77]. Indeed, cost has been cited as the main factor that prevented governments from switching to ACTs [78]. In addition to the social and cultural aspects described previously, market instability had a major influence in deterring widespread adoption and deployment of ACTs, and barriers were not restricted to supply and demand dynamics [79,80]. Lack of coordination in distribution chains and of private sector compliance have also posed additional challenges. Many stakeholders are involved in the decision to deploy new antimalarials and their views, attitudes, and concerns need to be assessed at an early stage in order to devise strategies for appropriate market positioning of TACTs. Manufacturers will have to be convinced that investments in developing and producing TACTs are warranted. There is a large market; over the period 2010–2018, ~3 billion treatment courses of ACTs were procured for the treatment of malaria, worldwide [15]. However, malaria is mainly a disease of underprivileged and poor populations and therefore antimalarial drug prices need to be kept very affordable for both the private and public sector. A dialogue between manufacturers and WHO, national malaria-control programmes, academic groups, civil society representation, Medicines for Malaria Venture (MMV), funding mechanisms such as Global Fund, Wellcome, Bill and Melinda Gates Foundation, and other stakeholders will be important to support manufacturers in this decision making.

Concluding Remarks

The underlying scientific rationale for TACTs is easily understood: if drugs with different targets and/or resistance mechanisms are combined, the probability that resistance will emerge to any of its components will be reduced. Effective antimalarials are the cornerstone of national malaria-control programmes, and prevention or delay of antimalarial resistance is essential for the longer-term goal of malaria elimination. In low-transmission zones with high ACT failure rates, such as Cambodia, effective treatment with TACTs will reduce transmission, which is amplified by the prevention of recrudescent infections that produce more gametocytes than primary infections and are more transmissible [81,82].

Outstanding Questions

Which antimalarial drugs should and should not be combined in triple artemisinin-based combination therapies (TACTs), considering pharmacokinetic interactions, efficacy, safety, drug resistance mechanisms, synergistic and antagonistic effects?

What is the optimum dose of individual drugs in TACTs? Does coformulation of drugs affect stability and absorption? If so, how can this be addressed?

What can be incentives for pharmaceutical companies to develop TACTs? What will be the regulatory requirements for TACTs with regard to drug licensing and registration?

What are the safety, tolerability, and efficacy of TACTs such as dihydroartemisinin–piperaquine plus mefloquine, artesunate–mefloquine plus piperaquine and artemether–lumefantrine plus amodiaquine, specifically in children in sub-Saharan Africa? Will they be safe for repeated use – which is particularly relevant for areas of high malaria transmission?

Standard ACTs could still have an acceptable efficacy (>90%) in the setting of artemisinin and/or partner-drug resistance. In this setting, are TACTs more effective?

Will deploying TACTs in areas with existing resistance affect the selection of multidrug-resistant malaria?

What is the effectiveness of TACTs in preventing emergence or importation of antimalarial resistance in areas where antimalarial resistance is not clearly established, and how will this vary based on coverage, adherence, and transmission intensity?

What is the cost-effectiveness of deploying TACTs, considering the additional costs of adding an active pharmaceutical ingredient, development and production of the combination, versus the potential important increase in the lifetime of the drug?

Will TACTs be accepted in areas where ACTs are still effective? Is it ethical to expose patients to TACTs that might have more side effects than ACTs to prevent emergence of resistance in the future?

How can barriers towards potential widescale deployment of TACTs be addressed at an early stage?

