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Monitoring antifolate resistance in intermittent preventive therapy for malaria

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Abstract

Mutations in the *Plasmodium falciparum* genes *Pfdhfr* and *Pfdhps* have rendered sulfadoxine-pyrimethamine (SP) ineffective for malaria treatment in most regions of the world. Yet, SP is efficacious as intermittent preventive therapy in pregnant women (IPTp) and infants (IPTi) and as seasonal malaria control in children (SMC). SP-IPTp is being widely implemented in sub-Saharan Africa. SP-IPTi is recommended where the prevalence of SP-resistant malaria parasites is low, while SMC is recommended for areas of intense seasonal malaria transmission. The continuing success of these interventions depends largely on the prevalence of *Pfdhfr* and *Pfdhps* resistance mutations in the target population. Here we review the relationship between resistance mutations and SP-IPT within target populations in the context of monitoring and informing implementation of this intervention.

SP as intermittent preventive therapy

The antifolate combination sulfadoxine-pyrimethamine (SP) has been used widely to treat falciparum malaria for nearly half a century. Resistance to SP first appeared in Southeast Asia and South America in the 1970s [1], and soon followed in Africa, reaching Tanzania in 1982 [2,3] and West Africa by the late 1980s [4,5]. By the late 1990s, SP was rendered ineffective as resistance reached intolerable levels (>10% clinical failures) in many regions [6]. As a result, most countries abandoned SP as first-line therapy in favor of artemisinin combination therapies (ACTs) [7].

Because of its low cost, safety, and prolonged post-treatment prophylactic effects, SP is currently being implemented as preventive therapy in vulnerable populations. In pregnant women, evidence from four trials showed that intermittent preventive therapy of two SP doses (SP-IPTp) significantly reduced placental malaria, low birth weight, and anemia [8].

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Based on this evidence, in 2004 the World Health Organization (WHO) recommended at least two doses of SP beginning in the second trimester of pregnancy [9]. IPTp is now part of antimalarial policy in 35 malaria-endemic countries in sub-Saharan Africa [10]. In infants, SP-IPTi consists of three to four doses given between the ages of two to 15 months, coinciding with childhood vaccinations [11]. Results of six trials in four countries showed significant reductions in the incidence of clinical malaria even in sites with more than 30% SP treatment failures [12]. SP-IPTi is currently recommended by WHO in moderate to high malaria transmission areas. In children, seasonal IPTc has been investigated using monthly or bimonthly administration of SP alone or in combination with other drugs. A meta-analysis in West Africa showed >80% protective efficacy of IPTc [13]. IPTc was recently replaced by seasonal malaria chemoprevention, or SMC, which consists of up to four monthly doses of SP + amodiaquine. SMC is now recommended by WHO in areas of intense seasonal malaria transmission [14].

The relationship between SP resistance and protective efficacy of IPTp, IPTi, and SMC requires continued investigation, particularly because the decline of malaria in parts of Sub-Saharan Africa [7,15] may affect the cost-effectiveness of these interventions. Because it is ethically questionable to conduct SP efficacy trials in regions with known high rates of SP treatment failure [16,17], the application of molecular markers of SP resistance may be the best approach. Point mutations in the *P. falciparum* dihydrofolate reductase (*Pfdhfr*) gene and dihydropteroate synthase gene (*Pfdhps*) confer resistance to pyrimethamine and sulfadoxine, respectively, decreasing susceptibility of blood stage parasites to each drug by up to many thousands-fold [18–25]. Accumulation of *Pfdhfr* and *Pfdhps* mutations leads to increasing levels of SP resistance *in vivo* [26,27], with the *Pfdhfr* 'triple mutant' (mutations at positions N51I + C59R + S108N) and the *Pfdhfr/Pfdhps* 'quintuple mutant' (two additional mutations in *Pfdhps* A437G and K540E) serving as significant predictors of SP treatment failure [28].

