Update on the in vivo tolerance and in vitro reduced susceptibility to the antimalarial lumefantrine

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Coartem®, the combination of artemether (an artemisinin derivative) and lumefantrine, has been adopted as the first-line treatment for uncomplicated malaria in many countries. The emergence of resistance to artemisinin derivatives has now been proven in South-East Asia, and there is concern that this may spread to other endemic areas. Strategies to contain and control the spread of artemisinin resistance have been proposed. On the other hand, not much attention has been given to lumefantrine. Indeed, for more than 7 years, reports have been emerging that the use of Coartem® is associated with rapid selection of lumefantrine-tolerant parasites. These parasites can survive in the presence of sub-therapeutic lumefantrine concentrations, and, interestingly, this in vivo phenotype is translated in vitro into reduced susceptibility to lumefantrine. As a result, such parasites could form the setting in which lumefantrine resistance would emerge. Thus, identifying genetic markers that reflect this phenotype (both in vitro and in vivo) could yield information on the mechanisms of lumefantrine resistance. More interestingly, lumefantrine tolerance is associated with an increase in chloroquine susceptibility, raising the possibility of re-introducing chloroquine. In this work, we have reviewed the current knowledge, and we present existing challenges and gaps with regard to the mechanisms of in vivo tolerance and in vitro reduced susceptibility to lumefantrine. The re-introduction of chloroquine in areas of high lumefantrine resistance is also discussed.

Keywords: malaria, drug resistance, lumefantrine tolerance, Pfmdr1 genotype, chloroquine

Introduction

In the early 2000s, the burgeoning problem of the rapid emergence and selection of malaria parasites resistant to monotherapy led the WHO to recommend that antimalarial treatment be based on the selection of two efficacious drugs that have different modes of action. The rationale behind this concept is that it is more difficult for a given cell or organism to develop resistance against two different drugs at the same time, thus delaying the onset of resistance. Artemisinin derivatives have been selected as drugs of choice in such combinations owing to their high efficacy and rapid onset of antiparasitic effect, leading to a rapid reduction of the parasite biomass.1,2 Such combinations are now known as artemisinin-based combination therapies (ACTs).3

The combination of lumefantrine and artemether (an artemisinin derivative), also known as Coartem®, was the first ACT to meet the WHO criteria for safety, efficacy and quality.4,5 In the mid-2000s, this drug was adopted as the first-line treatment for uncomplicated malaria by many African countries, including Kenya.4,5 In the Kingdom of Saudi Arabia, the combination of artesunate and pyrimethamine/sulfadoxine is the recommended first choice, and Coartem® is the second-line treatment.6

This combination was developed as a tablet with 80 mg of lumefantrine and 800 mg of artemether. Early investigations of a four-dose regimen (two doses per day for 2 days) were not efficacious, especially in multidrug-resistant areas such as Thailand.7 The treatment was modified to a 3 day course of six doses (one tablet per adult dose), with doses at 12 h intervals. With this regimen, this drug is efficacious, and information on most of the clinical studies has been summarized elsewhere.4,5

However, early studies indicated that the use of Coartem® selects quickly for lumefantrine-tolerant parasites. These are parasites that can grow in the presence of sub-therapeutic concentrations of lumefantrine.8,9 Interestingly, these lumefantrine-tolerant parasites have wild-type Pfmdr1 (asparagine at codon 86, Pfmdr1_86N) and, to some extent, wild-type Pfcr7 (lysine at position 76, Pfcr7_76K), the two alleles associated with chloroquine susceptibility.8,9 This raises the possibility of re-introducing chloroquine, the old and cheap antimalarial, in lumefantrine-resistant areas. In this paper, we have reviewed work carried out on lumefantrine tolerance in vivo and in vitro, and propose scenarios that could lead to the emergence of
lumefantrine resistance in vivo. We also discuss the concept of re-introducing chloroquine for the treatment of uncomplicated malaria in areas where lumefantrine resistance is dominant.

