Minutes of the Technical Expert Group (TEG) on

Drug Efficacy and Response

10–11 December 2015

Crowne Plaza Hotel, Geneva, Switzerland

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Abbreviations

ACT	artemisinin-based combination therapy
AL	artemether–lumefantrine
AQ+SP	amodiaquine + sulfadoxine–pyrimethamine
ASAQ	artesunate–amodiaquine
ASMQ	artesunate–mefloquine
ASPY	artesunate–pyronaridine
AS+SP	artesunate + sulfadoxine–pyrimethamine
DER	Drug Efficacy and Response Unit (part of GMP)
DP	dihydroartemisinin–piperaquine
ERG	evidence review group
GMP	Global Malaria Programme
GMS	Greater Mekong subregion
IPTp	intermittent preventive treatment in pregnancy
iRBC	infected red blood cell
K13	*P. falciparum Kelch 13*
KARMA	*K13* Artemisinin Resistance Multicenter Assessment
LBW	low birth weight
MDA	mass drug administration
MDR	multidrug resistant
MFLT	multiple first-line treatments
NMCP	national malaria control programme
NS	non-synonymous
OUCRU	Oxford University Clinical Research Unit
PCR	polymerase chain reaction
Pfcrt	P. falciparum *chloroquine resistance transporter*
Pfdhfr	P. falciparum *dihydrofolate reductase*
Pfdhps	P. falciparum *dihydropteroate synthase*
Pfmdr1	P. falciparum *multidrug resistance protein 1*
qPCR	quantitative PCR
$RSA_{0–3h}$	ring-stage survival assay
SEA	South-East Asia
SMC	seasonal malaria chemoprevention
SP	sulfadoxine–pyrimethamine
TEG	Technical Expert Group
WHO	World Health Organization

Summary and recommendations

The format of the summary and recommendations are similar to those of the previous Technical Expert Group (TEG) (*1*). The TEG's recommendations are made specifically in response to questions directed to the TEG from WHO (Annex 3).

Session 1 Update on artemisinin resistance

Definitions of *confirmed* and *associated K13* mutations are required. The criteria for determining whether a *K13* propeller mutation is confirmed or associated still follow the criteria suggested by the ERG on *K13* 2014; that is, one of the following:

- a statistically significant association ($p < 0.05$) between a *K13* mutation and either a half-life of the parasite clearance slope of ≥5 hours or positive parasitaemia at 72 hours (±2 hours) via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases; or
- >1% survival using the $RSA_{0–3h}$ (or >2 standard deviations above the mean value for *K13* wild-type parasites from the same area) in at least five individual isolates with a given mutation; or a statistically significant difference ($p < 0.05$) in the $RSA_{0–3h}$ assay between culture-adapted recombinant isogenic parasite lines, produced using transfection and gene-editing techniques, which express a variant allele of *K13* as compared to the wild-type allele.

A *K13* mutation is *confirmed* when both of these requirements are met, and *associated* when only one of these requirements is met. However, the $RSA_{0–3h}$ and thresholds for in vivo tests are currently only validated for South-East Asian parasites and patients.

The list of associated, confirmed and not associated mutations has been updated as shown in the table below.[1]

***K13* mutation**	**Classification**
E252Q	Not associated
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R539T	**Confirmed**
I543T	**Confirmed**
P553L	Associated
R561H	**Confirmed**
V568G	Associated

[1] Other rare variants were reported associated with in vivo or in vitro tests, or both: M476I; C469Y; A481V; S522C; N537I; N537D; G538V; M579I; D584V; H719N.

P574L	Associated
A578S	Not associated
C580Y	**Confirmed**
A675V	Associated

Investigation of associated mutations should be prioritized based on their prevalence, clinical evidence of resistance and the results of the $RSA_{0–3h}$, triggering subsequent transfection studies, if feasible.

A confirmed single *K13* propeller mutation at a threshold prevalence of ≥5% probably signifies selection of the genotype in the parasite population, which is an appropriate indirect measure of the partial artemisinin resistance phenotype. Current research focuses on identifying other possible parasite genetic variants that may facilitate the successful selection of *K13* mutants; however, at present, these possible permissive or compensatory background mutations are insufficiently defined or established.

Currently available tools are sufficient for the detection of artemisinin resistance in an area. The percentage of patients with positive parasitaemia at day 3 is a relevant and practical measure for routine surveillance. Blood filter papers should be routinely collected at day 0 in all studies for identification of *K13* mutations. In the research setting, the parasite clearance slope is currently most appropriate, but other tool including lag phase and tail should not be ruled out. In the context of potential drug resistance, tools to evaluate residual parasitaemia should be considered.