Given the limited antimalarial drug arsenal [29], particularly for safely preventing malaria in vulnerable populations, SP continues to be the best option for IPT. However, mounting antifolate resistance threatens its efficacy. Evidence of diminished SP-IPTi protection in populations carrying the *Pfdhfr/Pfdhps* quintuple mutant prompted the WHO to recommend limiting implementation to countries with ≤50% prevalence of the *Pfdhps* K540E mutation [30,31], a surrogate for the quintuple mutant. Important questions regarding the dynamics of resistance markers, marker cutoff levels for efficacy of SP-IPTp and SMC, and effects of IPT-related selection of mutations remain. The accompanying review by Naidoo and Roper [32] describes the spatial distribution of *Pfdhfr* and *Pfdhps* mutations across sub-Saharan Africa. Here, we synthesize what is currently known about *Pfdhfr* and *Pfdhps* markers and SP-IPT in pregnant women, infants, and children. Based on our findings, we present recommendations for effective molecular monitoring of IPT.

Pregnant women and IPTp

Prevalence of Pfdhfr/Pfdhps mutations

Baseline (pre-IPTp) prevalence of SP resistance markers in pregnant women has been recorded in a handful of studies. The prevalence of *Pfdhfr* triple mutants in early gestation antenatal care seekers in Agogo, Ghana, was >70% in 2006 [33]. A survey of Gabonese women in 2005–2006, prior to the implementation of SP-IPTp, indicated that 80% of isolates carried the *Pfdhfr* triple mutation, over 50% carried an additional *Pfdhps* A437G mutation, and <5% carried the *Pfdhfr/Pfdhps* quintuple mutant [34]. In Malawi, where SP had been first-line therapy since 1993, the quintuple mutant was seen in over 90% of infections in pregnant women [35]. The highly resistant, rare mutants *Pfdhps* A581G, *Pfdhps* A613S/T, and *Pfdhfr* I164L were also observed at low prevalence (<5%). In Kenya, over 60% and 40% of infections in pregnant women in 2002 were *Pfdhfr* triple and *Pfdhfr/Pfdhps*

quintuple mutants, respectively, at a time when SP-IPTp coverage was only 7% [36]. A prospective study in Tanzania detected near-fixation of three *Pfdhfr* and two *Pfdhps* mutations in pregnant women, regardless of whether they were receiving SP-IPTp [37]. These studies indicate that pregnant women in sub-Saharan Africa are often infected with parasites with four, five, or even six SP-resistance mutations before SP-IPTp, likely as a consequence of continuous antifolate pressure.

Resistance markers and efficacy of IPTp

Two-dose SP-IPTp remains efficacious in regions where SP treatment failure is up to 26% in children [8], and pregnant women are over twice as likely to be cured by SP treatment as children in the same location [38]. These findings suggest that treatment efficacy in children may not be an accurate predictor of preventive efficacy in women in malaria endemic areas who have acquired immunity. This observation has been corroborated by studies showing better treatment outcomes with SP in pregnant women than in children [39,40]. Because it has significant benefits in the face of clinical resistance, the WHO recommends SP-IPTp in regions with up to 50% SP treatment failure in children [41] and high prevalence of the quintuple mutant [42]. This recommendation is supported by recent studies showing no association between quintuple mutants and reduced SP-IPTp protection, higher parasite densities, placental inflammation, or poor maternal or fetal outcomes [43,44]. However, highly resistant genotypes may compromise IPTp efficacy. A study in Tanzania found that the quintuple mutant plus *Pfdhps* A581G was associated with increased placental parasitemia and inflammation [37]. Furthermore, SP-IPTp did not protect against low birth weight in the Democratic Republic of Congo where the prevalence of *Pfdhps* A581G was likely high (see review by Naidoo and Roper review [32]). Clarification of the relationship between highly resistant infections and SP-IPTp efficacy will be useful for monitoring areas without clinical data or with changing resistance patterns.