**Historical background on the use of lumefantrine as monotherapy and in combination**

Lumefantrine is the generic name for (dibutylaminomethyl)-2,7-dichloro-9-(p-chlorobenzylidene)-4-fluorenemethanol. This compound, originally known as benflumetol, was synthesized in the 1970s by the Chinese Academy of Military Medical Sciences, and registered for use as an antimalarial drug in 1987 in China. However, data on its in vivo efficacy as monotherapy are scarce. The only published information was obtained during the Scientific Working Group on the Chemotherapy of Malaria, held under the auspices of the WHO in April 1989 in Beijing. Based on this report, several clinical studies using benflumetol capsules were carried out in the 1980s. A dose of 2000 mg of lumefantrine given over 4 days (800 mg on day 0 and 400 mg daily on days 1 and 3) was used in 350 patients, with a cure rate of 96%, fever subsidence in 38–41 h and parasite clearance in 62–67 h. To the best of our knowledge, no other information on the efficacy of this drug was publicly available at that time. Figure 1 shows the chemical structures of lumefantrine and its metabolite, desbutyl-lumefantrine.

**Development of Coartem®**

In the late 1980s, lumefantrine was administered together with artemether for the treatment of *Plasmodium falciparum* infection in China. A dose of 160 mg of artemether plus 960 mg of lumefantrine on the first day, followed by 80 mg of artemether plus 480 mg of lumefantrine on days 2 and 3 was used to treat malaria. A cure rate of 92.5% was reported in 40 patients, with fever clearance and parasite clearance times of ~25 and ~43 h, respectively. In 1990, Chinese officials met with Novartis (then Ciba–Geigy) and ultimately agreed to develop and manufacture the combination lumefantrine and artemether, under the name of Coartem®, through a joint venture—the first collaboration of its kind in Chinese history. Novartis carried out drug development studies according to international standards, leading to the registration of this combination as an antimalarial in the early 2000s.

As stated above, in the early 2000s, artemisinin derivatives were singled out as drugs of choice to be used in combination therapy, and as at that time Coartem® was already in the later development stages, this new combination was chosen as the ACT to be deployed. Coartem® is now the most widely used antimalarial for the treatment of uncomplicated malaria.

**Use of Coartem® and lumefantrine tolerance**

**Pfcr and Pfmdr1 in chloroquine resistance**

As discussed below, lumefantrine tolerance is associated with polymorphisms in *Pfmdr1* and *Pfcr*, the two well-characterized chloroquine resistance genes. Therefore, we give a brief overview and a description of their role in chloroquine resistance.

*Pfcr* is a 45 kDa protein containing 10 transmembrane domains, and is located on the membrane of the digestive vacuole (DV). The DV is a compartment in which haemoglobin is degraded to haematin, a toxic agent resulting from a dimeric form of oxidized haem, and this agent is detoxified by conversion to haemozoin. Chloroquine, a weak base, concentrates in its diprotonated form in the DV, where it prevents haemozoin synthesis, leading to parasite death.

The analysis of malaria parasites from different endemic settings shows wide diversity in the *Pfcr* sequence. Single nucleotide polymorphisms (SNPs) or point mutations have been detected in 15 residues, located within codons (amino acids) 72–371. Of all these, the mutation *Pfcr_76T* (threonine at position 76) is the most critical determinant of chloroquine resistance in *in vitro* and *in vivo*, making it a useful marker to map and monitor the emergence and spread of chloroquine resistance.

The *P. falciparum* multidrug-resistance transporter gene, *Pfmdr1* (encoding Pglyh1, an orthologue of mammalian P-glycoprotein), was initially identified as a marker of mefloquine resistance. This gene is located on chromosome 5 and encodes a 162 kDa ABC-type transporter, expressed on the membrane of the DV. Six key mutations have been identified in this gene: N86Y, Y184F, S1034C, N1042D and D1246Y. However, the occurrence of *Pfmdr1* mutations alone does not confer a decrease in chloroquine activity; this gene only plays an ancillary role in chloroquine resistance. Chloroquine resistance occurs with the selection of the *Pfcr_76T* mutation; the additional mutations in *Pfmdr1* (mainly at codon 86) increase the resistance level.