Some key questions should be addressed to facilitate the development, deployment, and uptake of TACTs (see Outstanding Questions). Choosing the right combinations of drugs and the optimum dose of each individual component is important. Coformulating drugs in triple combinations requires investments by manufacturers. Early provision of a business case analysis for pharmaceutical companies on the economic viability of TACTs, which includes current funding structures for malaria drugs, such as the Global Fund, will likely help engage manufacturers. From the start of the development process it will be important to have insight in the regulatory requirements for licensing of TACTs through WHO prequalification or stringent regulatory authorities. For the potential adaptation of TACTs in treatment guidelines, it will be prudent that study plans, and results, are shared between academia and nonacademic stakeholders, and early involvement of policy makers and the WHO is important. More information on the safety, efficacy, and tolerability of TACTs is needed, especially in children in sub-Saharan Africa. Additionally, the safety of repeated treatments with TACTs should be assessed to support their deployment in high-transmission areas. In areas like the GMS, where artemisinin and partner drug resistance has emerged, TACTs outperforming the efficacy of standard ACTs will be a strong argument for their deployment. For areas not yet affected by these resistance problems, a continued discussion will be needed on the pros and cons of TACT as a new paradigm to break the ever-returning chain of events associated with drug resistance that severely reduces the useful lifetime of valuable antimalarial drugs [83,84]. With reduced *P. falciparum* transmission in many parts of South America and sub-Saharan Africa the chance of artemisinin and partner drug emergence and spread increases for a variety of reasons. Reduced transmission, which involves better treatment coverage, will decrease population level immunity, which will increase the proportion of symptomatic patients seeking treatment, and will increase further antimalarial drug pressure on the parasite population. Higher drug pressure increases the chance for antimalarial drug resistance to emerge, and reduced immunity increases the probability of survival of low-grade resistant parasites. With reduced transmission, there will be fewer multiclonal infections with subsequent recombination events during the sexual stage of the parasite in the *Anopheles* mosquito, increasing the chance that resistant haplotypes remain intact. When transmission further decreases, genetic diversity will decline, also translating to fewer recombination events between artemisinin- (or partner drug-) sensitive and resistant strains. The increased ACT coverage, and the reduction in *P. falciparum* malaria transmission, might thus have been drivers for the recently described emergence and local spread of artemisinin resistance in Rwanda [39]. To prevent the further spread or emergence of multidrug-resistant falciparum malaria the deployment of TACTs, worldwide, deserves serious consideration.

As described, several important issues around deployment of TACTs need to be addressed; this will require a multifaceted approach to identify and overcome barriers and provide convincing evidence of the potential benefit of TACTs in order to gain support and endorsement by policy makers. This is being addressed by the Development of Triple Artemisinin-based Combination Therapies (DeTACT) project, which takes a holistic approach by partnering with pharmaceutical companies, MMV, collaborators in Asia and Africa to conduct clinical trials, and experts in the fields of mathematical modelling, market positioning, bioethics, and engagement. Realizing the vision of a world free of malaria might become a reality if we act now [85,86].