Selection of resistance markers by IPTp

In a study measuring *Pfdhfr* and *Pfdhps* mutations in infections in women before starting SP-IPTp and after delivery, prevalence of the *Pfdhfr/Pfdhps* quintuple mutation increased significantly from 81% to 100% [45]. Four studies have compared resistance markers in women receiving SP-IPTp with those not receiving treatment. Mockenhaupt [33] compared the prevalence of *Pfdhfr* mutations in infections in women at early gestation who had not received SP-IPTp with that in delivering women, nearly all of whom had received at least one dose. Prevalence of the *Pfdhfr* triple mutant was similar between groups and did not increase with an increasing number of SP-IPTp doses. Therefore, although the overall prevalence of *Pfdhfr* mutations in the study population doubled between 1998 and 2006, concomitant with the implementation of SP-IPTp, the authors suggest that SP-IPTp may not be responsible for this increase. Similarly, examination of peripheral and placental samples from pregnant women over a 13-year span in Kenya indicated that prevalence of the quintuple mutant increased significantly, contemporaneous with the implementation of SP-IPTp [36]. However, presence of the quintuple mutant was not associated with SP-IPTp use in a multivariate analysis, suggesting that other factors were chiefly responsible for its rise [36].

A study investigating marker selection and the timing of SP-IPTp doses found that prevalence of the quintuple mutant was higher in placentas of women receiving SP-IPTp than those receiving a placebo; however, this relationship was only significant for women who received a dose within 2.5 months before delivery [43], suggesting that the increase in resistant parasites disappears once SP is cleared from the blood.

In Tanzania, prevalence of *Pfdhps* A581G was significantly higher in SP-IPTp recipients compared to those not receiving treatment [37]. The investigators also reported an association

between IPTp and higher parasite density, suggesting that highly resistant parasites (quintuple mutant + *Pfdhps* A581G) may spread more rapidly than less resistant counterparts. Further study is required to investigate this potentially troubling observation and to establish the effect of SP-IPTp on the selection of drug resistance.

IPTp and HIV

Pregnant women with both HIV and malaria are at greater risk of placental parasitemia, clinical malaria, anemia, and delivering low birth weight infants [46]. Because of this increased risk, they require more frequent IPT dosing to benefit from its protection [8]. In addition to a greater risk of poor malaria-related outcomes, it has been hypothesized that reduced immunity in pregnant HIV-infected women may also allow drug-resistant parasites to persist. A study of SP-IPTp in Mozambique indicated that quintuple mutants were more prevalent in the peripheral blood and placentas of HIV-infected compared to HIV-uninfected women, suggesting that HIV-associated dampening of adaptive immunity may hinder clearance of resistant parasites [43]. A second study in Kenya found no association between presence of the quintuple mutant in pregnant women with HIV infection [36], although the analysis did not differentiate among women using SP-IPTp, trimethoprim-sulfamethoxazole prophylaxis, or no antifolate treatment. Information on the relationship between HIV and antifolate resistance in pregnant women remains limited and requires further investigation.

In many malaria-endemic countries, HIV-infected women receive daily prophylaxis with trimethoprim-sulfamethoxazole (TS), an antifolate combination with cross-resistance to SP [24,47], and are not given SP-IPTp because TS is thought to provide adequate antimalarial prophylaxis. A comparison of HIV-infected mothers on TS with HIV-uninfected mothers on SP-IPTp in Uganda found no difference in the prevalence of placental malaria or the prevalence of *pfdhfr* C59R, *Pfdhps* A437G or *Pfdhps* K540E [48]. However, differences in selection may not have been detectable because the baseline prevalence of resistance markers in both populations was very high [48]. Studies of TS prophylaxis in healthy Malian children [49] and in HIV-infected Ugandans [50] and their household contacts [51] similarly failed to detect selection of resistance mutations in *Pfdhfr* or *Pfdhps* by TS, mitigating concerns that SP efficacy might be impaired in persons receiving TS prophylaxis.