The definition of partial artemisinin resistance has not been amended from TEG 2014 except for the specification of day 3 being 72 hours (±2 hours) after the start of a full artemisinin-based treatment course. At this time, there should be a single global definition of artemisinin resistance.

Suspected endemic artemisinin resistance is defined as:

- ≥5% of patients carrying *K13* resistance-confirmed mutations; or
- ≥10% of patients with persistent parasitaemia by microscopy at 72 hours (±2 hours; i.e. day 3) after treatment with ACT or artesunate monotherapy; or
- ≥10% of patients with a half-life of the parasite clearance slope ≥5 hours after treatment with ACT or artesunate monotherapy.

Confirmed endemic artemisinin resistance is defined as:

- ≥ 5% of patients carrying K13 resistance-confirmed mutations, all of whom have been found, after treatment with ACT or artesunate monotherapy, to have either persistent parasitaemia by microscopy on day 3, or a half-life of the parasite clearance slope ≥ 5 hours.

The detection of artemisinin resistance signifies an epidemiological threat, but does not necessarily signify reduced ACT efficacy as a manifest public health problem. The immediate consequences should be the investigation of possible causes, such as irrational drug use, substandard antimalarial drugs or the importation of resistant genotypes. Detection of resistance must also prompt surveillance and evaluation of ACT efficacy, to assess potential concomitant partner drug resistance (for some partner drugs, molecular markers are available). The priority in such areas is to ensure that antimalarial treatment is based on a definitive diagnosis, that drugs are of good quality, and that there is a good clinical provider and patient adherence. Based on the local epidemiological

situation, capacity for intensifying vector-control efforts to interrupt transmission should be investigated, including the potential for malaria elimination. In countries where targeting of malaria control and treatment interventions is directed by risk stratification, the presence of artemisinin resistance is clearly a criterion for upgrading risk.

Session 2 Intermittent preventive treatment in pregnancy (IPTp-SP)

At a population level, IPTp-SP is associated with improved birth outcomes (fewer LBW), irrespective of SP's failure to clear or prevent parasitaemia, in all settings where the prevalence of sextuple mutant haplotype containing *Pfdhps* A581G is below 5%. The presence of parasites bearing the sextuple mutant haplotype containing *Pfdhps* A581G at a prevalence of >35% appears to negate the benefits of IPTp-SP on birth outcomes. Overall, the evidence suggests that IPTp-SP given to women with the sextuple mutant is not harmful. This concern was suggested in a single study, but was not confirmed by later studies. There are no data at present on the effectiveness of IPTp-SP at the prevalence of sextuple mutant haplotype containing *Pfdhps* A581G of 5–35%.

For national malaria control programme (NMCP) settings, molecular surveillance should be used to guide routine assessment of IPTp-SP effectiveness. IPTp-SP should be continued or implemented in areas of unknown, low or moderate SP resistance. In areas of high SP resistance, IPTp-SP may be of more limited benefit, and this benefit is primarily associated with the specific prevalence of the *Pfdhps* A581G sextuple mutation. The threshold of A581G prevalence at which IPTp-SP is no longer of benefit is unclear, but the evidence suggests that there will be no benefit of IPTp-SP at >35% A581G prevalence. However, IPTp in areas with a high prevalence of A581G is not thought to cause harm. Therefore, even in settings of high SP resistance, molecular monitoring of the prevalence of A581G mutations can be used as a proxy for IPTp-SP effectiveness. Molecular surveillance should focus on the *Pfdhps* gene, and in particular on the mutations occurring at codons S436A/F, A437G, K540E, A581G and A613S/T. Methods include aggregate genotyping by sequencing of pooled samples (frequency) or individual-level genotyping by polymerase chain reaction (PCR) and sequencing, or through allele-specific assays such as PCR-RFLP (restriction fragment length polymorphism), PCR-SSOP (sequence specific oligonucleotide probe) and real-time PCR (prevalence). Genotyping may be carried out on parasite samples if collected from a population that has not recently (i.e. in the previous 6 weeks) been treated with antifolates. Sampling should take place every 3 years in areas of low SP resistance, every 2 years in moderate areas, and every year in high areas. Quality control of genotyping should be implemented whether molecular data are generated in-country, in regional laboratories or with international partners.