**Landmark work on lumefantrine tolerance in vivo**

The landmark investigation of the in vivo mechanisms of lumefantrine tolerance was provided by two studies in 2005 in Zanzibar, East Africa. In the first study, the authors analysed polymorphisms at codon 76 of *Pfcr* and at codon 86 of *Pfmdr1* in parasites collected before treatment, and within 42 days following Coartem® treatment, whenever parasitaemia recrudesced. Before treatment, most parasites had *Pfcr_76T* (mutant), and this proportion did not change significantly after treatment. However, the proportion of parasites carrying *Pfmdr1_86N* (wild-type)
increased significantly, from 15% to almost 41% after treatment ($P=0.0075$). Interestingly, most of these wild-type parasites were new infections (as opposed to recrudescence infections).

In the second study, which was carried out in the same area, breakthrough parasites following treatment with Coartem® had significantly higher proportions of Pfmdr1_86N than those before treatment (23.5% versus 47.8%, $P<0.001$), and the majority of these wild-type parasites were new infections, as in the previous study. Interestingly, in the second study, Coartem® was evaluated in comparison with amodiaquine/artesunate; no change was observed in the proportion of wild-type Pfmdr1 (and Pfcr) before and after amodiaquine/artesunate treatment, a clear indication that the selection of wild-type codon Pfmdr1_86N is associated with the use of Coartem®.

For the first time, these reports provided evidence that the use of Coartem® was associated with the selection of wild-type (Pfmdr1_86N) parasites, and they were named ‘lumefantrine-tolerant’ parasites. The publication of these results caused concern. At the time these clinical trials were carried out in Zanzibar, Coartem® was not commonly used as an antimalarial; indeed, at that time the first-line treatment was amodiaquine/artesunate, and, as a result, prior exposure of parasites to lumefantrine before these trials was limited. Thus, these results pointed to rapid selection of lumefantrine-tolerant parasites, which would lead to quick selection of lumefantrine resistance. The second reason for concern was that most of the Pfmdr1_86N parasites were new infections (as opposed to recrudescence infections). This observation lent support to early concerns on the shortfall of the ACT concept: that there was a pharmacokinetic mismatch between artemisinin derivatives and the partner drugs, such as lumefantrine. Indeed, artemisinin derivatives are short-acting drugs with half-lives of <2 h, while lumefantrine is a long-acting drug with a half-life of 4–5 days. Thus, new infections will occur not only during the time that lumefantrine alone is in the body, but also during the time that the lumefantrine level is sub-therapeutic—the ideal situation to select for lumefantrine-tolerant parasites. This observation sparked off debates that Coartem® may have a relatively short therapeutically lifetime, especially in high transmission areas such as in Africa. However, it is worth noting that, 7 years after this report, Coartem® is still efficacious in Africa. We discuss the possible reasons for the good efficacy of Coartem®.

**Subsequent work on lumefantrine tolerance in vivo**

**Confirmation of selection of Pfmdr1_86N**

Following the aforementioned work, several investigations have explored further the role of Pfmdr1 in lumefantrine tolerance. Overall, these investigations have confirmed the existence of lumefantrine-selective pressure for Pfmdr1_86N (wild-type) in isolates collected in Uganda, Tanzania, Burkina Faso, Sudan, and Nigeria (though the difference was not statistically significant). In addition, the role of mutations at codons 184 and 1246 of Pfmdr1 was investigated. An increase in the selection for Pfmdr1_184F (mutant) following Coartem® treatment was observed in most studies in Tanzania, Nigeria, Uganda, and Burkina Faso. No change was observed in another Tanzanian study. With regard to codon 1246, no clear-cut pattern of selection was observed in these studies.

**Selection of Pfmdr1 haplotype**

Attempts have been made to analyse haplotypes encompassing codons 86, 184 and 1246. However, this approach is limited by the high number of possible haplotypes ($2 \times 2 \times 2 = 8$), and also by the small number of breakthrough parasites. Nevertheless, several studies have investigated the role of such haplotypes in lumefantrine tolerance.

Happi et al. reported that haplotype Pfmdr1_86N_184F_1246D (NFD) rose from 10% (9/90) in baseline samples to 75% (12/16) in breakthrough parasites, an indication of its enhanced survival fitness. Similar observations were reported in two different studies, and in the Sudanese study, the NFD haplotype was predominant in recurrent parasites.