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Resources

[i] www.mmv.org/access/products-projects/pyramax-pyronaridine-artesunate

References

1. Dondorp, A.M. *et al.* (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 361, 455–467
2. Meshnick, S.R. *et al.* (1996) Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol. Rev.* 60, 301–315
3. Okell, L.C. *et al.* (2008) Modelling the impact of artemisinin combination therapy and long-acting treatments on malaria transmission intensity. *PLoS Med.* 5, e226 discussion e226
4. Li, G.Q. *et al.* (1984) Randomised comparative study of mefloquine, qinghaosu, and pyrimethamine-sulfadoxine in patients with falciparum malaria. *Lancet* 2, 1360–1361
5. Li, G.Q. *et al.* (1994) Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans. R. Soc. Trop. Med. Hyg.* 88, S5–S6
6. Teuscher, F. *et al.* (2010) Artemisinin-induced dormancy in *Plasmodium falciparum*: duration, recovery rates, and implications in treatment failure. *J. Infect. Dis.* 202, 1362–1368
7. Witkowski, B. *et al.* (2010) Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. *Antimicrob. Agents Chemother.* 54, 1872–1877
8. Nosten, F. *et al.* (1994) Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J. Infect. Dis.* 170, 971–977
9. White, N.J. (2013) Pharmacokinetic and pharmacodynamic considerations in antimalarial dose optimization. *Antimicrob. Agents Chemother.* 57, 5792–5807
10. White, N.J. (2011) The parasite clearance curve. *Malar. J.* 10, 278
11. World Health Organization (2015) *Guidelines for the Treatment of Malaria* (3rd edition), WHO
12. World Health Organization (2019) *Status Report on Artemisinin Resistance and ACT Efficacy*, WHO
13. Adjuik, M. *et al.* (2004) Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 363, 9–17
14. Bhatt, S. *et al.* (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207–211
15. World Health Organization (2019) *World Malaria Report*, WHO
16. Flegg, J.A. *et al.* (2011) Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. *Malar. J.* 10, 339
17. van der Pluijm, R.W. *et al.* (2019) Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect. Dis.* 19, 952–961
18. Ariey, F. *et al.* (2014) A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505, 5055
19. Witkowski, B. *et al.* (2013) Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: *in-vitro* and *ex-vivo* drug-response studies. *Lancet Infect. Dis.* 13, 1043–1049
20. Straimer, J. *et al.* (2015) Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. *Science* 347, 428–431
21. Iwagami, M. *et al.* (2018) Heterogeneous distribution of k13 mutations in *Plasmodium falciparum* in Laos. *Malar. J.* 17, 483
22. Dondorp, A.M. *et al.* (2017) How to contain artemisinin- and multidrug-resistant falciparum malaria. *Trends Parasitol.* 33, 353–363
23. Phyo, A.P. *et al.* (2016) Declining efficacy of artemisinin combination therapy against *P. falciparum* malaria on the Thai-Myanmar border (2003–2013): the role of parasite genetic factors. *Clin. Infect. Dis.* 63, 784–791
24. Amaratunga, C. *et al.* (2016) Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. *Lancet Infect. Dis.* 16, 357–365
25. Phuc, B.Q. *et al.* (2017) Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria, Vietnam. *Emerg. Infect. Dis.* 23, 715–717
26. Mairet-Khedim, M. *et al.* (2020) Clinical and *in vitro* resistance of *Plasmodium falciparum* to artesunate-amodiaquine in Cambodia. *Clin. Infect. Dis.* Published online May 27, 2020. https://doi.org/10.1093/cid/ciaa628