In research settings, additional considerations are mutations in *Pfdhfr* and the sequencing of each locus, which may identify new mutations in the gene targets of interest. The impact of I431V, which is emerging in Nigeria, needs to be investigated. Although there are sufficient data from areas with low, moderate and high SP resistance, more information is needed from areas with the highest levels of SP resistance, defined by the presence of *Pfdhps* A581G mutants; when these data become available, they will help to define the thresholds at which alternative strategies are needed. In these areas with the highest levels of SP resistance, priority research themes include:

- ecological studies of the impact of IPTp-SP on birth outcome (birth weight), maternal anaemia, and maternal and placental malaria measured at the time of delivery;

- individual-level studies of the impact of *Pfdhps* A581G-bearing parasites on birth outcomes, and of whether this relationship is modified by IPT-SP; and
- effective, well-tolerated, feasible alternatives for prevention.

Session 2 Seasonal malaria chemoprevention (SMC)

The ongoing ACCESS-SMC study will provide more robust information about which SMC measures will be most appropriate – an issue that will need to be revisited by the TEG once the data are available. Both molecular markers and efficacy evaluation are required and, ideally, some measure of transmission intensity. In addition to the protocol for monitoring drug resistance of the ACCESS-SMC study presented at the TEG meeting, the following parameters should be explored:

- Efficacy evaluation – the ratio of malaria cases in children aged under 5 years versus those aged over 10 years; the occurrence of clinical malaria relative to the time of the previous SMC dose; the incidence of severe malaria at sentinel sites; case–control sampling before each dose for microscopy, gametocytaemia and PCR positivity relative to the time of previous SMC dose.
- Molecular markers – at least Pfcrt K76T and Pfmdr1 N86Y, Y184F and D1246Y (still rare in west Africa) should be determined routinely to track any changes in their prevalence, as an indicator of changes in amodiaquine efficacy. In particular, the prevalence of the Pfcrt codon 72 to 76 haplotype SVMNT (Ser-Val-Met-Asn-Thr) should be determined. Also Pfdhfr and Pfdhps should be included in the molecular markers to be tested.
- Capacity-building – local capacity-building for the monitoring of molecular markers is needed.
- Impact on transmission – assessed through standard membrane feeding assay, if feasible, and parasite genetic indicators of complexity of infection and overall changes in parasite diversity levels, where possible.
- Drug policy effects – the impact of SMC on first-line ACT diversity (and thus drug pressure) should be monitored.

Session 3 Safe and effective treatment in areas of confirmed multidrug resistance (MDR) malaria

Rotational first-line treatment (where the first-line treatment is changed based on updated surveillance data, which can include molecular markers) is already being implemented in Cambodia. However, operational issues in switching therapies are challenging. At present, there is no alternative in Cambodia other than to be flexible, and use rotational first-line treatment.

Information is limited on the efficacy and safety of prolonged treatment with an ACT, triple combination treatment containing an artemisinin and two partner drugs, or sequential ACTs. Once more data are available, it is recommended that the DER TEG should hold a joint session with the Chemotherapy TEG to evaluate the information emerging from these studies.

ASAQ may have a role to play in Cambodia. As a first step, resistance markers for amodiaquine (single nucleotide polymorphism alleles *Pfcrt* 72-76, *Pfmdr1* N86Y, Y184F and D1246Y) and in vitro susceptibility should be examined in GMS isolates. This should be followed by a therapeutic efficacy

study of a fixed-dose ASAQ combination in Cambodia in 2016, if molecular marker data suggest reasonable amodiaquine efficacy.

Session 3Prevention or delay of MDR where it has not been identified

In areas where there is no established MDR, simultaneous deployment of multiple effective ACT first-line treatments (MFLT) is unlikely to hasten, and may actually delay, the emergence of drug resistance, according to modelling studies. Therefore:

- countries that presently have multiple approved ACT first-line treatments should continue to use them; and
- countries that rely on a single ACT first-line treatment are encouraged to add additional effective ACT treatments to the national treatment guidelines, both to potentially delay the onset of resistance and to be better prepared to respond to failure (or stock-outs) of the current first-line treatment.

Because modelling is the only means of evaluating the impact of MFLT on delaying resistance, the TEG recommends that the Malaria Modelling Consortium be asked to further develop these modelling approaches. Implementation issues should also be considered. The DER TEG is ready to examine outputs from the Malaria Modelling Consortium and any supporting clinical data.