In another study, haplotypes Pfmdr1_86N_184F (NF), and Pfmdr1_86N_1246D (ND) were significantly selected following Coartem® treatment. The roles of these haplotypes in lumefantrine tolerance were supported by linkage tests showing that codons 86 and 184 on one side and codons 86 and 1246 on the other side were significantly associated; however, no association was found between codons 184 and 1246. This clearly underscores the predictive value of the 86 locus in lumefantrine tolerance: parasites with codons 184F or 1246D pre-dominantly have codon 86N. However, two studies carried out in Burkina Faso showed that the use of Coartem® is associated with the selection of 184Y (instead of 184F). Thus, the pattern of selection at codon 184 may not be the same in all geographical sites or parasite populations. Clearly, more studies are needed to explore this further.

**Role of increase in Pfmdr1 copy number**

In early 2006, in Thailand (South-East Asia), Price et al. provided evidence that an increase in Pfmdr1 copy number was also associated with a high hazard ratio risk of treatment failure (4; 95% CI, 1.8–11, $P=0.008$), following the use of a four-dose regimen of Coartem®, a clear indication that lumefantrine efficiency is reduced in parasites with high Pfmdr1 copy number. This has also been confirmed in vitro (discussed below).

Parasites with increased Pfmdr1 copies are common in South-East Asia, as a result of the use of mefloquine. In Africa, such parasites are rare; indeed, most studies have not found any increase in copy number, apart from one where the increase was associated with the use of mefloquine. In relation to the use of Coartem®, three African studies investigated Pfmdr1 copy number variation, but all parasites were found to have a single copy. However, a recent report has indicated that parasites with Pfmdr1 copy number >1 were more likely to break through within 42 days following Coartem® treatment, an indication that African parasites with increased Pfmdr1 copy number would survive in the presence of sub-therapeutic lumefantrine levels. In support of this, a recent study has shown that selection of lumefantrine resistance is associated with increased mdr1 copy number in the murine malaria agent Plasmodium chabaudi.
Pfcrt and lumefantrine tolerance

Several studies have also investigated a role for Pfcrt codon 76 in lumefantrine tolerance. In most studies, the proportion of Pfcrt_76K (wild-type) did not change significantly after treatment,8,9,20,22 apart from in two studies, in Burkina Faso and Tanzania, where an increase in wild-type parasites was observed, from 22.3% to 47.9% (P = 0.008) and 48% to 86.5% (P < 0.001), respectively.23,24 In the Tanzanian study, further genetic studies of Pfcrt confirmed the role of Pfcrt_76K in modulating the activity of lumefantrine in vitro.23 Thus, Pfcrt_76K is a contributory factor in lumefantrine tolerance, though this needs to be confirmed by additional studies.

Translation of in vivo ‘lumefantrine tolerance’ to ‘lumefantrine decreased in vitro susceptibility’ (LMDS)

Early evidence

An early study provided evidence that Pfmdr1 polymorphisms modulate the in vitro activity of lumefantrine. This study was based on a genetic cross between strains HB3 and 3D7. HB3 and 3D7 have wild-type Pfcrt (and are fully chloroquine susceptible), but differ in their Pfmdr1 polymorphisms, and their lumefantrine IC50 (inhibitory concentration that kills 50% of parasitaemia) values were 37 and 87 nM, respectively. Interestingly, progeny lines with reduced lumefantrine activity inherited 3D7 Pfmdr1, whereas more lumefantrine-susceptible progeny lines had an HB3 Pfmdr1 allele.33 Evidence that lumefantrine activity segregated with Pfmdr1 following the use of Coartem® treatment, which led to the identification of lumefantrine-tolerant parasites. These two early observations also indicated that parasites with an LMDS phenotype would be lumefantrine-tolerant in vivo.

To gain more insight on LMDS, we have reviewed work published so far on lumefantrine in vitro activity, with the aim of establishing the following: (i) the range in variation of lumefantrine in vitro in malarial endemic areas; (ii) the existence of an inverse relationship between lumefantrine and chloroquine resistance; and (iii) the relationship between lumefantrine IC50 values and Pfmdr1 and Pfcrt alleles.