27. Denis, M.B. *et al.* (2006) Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Cambodia. *Tropical Med. Int. Health* 11, 1800–1807
28. Denis, M.B. *et al.* (2006) Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. *Tropical Med. Int. Health* 11, 1360–1366
29. Leang, R. *et al.* (2016) Efficacy and safety of pyronaridine-artesunate for treatment of uncomplicated *Plasmodium falciparum* malaria in Western Cambodia. *Antimicrob. Agents Chemother.* 60, 3884–3890
30. Roper, C. *et al.* (2004) Intercontinental spread of pyrimethamine-resistant malaria. *Science* 305, 1124
31. Trape, J.F. (2001) The public health impact of chloroquine resistance in Africa. *Am. J. Trop. Med. Hyg.* 64, 12–17
32. Wootton, J.C. *et al.* (2002) Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. *Nature* 418, 320–323
33. Das, S. *et al.* (2019) Novel pfkelch13 gene polymorphism associates with artemisinin resistance in Eastern India. *Clin. Infect. Dis.* 69, 1144–1152
34. Das, S. *et al.* (2018) Evidence of artemisinin-resistant *Plasmodium falciparum* malaria in Eastern India. *N. Engl. J. Med.* 379, 1962–1964
35. Bwire, G.M. *et al.* (2020) Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of Tanzania. *Sci. Rep.* 10, 3500
36. Menard, D. *et al.* (2016) A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. *N. Engl. J. Med.* 374, 2453–2464
37. Ikeda, M. *et al.* (2018) Artemisinin-resistant *Plasmodium falciparum* with high survival rates, Uganda, 2014–2016. *Emerg. Infect. Dis.* 24, 718–726
38. Mathieu, L.C. *et al.* (2020) Local emergence in Amazonia of *Plasmodium falciparum* k13 C580Y mutants associated with *in vitro* artemisinin resistance. *eLife* 9, e51015
39. Uwimana, A. *et al.* (2020) Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat. Med.* Published online August 3, 2020. https://doi.org/10.1038/s41591-020-1005-2
40. Ashley, E.A. and Phyo, A.P. (2018) Drugs in development for malaria. *Drugs* 78, 861–879
41. Tse, E.G. *et al.* (2019) The past, present and future of anti-malarial medicines. *Malar. J.* 18, 93
42. White, N.J. (2016) Can new treatment developments combat resistance in malaria? *Expert. Opin. Pharmacother.* 17, 1303–1307
43. Phyo, A.P. *et al.* (2018) Poor response to artesunate treatment in two patients with severe malaria on the Thai–Myanmar border. *Malar. J.* 17, 30
44. Tilley, L. *et al.* (2016) Artemisinin action and resistance in *Plasmodium falciparum*. *Trends Parasitol.* 32, 682–696
45. Ashley, E.A. *et al.* (2014) Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 371, 411–423
46. Boni, M.F. *et al.* (2016) The community as the patient in malaria-endemic areas: preempting drug resistance with multiple first-line therapies. *PLoS Med.* 13, e1001984
47. Nosten, F. and White, N.J. (2007) Artemisinin-based combination treatment of falciparum malaria. *Am. J. Trop. Med. Hyg.* 77, 181–192
48. White, N.J. (1999) Delaying antimalarial drug resistance with combination chemotherapy. *Parassitologia* 41, 301–308
49. World Health Organization (2016) *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection*, WHO
50. World Health Organization (2019) *WHO Consolidated Guidelines on Drug-resistant Tuberculosis Treatment*, WHO
51. van der Pluijm, R.W. *et al.* (2020) Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated *Plasmodium falciparum* malaria: a multicentre, open-label, randomised clinical trial. *Lancet* 395, 1345–1360
52. Lim, P. *et al.* (2015) Decreasing pfmdr1 copy number suggests that *Plasmodium falciparum* in Western Cambodia