Infants and IPTi

Prevalence of Pfdhfr/Pfdhps mutations

While prevalence surveys are often conducted in children aged 6–59 months, little is known about resistance in infants aged <12 months. The *Pfdhfr* triple mutant and *Pfdhps* double mutant were both more prevalent in infants versus older children in a baseline survey in Tanzania, but the differences were not significant across all arms [52]. Near 100% saturation of *Pfdhfr/Pfdhps* quintuple mutants was reported in both symptomatic 6–59 month old children and in asymptomatic infants aged 2–10 months in Tanzania [10], but the number of infants studied was small. In Mali, the prevalence of *Pfdhfr* triple and *Pfdhps* A437G mutations was similar among children aged 2–24 months and 25–60 months [53]. These limited data suggest that molecular resistance levels are comparable among infected infants and children.

Resistance markers and efficacy of IPTi

The relationship between molecular markers and protective efficacy of IPTi was investigated in six pilot studies in Tanzania, Ghana, Mozambique, and Gabon [12,54]. SP-IPTi delivered 20 – 30% protective efficacy for infants during the first year of life, a range deemed ‘worthy of further investment’ [54] for reducing malaria morbidity in infants. This level of protection was achieved against a backdrop of 44–77% prevalence of the *Pfdhfr*

triple mutant among the six sites, with three sites also reporting *Pfdhps* mutations. In Ashanti, Ghana, over 60% of baseline infections in infants had four or more *Pfdhfr/Pfdhps* mutations [55] and in Tamale, Ghana, the *Pfdhfr* triple mutant plus *Pfdhps* A437G (the 'quadruple mutant') was found in 44% of infected children under 5 years [56]. The highest level of resistance was reported in Manhica, Mozambique, where the placebo arm had a 44% prevalence of the *Pfdhfr/Pfdhps* quintuple mutant [57].

Subsequent studies have shown that efficacy of SP-IPTi diminishes with prevalence of the quintuple mutant. Trials in Korogwe and Same, Tanzania, did not show any protective efficacy of SP-IPTi [58]. Pre-treatment infections in all nine of the infants genotyped in Korogwe bore the quintuple mutant and four (44%) also carried the *Pfdhps* A581G mutation [16]. Although marker prevalence was not reported for infants in Same, a 2001 survey of two sites nearby found a 60–75% prevalence of the *Pfdhfr* triple mutant and a 43–55% prevalence of the *Pfdhps* double mutant [59].

Results from seven IPTi trials indicated that the prophylactic time period was greatly shortened in the presence of quintuple mutant parasites, with protective efficacy falling from 42 days in Navrongo, Ghana (no *Pfdhfr/Pfdhps* quintuple), to 21 days in Korogwe, Tanzania (89% prevalence quintuple) [60]. The analysis also showed that protective efficacy in the 35-day period after the 9-month dose of SP-IPTi decreased with an increasing number of resistance markers, although there were not enough data points to determine the effects of specific markers.

Given that protective efficacy of SP-IPTi is significant in areas with high prevalence of *Pfdhfr* triple and *Pfdhps* A437G mutations, but wanes as prevalence of the quintuple mutant increases, the WHO has recommended a threshold for implementation of SP-IPTi at 50% prevalence of *Pfdhps* K540E mutation [30].

Selection of resistance markers by IPTi

The impact of single-dose SP-IPTi on selection of *Pfdhfr/Pfdhps* mutations was assessed in Ghana [61]. The observed period between treatment and the first detection of *P. falciparum* infections with the *Pfdhfr/Pfdhps* quadruple mutant was significantly shorter in the SP treatment group when compared to placebo. Furthermore, the incidence rate of infections with quadruple mutants was twice as high in the treatment group as compared to the placebo group after week 8. Similarly, in Mozambique [57], the prevalence of quintuple mutants nearly doubled in the SP group compared to the placebo group within the first two months after the third SP IPTi dose. However, this trend reversed by the time the children reached two years of age. The prevalence of resistance markers also did not increase over a one-year period of SP-IPTi in Mali [53], which had comparable pre- and post-intervention levels of *Pfdhfr* triple and *Pfdhfr/Pfdhps* quadruple mutants.