1 Welcome and introduction of guest speakers

The list of participants is provided in Annex 1. All members except J. Thwing and N. Q. Thieu attended the meeting. Organizations invited as observers were the Bill & Melinda Gates Foundation; the Global Fund to Fight AIDS, Tuberculosis and Malaria; the United Kingdom of Great Britain and Northern Ireland (United Kingdom) Department for International Development; the Medicines for Malaria Venture; and the United States (US) Agency for International Development. The meeting agenda is provided in Annex 2.

Membership of the Technical Expert Group (TEG) is rotated every 3 years. Thanks were extended to L. Conteh, C. Karema, C. Rogier and S. Vreden, who have left the TEG; D. Ménard and S. Volkman were welcomed as new members. A further five TEG members will rotate out next year, to maintain a core membership of 15.

The remit of this TEG has changed from "drug resistance and containment" to "drug efficacy and response", to reflect reorganization within the Global Malaria Programme (GMP). The role of this TEG is to advise the GMP's Drug Efficacy and Response Unit (DER) on policy and recommendations regarding drug efficacy and response. Questions directed to the TEG from DER are listed in Annex 3.

2 Declarations of interest

All TEG members participating in the meeting submitted a declaration of interest, which was assessed by DER at GMP and by the Legal department at WHO. WHO policy on how to report conflicts of interest has changed. In the future, TEG member biographies and conflict of interest forms will be placed on the GMP website 2 weeks before the meeting, to comply with the public notice and comment requirements set by WHO.

3 Minutes and action points of TEG 2014

The minutes of the TEG 2014 were accepted (*1*). The chair also summarized the key recommendations of the evidence review group (ERG) on *K13* held in September 2015 in Geneva (Annex 4).

4 Update on drug resistance and new WHO policies

4.1 WHO policies

Presentation

The *Global technical strategy for malaria 2016–2030* (*2*) was adopted by the World Health Assembly in May 2015.

The *Strategy for malaria elimination in the Greater Mekong subregion (2015–2030)* (*3*) was developed in collaboration with six countries, WHO and multiple development partners, and was launched during a side event at the World Health Assembly in May 2015.

Based on available clinical trial data and input from the Malaria Modelling Consortium, the Malaria Policy Advisory Committee (MPAC) made recommendations on mass drug administration (MDA). A policy brief is available on the GMP website (*4*).

Discussion

The highlighting of the Greater Mekong subregion (GMS) as a special situation where MDA is warranted was welcomed; WHO is meeting with GMS partners to reinforce the recommendations for MDA in the region. The implications for MDA of the rapid emergence of resistance to dihydroartemisinin–piperaquine (DP) in Cambodia need to be considered. The TEG commented that MDA should not be used as a general tool to reduce malaria prevalence, and that the focus on areas approaching elimination, epidemics and complex emergencies is appropriate, although "approaching elimination" requires more specific guidance.

4.2 Drug resistance

Presentation

Following detection of the *Plasmodium falciparum Kelch 13* (*K13*) C580Y mutant in Guyana in 2010, a 7-day artesunate and single-dose primaquine clinical trial found no evidence of artemisinin resistance. The next step is a survey comprising 800 samples. No C580Y has been reported from elsewhere in the region.

Artemether–lumefantrine (AL) and artesunate–amodiaquine (ASAQ) remain efficacious in Africa.

Currently, nine countries in the Middle East, eastern Africa and India have recommended artesunate+sulfadoxine–pyrimethamine (AS+SP) as their first-line treatment. However, studies with elevated treatment failure rates have been observed in Somalia, Sudan and north-east India near the Myanmar border, leading to a treatment policy change to AL in this region of India. AS+SP treatment failures are associated with *Pfdhfr* and *Pfdhps* quadruple and quintuple mutants, in the absence of artemisinin resistance. In India, only four isolates with *K13* mutations have been identified so far.

In the GMS, high rates of treatment failure have been reported for DP in western, northern and eastern Cambodia. Artesunate–mefloquine (ASMQ) is 100% efficacious in areas where DP treatment failure is common. Artesunate–pyronaridine (ASPY) failure rates are about 10–15% in western Cambodia. Preliminary results report treatment failures with AL in southern Laos and with DP in Binh Phuoc province of Viet Nam. Investigations are ongoing.