is regaining *in vitro* susceptibility to mefloquine. *Antimicrob. Agents Chemother.* 59, 2934–2937
53. Veiga, M.I. *et al.* (2016) Globally prevalent PfMDR1 mutations modulate *Plasmodium falciparum* susceptibility to artemisinin-based combination therapies. *Nat. Commun.* 7, 11553
54. Venkatesan, M. *et al.* (2014) Polymorphisms in *Plasmodium falciparum* chloroquine resistance transporter and multidrug resistance 1 genes: parasite risk factors that affect treatment outcomes for *P. falciparum* malaria after artemether-lumefantrine and artesunate-amodiaquine. *Am. J. Trop. Med. Hyg.* 91, 833–843
55. Folarin, O.A. *et al.* (2011) *In vitro* amodiaquine resistance and its association with mutations in pfcrt and pfmdr1 genes of *Plasmodium falciparum* isolates from Nigeria. *Acta Trop.* 120, 224–230
56. Price, R.N. *et al.* (2004) Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. *Lancet* 364, 438–447
57. Sisowath, C. *et al.* (2005) *In vivo* selection of *Plasmodium falciparum* pfmdr1 86N coding alleles by artemether-lumefantrine (Coartem). *J. Infect. Dis.* 191, 1014–1017
58. Croft, S.L. *et al.* (2012) Review of pyronaridine anti-malarial properties and product characteristics. *Malar. J.* 11, 270
59. Nixon, G.L. *et al.* (2013) Antimalarial pharmacology and therapeutics of atovaquone. *J. Antimicrob. Chemother.* 68, 977–985
60. Mwesigwa, J. *et al.* (2010) Pharmacokinetics of artemether-lumefantrine and artesunate-amodiaquine in children in Kampala, Uganda. *Antimicrob. Agents Chemother.* 54, 52–59
61. Tarning, J. *et al.* (2012) Population pharmacokinetics and pharmacodynamics of piperaquine in children with uncomplicated falciparum malaria. *Clin. Pharmacol. Ther.* 91, 497–505
62. WorldWide Antimalarial Resistance Network, D.P.S.G (2013) The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin-piperaquine: a pooled analysis of individual patient data. *PLoS Med.* 10, e1001564 discussion e1001564
63. Barnes, K.I. *et al.* (2008) Antimalarial dosing regimens and drug resistance. *Trends Parasitol.* 24, 127–134
64. White, N.J. *et al.* (2009) Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. *Malar. J.* 8, 253
65. Barnes, K.I. *et al.* (2006) Sulfadoxine-pyrimethamine pharmacokinetics in malaria: pediatric dosing implications. *Clin. Pharmacol. Ther.* 80, 582–596
66. Hanboonkunupakarn, B. *et al.* (2019) Sequential open-label study of the safety, tolerability, and pharmacokinetic interactions between dihydroartemisinin-piperaquine and mefloquine in healthy Thai adults. *Antimicrob. Agents Chemother.* 63, e00060-19
67. World Health Organization (2017) *The Cardiotoxicity of Antimalarials*, WHO
68. Haeusler, I.L. *et al.* (2018) The arrhythmogenic cardiotoxicity of the quinoline and structurally related antimalarial drugs: a systematic review. *BMC Med.* 16, 200
69. Hastings, I.M. *et al.* (2002) The evolution of drug-resistant malaria: the role of drug elimination half-life. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 357, 505–519
70. Slater, H.C. *et al.* (2016) Assessing the potential impact of artemisinin and partner drug resistance in sub-Saharan Africa. *Malar. J.* 15, 10
71. Slater, H.C. *et al.* (2017) Mathematical modelling to guide drug development for malaria elimination. *Trends Parasitol.* 33, 175–184
72. Lubell, Y. *et al.* (2014) Artemisinin resistance–modelling the potential human and economic costs. *Malar. J.* 13, 452
73. Dini, S. *et al.* (2018) Investigating the efficacy of triple artemisinin-based combination therapies for treating *Plasmodium falciparum* malaria patients using mathematical modeling. *Antimicrob. Agents Chemother.* 62, e01068-18
74. Jamrozik, E. *et al.* (2015) Ethical aspects of malaria control and research. *Malar. J.* 14, 518
75. Cheah, P.Y. and White, N.J. (2016) Antimalarial mass drug administration: ethical considerations. *Int. Health* 8, 235–238
76. Maslove, D.M. *et al.* (2009) Barriers to the effective treatment and prevention of malaria in Africa: A systematic review of qualitative studies. *BMC Int. Health Hum. Rights* 9, 26
77. Bosman, A. and Mendis, K.N. (2007) A major transition in malaria treatment: the adoption and deployment of artemisinin-based combination therapies. *Am. J. Trop. Med. Hyg.* 77, 193–197
78. Maximizing the effective use of antimalarial drugs. In *Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance* (Arrow, K.J. *et al.*, eds), pp. 312–324, National Academies Press
79. Williams, H.A. *et al.* (2004) The process of changing national malaria treatment policy: lessons from country-level studies. *Health Policy Plan.* 19, 356–370
80. Luiza, V.L. *et al.* (2017) Challenges in a product development partnership: a malaria treatment case study. *Cien Saude Colet* 22, 2197–2211
81. WWARN Gametocyte Study Group (2016) Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. *BMC Med.* 14, 79
82. Barnes, K.I. and White, N.J. (2005) Population biology and antimalarial resistance: The transmission of antimalarial drug resistance in *Plasmodium falciparum*. *Acta Trop.* 94, 230–240
83. Krishna, S. (2019) Triple artemisinin-containing combination antimalarial treatments should be implemented now to delay the emergence of resistance: the case against. *Malar. J.* 18, 339
84. White, N.J. (2019) Triple artemisinin-containing combination anti-malarial treatments should be implemented now to delay the emergence of resistance. *Malar. J.* 18, 338
85. World Health Organization (2015) *Global Technical Strategy for Malaria*, WHO, pp. 2016–2030
86. Chen, I. *et al.* (2018) The Lancet Commission on malaria eradication. *Lancet* 391, 1556–1558
87. Plowe, C.V. (2009) The evolution of drug-resistant malaria. *Trans. R. Soc. Trop. Med. Hyg.* 103, S11–S14
88. Dondorp, A.M. *et al.* (2010) Artemisinin resistance: current status and scenarios for containment. *Nat. Rev. Microbiol.* 8, 272–280
89. Hamilton, W.L. *et al.* (2019) Evolution and expansion of multidrug-resistant malaria in southeast Asia: a genomic epidemiology study. *Lancet Infect. Dis.* 19, 943–951
90. Imwong, M. *et al.* (2020) Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study. *Lancet Infect. Dis.* Published online July 14, 2020. https://doi.org/10.1016/S1473-3099(20)30228-0