The effect of two consecutive intervention seasons was investigated in Tanzania [52] and Senegal [62]. In both countries, prevalence of the *Pfdhfr* triple mutant was significantly higher in the intervention arm compared to the control arm two years after implementation of SP-IPTi. Prevalence of *Pfdhps* 437 was also significantly higher in the intervention arm in Senegal, and while the prevalence of the *Pfdhps* double mutant was also higher in the intervention arm in Tanzania, this difference was not significant. Thus, limited evidence suggests that resistance mutations are not lastingly selected after a single SP-IPTi season, but cumulative effects may be observed.

Children and SMC/IPTc

Prevalence of Pfdhfr/Pfdhps mutations

In regions of high and stable malaria transmission, the prevalence of asymptomatic malaria increases with age due to continual infections that confer partial immunity [63]. Because asymptomatic individuals are seldom treated, their infections may contain a higher proportion of drug-sensitive parasites. By contrast, young non-immune children suffer repeated symptomatic episodes, undergo consecutive drug treatment, and are more likely to carry drug-resistant parasites. Corroborating this hypothesis, studies in areas of high malaria transmission found that prevalence of SP resistance markers declines with age [64,65] and that quintuple mutants are more prevalent in children under five years of age than in older children [66]. Conversely, prevalence of resistance mutations may not vary by age in areas of low or unstable malaria transmission with less immunity, as was observed when comparing children under and over the age of 4 years in Northern Tanzania [59].

The value of *Pfdhfr* and *Pfdhps* mutations in predicting treatment outcome also varies by age. In Uganda [67] and Nigeria [68], two to four mutations were predictive of treatment failure only in children under five years but not in older patients, presumably because they were able to clear these infections. By contrast, the *Pfdhfr/Pfdhps* quintuple mutant was equally predictive of treatment failure in both younger and older children, suggesting that even semi-immunes are unable to clear these parasites.

Resistance markers and efficacy of SMC/IPTc

SMC (previously IPTc) has often been tested as a combination of SP with another drug, and its evaluation has been restricted to regions of West Africa with intense seasonal malaria. While the *Pfdhfr/Pfdhps* quadruple mutant is prevalent in this region, *Pfdhps* K540E and thus the quintuple mutant are rare [32]. Protective efficacy of SP combined with either amodiaquine or artesunate ranged from 70–87% at sites in Senegal, Mali, and Burkina Faso with baseline prevalences of 32–58% of the *Pfdhfr* triple mutant and 22–29% for *Pfdhps* A437G [69–71], suggesting that IPTc is effective against moderate levels of the quadruple mutant. A meta-analysis of IPTc trials was conducted [13], but because baseline prevalence of resistance markers prior to implementation was generally not reported, marker prevalence could not be associated with efficacy of the intervention, nor could selection be measured.

Selection of resistance markers by SMC/IPTc

The prevalence of *Pfdhfr/Pfdhps* quadruple mutants after three months of SP + amodiaquine IPTc in Burkina Faso was comparable in the treatment and placebo arms, with an overall increase in both groups from baseline levels [71]. By contrast, the post-intervention prevalence of quadruple mutants in Mali was significantly higher in the SP + amodiaquine-IPTc group than the placebo group, and prevalence also increased from baseline in the IPTc group but not in the placebo group [70]. In Senegal, post-intervention prevalence of triple *Pfdhfr* and *Pfdhps* A437G mutants was also significantly higher in the SP + artesunate treatment arm than the placebo arm, despite an increase in both groups from baseline [69]. Prevalence of resistance markers continued to rise in both groups, and the difference between the intervention and placebo arms was no longer detected after the second year of follow up, perhaps owing to increased SP use in the general population after a shift in national treatment policy to SP + amodiaquine [69]. Based on this small number of studies, it appears that at least short-term selection of resistance markers may follow administration of SMC/IPTc.

Recommendations for molecular monitoring of IPT

Authors of a recent multi-site analysis of resistance markers and SP-IPTi studies have recommended that where prophylactic endpoints cannot be measured to evaluate protective efficacy, assessment of molecular markers is useful in guiding implementation [60]. Here we have identified several knowledge gaps in the baseline prevalence of molecular markers in IPT target populations, the relationship between molecular markers and the efficacy of IPT, and the effect of IPT on selection of resistance. We suggest that targeted monitoring based on known molecular marker distributions across populations, described here, and geographic regions, described by Naidoo and Roper [32], will guide strategies for addressing these gaps.