Lumefantrine in vitro activity

In total, we reviewed 25 publications (12 from Africa and 13 from South-East Asia), from the first report of lumefantrine in vitro activity in 1992 to the latest in 2011; more than 75% (20/26) of the studies were carried out after 2000. In most of these studies, chloroquine was used as comparator, thus we have included it in our analysis. Data are presented as mean (arithmetic or geometric) or median IC50 values (Figure 2). Since there is no statistical way of transforming or converting these measures of central tendency to make them objectively comparable, we analysed them as they were given. This represents a limitation that we could not overcome. In total, data on 2135 isolates were analysed. Chloroquine IC50 values were higher in Asian isolates than those from Africa (100–350 nM and 20–180 nM, respectively). In Africa, the lumefantrine IC50 range was 5–80 nM, similar to that in Asia, apart from two reports where lumefantrine IC50 fell between 100 and 150 nM in South-East Asia (Figure 2). Thus, lumefantrine IC50 values were relatively low (compared with chloroquine), with most values <100 nM, in both Africa and South-East Asia. Detailed information on sample size and location, sampling year, and individual IC50 values are summarized in Table S1 (available as Supplementary data at JAC Online).

We also carried out a correlation analysis between lumefantrine and chloroquine activities; the results indicate that, among the African isolates, chloroquine activity may be inversely correlated with lumefantrine (r = –0.64, P = 0.01), while in South-

Figure 2. Lumefantrine (LM) and chloroquine (CQ) in vitro activity ranges of African (a) and Asian (b) P. falciparum isolates. Values are represented as the inhibitory concentration (nM) that kills 50% of parasitaemia (IC50). Each dot is a geometric or arithmetic mean, or median of 12–200 isolates, representing a total of more than 2135 isolates. Detailed information on these isolates is summarized in the Table S1 (available as Supplementary data at JAC Online).
East Asia, no significant correlation was observed ($r=0.32$, $P=0.34$). In Africa, this inverse relationship of chloroquine and lumefantrine activities was associated with the selection of wild-type parasites harbouring Pfmdr1_86N and Pfcrt_76K following Coartem® treatment (as discussed above) and an increase in chloroquine susceptibility in LMDS parasites. We discuss the implications of these findings later.

**Impact of mutations in Pfmdr1 (at codon 86) and in Pfcrt (at codon 76) on lumefantrine IC$_{50}$**

So far, to the best of our knowledge, only three studies have addressed this issue. Mwai et al.\textsuperscript{34} reported a significant decrease in lumefantrine activity against parasites harbouring Pfmdr1_86N (wild-type) compared with mutant (124 versus 43 nM, $P<0.001$); similar results were reported elsewhere,\textsuperscript{35} while no difference was found in a study in Uganda.\textsuperscript{36}

Veiga et al.\textsuperscript{37} explored mutations at codons 1226 (from F to Y) and 1390 (from F to I). At both codons, mutations were significantly associated with a decrease in lumefantrine activity. In the case of polymorphisms at codons 1226 and 1390, more studies are needed to confirm their role, since these codons have not yet been studied in relation to in vivo lumefantrine tolerance.

Mwai et al.\textsuperscript{34} also explored the impact of Pfcrt_76K in lumefantrine in vitro activity. In this study, parasites harbouring Pfcrt_76K (wild-type) were significantly less susceptible to lumefantrine than mutant (IC$_{50}$ of 67 versus 43 nM, $P<0.05$). More interestingly, when the effects of Pfcrt and Pfmdr1 genes were analysed together, lumefantrine IC$_{50}$ values were significantly higher in wild-type parasites (Pfmdr1_86N and Pfcrt_76K) and lowest in mutants (173 versus 31 nM, $P<0.05$), and parasites with the other two combinations (Pfmdr1_86N and Pfcrt_76T; Pfmdr1_86Y and Pfcrt_76K) had values falling between these extremes.\textsuperscript{34} Thus, if these results are confirmed, this would indicate that LMDS emerges on a background of Pfmdr1_86N, and the presence of Pfcrt_76K enhances this phenotype. Thus, LMDS parasites would be highly susceptible to chloroquine,\textsuperscript{34} highlighting the inverse relationship between lumefantrine and chloroquine activity.