Box 1 outlines recommendations for improved and targeted molecular monitoring of SP-IPT. Currently, surveillance of resistance mutations in Africa is often conducted via convenience sampling at health centers. Variations in sampling and methodology reduce comparability across data sets and therefore utility for informing policy. Future surveillance should employ standard epidemiological methods including systematic, periodic sampling at sentinel sites [72–75] and calculations of statistical power to detect differences in marker prevalence. A 2010 WHO report [76] identified aspects that have not yet been standardized for surveillance, notably sample collection, reporting of mixed genotype infections, and laboratory methods. Standardization of these methods along with guidelines for frequency of sampling, distance among sample sites, and characteristics of sample populations (age groups, symptomatic status) will further improve the usefulness of data across regions and over time.

Despite differences in sampling strategies, most data on drug resistance mutations in Africa are collected in children aged 6–59 months. Limited information suggests that the pre-IPTi prevalence of resistance mutations in infants is comparable to that in children, indicating that monitoring resistance markers in children may be an effective proxy for infants. By contrast, the level of resistance in pregnant women may not correlate with that in children because acquired immunity may clear a higher proportion of resistant parasites in women compared to children. As successful control of malaria continues to reduce exposure [15], this difference in immunity and the ability to clear resistant parasites will likely decline. However, it may be counterbalanced by SP-IPTp-induced selection of highly resistant parasites [37,45] and diminished clearance of resistant parasites in immune-suppressed HIV-positive women. Despite these complexities and epidemiological changes, prevalence of SP resistance markers is rarely investigated in pregnant women and should be made a priority as continued efficacy of SP-IPTp is evaluated. Areas of focus should include regions where HIV infection and IPTp uptake are high [77].

The limited recommendation of SP-IPTi in regions with <50% prevalence of *Pfdhps* K540E indicates that it is not suitable for most of East Africa [78]. Such resistance thresholds have not yet been identified for SP-IPTp or SMC/IPTc. In order to determine a molecular threshold, efficacy from regions with different patterns of *Pfdhfr/Pfdhps* haplotypes is required. For example, parts of Tanzania and Ethiopia [32], may be selected as representative of regions with moderate and high levels of *Pfdhfr/Pfdhps* quintuple mutants, respectively, and monitored for SP-IPTp protective efficacy. Regions with highly resistant alleles like *Pfdhps* 581G and 613S/T, such as Uganda and Kenya [32], may be monitored to determine the effects of six or more mutations. Similarly, parts of West Africa with varying levels of the *Pfdhfr/Pfdhps* quadruple mutant in children (reviewed in [32]), could be monitored to reflect the range of SMC efficacy throughout the region.

The effect of IPT on selection of drug resistance in the intervention and general population is also largely unknown. The effect of drug pressure is difficult to predict because as SP and

other antimalarials are replaced with ACTs for treatment of uncomplicated malaria, a concomitant scaling up of SP-IPT is currently underway. Thus, while highly resistant mutations may begin to disappear from the general population as a result of decreased overall SP drug pressure, as seen in the Peruvian Amazon [79,80] and Mozambique [64], they may continue to be selected in populations receiving SP-IPT. How these interactions and changes affect the spread of drug resistance require further investigation, beginning with studies such as that conducted by Pearce *et al.* in Tanzania [52], which detected pre- and post-intervention differences in marker prevalence, both in the target population and in the community. When possible, information on changes in prevalence in comparator sites not receiving the intervention is helpful in identifying larger patterns such as a general decline in SP resistance with the rise of ACT adoption. Lastly, periodic sampling of resistance markers will be crucial in determining the longer-term effects (if any) of multiple intervention seasons.