In the case of chloroquine resistance, Pfcrt_76T (the mutant codon) is the main determinant, and Pfmdr1 polymorphism increases this resistance. These data support an opposite scenario in LMDS: that Pfmdr1_86N is the main factor, and Pfcrt_76K polymorphism plays an ancillary role in this phenotype. This would then explain why Pfcrt_76K is not consistently found in lumefantrine-tolerant parasites, as Pfmdr1 polymorphisms do not always correlate with chloroquine resistance.\textsuperscript{13,16}

**Asian isolates and Pfmdr1 point mutation**

In the study mentioned above in which increased copy number was associated with a higher risk of Coartem® treatment failure,\textsuperscript{29} the authors also measured the in vitro activity of lumefantrine associated with Pfmdr1 polymorphism at codons 86, 184, 1042 (wild-type 1042N, mutant 1042D), 1034 and 1246, and copy number variation.\textsuperscript{29} No polymorphisms were observed at codons 1034 or 1246. Isolates harbouring the mutation Pfmdr1_1042D had significantly lower lumefantrine IC$_{50}$ values than wild-type (geometric means 7.8 versus 16.9 ng/L, $P<0.001$). Interestingly, the presence of Pfmdr1_86Y did not alter lumefantrine in vitro susceptibility. Parasites with haplotype Pfmdr1_86N_1042N (wild-type at both codons) showed a decrease in lumefantrine susceptibility, and the increased Pfmdr1 copy number of this haplotype was associated with further decreased lumefantrine susceptibility. Thus, based on this study, the selection of wild-type codons 86 and 1042 and the increase in Pfmdr1 copy numbers are the main determinants for the LMDS phenotype in South-East Asia. However, more studies are needed to confirm this pattern.

**Clinical implications of the inverse relationship between lumefantrine and chloroquine**

The information that LMDS (and thus lumefantrine tolerance) is associated with a significant increase in chloroquine susceptibility in African isolates raises the possibility of re-introducing chloroquine, a solution that was proposed in an early study.\textsuperscript{38}

In relation to this, the situation in Malawi is the most informative. Indeed, in this country, chloroquine was withdrawn in the early 1990s as a result of the spread of chloroquine-resistant parasites. However, within a decade, most parasites became susceptible to chloroquine again, leading to the concept of re-introducing this old antimalarial.\textsuperscript{37} Two clinical evaluations of chloroquine in combination with other antimalarials have been completed,\textsuperscript{39,40} and another is underway.\textsuperscript{41} Based on these, chloroquine could then be re-introduced into areas where lumefantrine resistance is common. However, the following needs to be considered. In the case of Malawi, the increase in chloroquine susceptibility was the result of the emergence of parasites with Pfcrt_76K, which are fully susceptible to chloroquine. However, so far the data indicate that lumefantrine tolerance is primarily associated with Pfmdr1_86N, while the Pfmdr1 gene only plays an ancillary role in chloroquine susceptibility. It still remains to be seen whether in vivo lumefantrine resistance would select for Pfcrt_76K, a feature which would warrant the re-introduction of chloroquine.

The corollary of the aforementioned argument (the inverse relationship between chloroquine and lumefantrine susceptibility) is that Coartem® would be more effective in areas where chloroquine resistance is high. Interestingly, most sites in which Coartem® has been tested and used are generally chloroquine-resistant. Thus, if this inverse relationship can be translated in vivo, Coartem® efficacy could be explained, at least partly, by chloroquine resistance. However, such a hypothesis is difficult to prove since this requires comparative studies in chloroquine-resistant and -susceptible areas. Nevertheless, the case of Malawi could be of interest. Indeed, chloroquine-susceptible parasites are now common in this country; however, clinical trials conducted in this area do not show a low Coartem® efficacy—on the contrary, this drug has proven to be efficacious.\textsuperscript{42} Thus, Coartem® may still be effective for chloroquine-susceptible parasites. In any case, if lumefantrine resistance is associated with chloroquine-susceptibility, the first cases of resistance would emerge in areas of chloroquine susceptibility such as in Malawi. Thus, it would be of interest to compare Coartem® efficacy in Malawi over time versus other African countries.

If the inverse relationship is proven, this could not only lead to the re-introduction of chloroquine, but also lend support to the ‘drug shift’ concept. Indeed, based on the lumefantrine and chloroquine inverse relationship, an increase in chloroquine resistance would be associated with an increase in lumefantrine susceptibility. Parasites with haplotype Pfmdr1_86N_1042N (wild-type at both codons) showed a decrease in lumefantrine susceptibility, and the increased Pfmdr1 copy number of this haplotype was associated with further decreased lumefantrine susceptibility. Thus, based on this study, the selection of wild-type codons 86 and 1042 and the increase in Pfmdr1 copy numbers are the main determinants for the LMDS phenotype in South-East Asia. However, more studies are needed to confirm this pattern.
susceptibility. Thus, the re-introduction of chloroquine after the spread of lumezantrine resistance would lead to the restoration of lumezantrine susceptibility. Then, lumezantrine could be re-introduced after chloroquine, and the process could be repeated if this inverse relationship endures as these drugs are used. Thus, this ‘drug shift’ concept could allow the re-introduction of old antimalarials, a possibility that could contribute to counterbalancing the burgeoning problem of drug resistance in Africa. However, as we have discussed, more investigations are needed to support this concept.

Finally, despite the propensity of lumezantrine to select for lumezantrine-tolerant parasites, we also argue that the high efficacy of Coartem® may be explained, at least in part, by desbutyl-lumezantrine, the main lumezantrine metabolite (Figure 1). Desbutyl-lumezantrine is more active than lumezantrine, and when a normal Coartem® dose is used, the in vivo achieved concentration can clear malaria parasites.13 Thus, in vivo it could efficiently clear lumezantrine-tolerant parasites, delaying the onset of resistance. However, this hypothesis would carry weight only once it is proven that desbutyl-lumezantrine activity is unchanged against lumezantrine-tolerant or LMDS parasites. To fully understand the dynamics of selection of Coartem® resistance, the role of desbutyl-lumezantrine needs to be investigated.

Concluding remarks

It is now almost 7 years since the rapid emergence of lumezantrine-tolerant parasites following Coartem® treatment was reported in isolates from Zanzibar (Tanzania), and now this phenotype has been confirmed in many other endemic areas. Detailed analysis of the data indicates that this phenotype can be translated in vitro into the LMDS phenotype. Both of these phenotypes are associated with wild-type Pfmdr1_B6N and wild-type Pfcrt_76K alleles associated with chloroquine susceptibility, thus explaining the inverse relationship between lumezantrine and chloroquine susceptibility. These lumezantrine-tolerant parasites would form the backdrop on which lumezantrine resistance could emerge.

However, the drug is still effective in treating malaria, and resistance, which had been predicted to emerge rapidly, has not been observed yet. We argue that the shift from lumezantrine tolerance to lumezantrine resistance may be more complex, involving multiple genetic factors. Another possible explanation is that the normal lumezantrine level can efficiently clear lumezantrine-tolerant parasites (though they are less susceptible), and/or that the lumezantrine metabolite, desbutyl-lumezantrine, may retain efficacy against lumezantrine-tolerant parasites, thus restoring the overall drug efficacy when normal doses are used. Whichever is the correct scenario, clearly polymorphisms in Pfmdr1 and Pfcrt alone do not account for lumezantrine resistance, thus more work needs to be undertaken to identify genes that could be used as markers of lumezantrine resistance.

Lumezantrine tolerance and LMDS is associated with an increase in chloroquine susceptibility, raising the possibility of re-introducing this old antimalarial. However, detailed examination of data published so far indicates that lumezantrine tolerance and LMDS do not primarily select for Pfcrt_76K, the codon associated with chloroquine susceptibility. Only once this happens could the re-introduction of chloroquine be considered. Thus, continuous monitoring of Pfcrt and Pfmdr1 should be carried out at sites where Coartem® is used.

As artemisinin resistance is now emerging in South-East Asia, there is concern that this resistance will spread to Africa—a worst-case scenario. On the other hand, the emergence of resistance to a drug such as lumezantrine could lead to re-introducing old drugs such as chloroquine. If proven, this could lead to ‘drug shift’, a concept that could counterbalance the burgeoning problem of antimalarial resistance. However, more work needs to be undertaken to understand the mechanisms of lumezantrine tolerance since this could inform not only the drug-shift concept, but also strategies on how best to monitor the emergence and spread of resistance of this important drug.

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Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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