As a complement to individual surveillance efforts, the WorldWide Antimalarial Resistance Network (WWARN) is working to harmonize drug resistance data and provide open access to summary information and visualization tools (see: http://www.wwarn.org), with a goal of facilitating global monitoring of resistance markers over space and time and understanding the relationship between molecular resistance and clinical outcomes [81]. Use of WWARN maps and tools by both scientific investigators and malaria control teams can aid in determining when and whether IPT is appropriate and with what combination, identifying the level of resistance markers in neighboring regions, and monitoring the dynamics of resistance in different populations.

Concluding remarks and future perspectives

With few options available for malaria chemoprevention, monitoring the efficacy of SP-IPT in the face of antifolate resistance is crucial for maintaining the public health benefits of Intermittent Preventive Therapy. Threshold levels of molecular resistance are required for SP-IPTp, particularly with the emergence of high-level resistance alleles in East Africa, and for SMC/IPTc as it is scaled up in West Africa. Standardized approaches for baseline surveillance of resistance markers, periodic monitoring, and prospective molecular sampling during interventions will ensure the effective and continued use of SP as a preventive tool against malaria.

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Box 1: How does SP-IPT work?

Sulfadoxine-pyrimethamine (SP)

Sulfadoxine and pyrimethamine target two enzymes in the parasite's folate synthesis pathway, acting against both the liver and blood stages of *Plasmodium falciparum* in the human host. The liver stages are thought to be sensitive to antifolates because they heavily synthesize folate while undergoing mitosis to produce thousands of merozoite progeny. Results from *in vivo* and *in vitro* mouse models for malaria support the hypothesis that pyrimethamine blocks parasite replication in the liver as well as in the blood [82]. Infection studies with pyrimethamine-resistant parasites in the mouse model also found that while blood stage parasites were fully resistant, liver stage resistant parasites remained partially susceptible to the drug, exhibiting attenuated replication. Although it has not been investigated in human malaria, a similar mechanism in *P. falciparum* could help explain why IPT remains partially effective in settings with high SP resistance [82].

Intermittent Preventive Therapy (IPT)

IPT reduces the adverse effects of malaria infection. IPTp reduces placental parasitemia and protects against maternal anemia and low birth weight in infants. IPTi and IPTc reduce the number of clinical malaria episodes in infants and children. IPT protects in two ways: clearance of existing parasites during treatment and prevention of future clinical episodes during a period of post-treatment prophylaxis [83]. The prophylactic period can last as long as 60 days against SP-sensitive parasites, but is shortened for resistant infections [83].

Box 2. Recommendations for molecular monitoring of SP-IPT in target populations

1. Conduct molecular surveillance using standardized epidemiological approaches to aid in informing policy
    - Systematic, periodic sampling; consideration of sample size
    - Further standardization may be required for sample collection and analysis [76]
    - Guidelines for sampling frequency, sites, and population characteristics
2. Determine baseline level of SP resistance markers in pregnant women/women of child-bearing age
    - For comparison with prevalence in children, to account for differences in immunity and selective pressure
    - Particularly important in regions where IPTp uptake is high [77], recent molecular data are not available [32], and with high HIV prevalence
3. Establish molecular resistance thresholds for efficacy of IPTp and SMC
    - Regions representative of populations with different *Pfdhfr/Pfdhps* haplotypes (4, 5, or 6 mutations) should be monitored in regions with varying transmission intensities [77] to determine which marker combinations undermine IPTp protection
    - Evaluation of resistance markers and protective efficacy of SP + amodiaquine for SMC
4. Determine effects of IPT on selection of drug resistance
    - Baseline and post-investigation surveys in intervention and non-intervention (if available) regions to investigate the effects of IPT on selection of drug resistance
    - Periodic follow-up surveys to determine cumulative and long-term selection in the intervention and general populations
5. Disseminate and pool information to improve global monitoring
    - Timely sharing of regional surveillance data with global networks such as WWARN
    - Pooled analysis of standardized data sets to aid in establishing relationship between markers and clinical outcomes, determination of marker cutoffs

Highlights

- We identify gaps in surveillance of SP resistance to inform IPT policy
- Molecular resistance thresholds for SP-IPTp and SMC are needed
- IPT selection of drug resistance mutations should be monitored
- Data on SP resistance should be widely shared to improve global monitoring