5 Session 1: Update on artemisinin resistance

5.1 Current definition of artemisinin resistance and tools to monitor

Presentation

The current definition of partial artemisinin resistance developed from the TEG 2014 and ERG *K13* 2014 is complex. The list of associated and confirmed *K13* resistance mutations may require

expansion, and additional mutations outside *K13* may also be relevant. On average, the parasitaemia at day 3 represents 1–2% of the initial parasitaemia, and the trend does not vary over time. There is no evidence for the recent emergence of a higher level of artemisinin resistance. The phenotype is confined to a delay in parasite clearance that results from a reduction in ring-stage sensitivity, which seems to be associated with decelerated parasite ring-stage development. Slow parasite clearance in patients treated with an artemisinin-based combination therapy (ACT) causes more parasites to be exposed to the partner medicine alone, increasing the risk of de novo resistance to the partner medicine. Selection of resistance to the partner drug is correlated with the half-life of the partner drug, prolonging the period of subtherapeutic drug levels. In two comparative studies (one in Democratic Republic of Congo and one in Viet Nam), the ACT partner did not affect the clearance time compared to the artesunate monotherapy. The tools used to define artemisinin resistance all have strengths and limitations. Day-3 parasitaemia is highly dependent on the initial parasitaemia, immunity of the patient, skill of the microscopist and method used for slide reading. Although the half-life of the parasite clearance slope is not influenced by the initial parasitaemia, it does not take into consideration the lag phase and the tail, cannot be used for low parasitaemia levels (i.e. it requires at least 1000 parasites/µL) and is difficult to implement in a routine surveillance. In addition, the half-life of the parasite clearance slope also depends on the skill of the microscopist and the method used for slide reading.

Discussion

There should be a single global definition of artemisinin resistance; it should not depend on the region. Although the ring-stage survival assay ($RSA_{0–3h}$) and thresholds for in vivo tests are currently only validated for parasites and patients in South-East Asia (SEA), there are not enough data from other regions to justify the additional complexity of regional definitions of artemisinin resistance.

By focusing the criteria for confirmed artemisinin resistance on *K13* mutations, there was concern that *K13*-independent resistance mutations may be overlooked. It was also noted that the $RSA_{0–3h}$ will only detect changes in drug susceptibility in ring-stage parasites, and that additional in vitro testing may be needed if there are discrepancies between $RSA_{0–3h}$ findings, molecular data and clinical observations. However, these two tools remain fully effective to detect the spread of South-East Asian parasites outside SEA.

As a general recommendation, sample sizes should be sufficient to reliably determine the prevalence of *K13* mutations in a population. However, there may be cases where the identification of a *K13* mutation at a low frequency will require further investigation.

It needs to be explicit that parasite positivity at day 3 means at 72 hours post-treatment (±2 hours). If sampling at this time is not feasible, then the actual sampling time should be recorded. The positivity at day 3 should be corrected for parasitaemia of >1000 to <100 000 parasites/µL if patients with lower or higher parasitaemia levels are included in a study.

5.2 KARMA project

Presentation

The *K13* Artemisinin Resistance Multicenter Assessment (KARMA) project aims to construct a global map of *K13* propeller sequence polymorphisms. Over 14 000 samples from 59 countries (163 sites) have been analysed, yielding 108 non-synonymous (NS) mutations, among which 70 had never been described before and nine had been reported with >1% frequency. In SEA and China, *K13* mutants have reached intermediate frequency to fixation. There was no overlap between the sets of mutations and haplotypes in the Cambodia–Viet Nam region versus the China–Myanmar region. Eight NS mutations observed in SEA and China were associated with day-3 positivity cases (F446I, N458Y, N537D, R539T, I543T, P553L, P574L and C580Y).

South America, Oceania, the Philippines and central and south Asia are free of NS mutations. In Africa, NS mutations are generally uncommon, except in the Central African Republic, Chad, Comoros, Gambia, Guinea, Kenya and Zambia (>3%), where the mutation is mainly A578S. However, the A578S allele does not spread and does not confer in vitro artemisinin resistance; there is no evidence of resistance-conferring alleles in Africa.

Discussion

Not all *K13* mutations are relevant to artemisinin resistance; a signal for selection (e.g. frequency or evidence of spreading) is also required. A prevalence of at least 5% can be considered evidence of selection for a confirmed *K13* mutation. The inclusion of additional data in KARMA from areas of low transmission in Africa would be desirable.

The *K13* F446I mutation has been much more refractory to gene editing. This mutation was engineered into a *K13* donor plasmids for gene editing, and two to three independent transfections were attempted with different parasite strains; no edited parasites were observed. This mutation may carry a fitness cost that precludes it from being readily introduced into a wild-type parasite. $RSA_{0–3h}$ will be conducted by Institut Pasteur and the University of Maryland on culture-adapted strains carrying this specific mutation.

5.3 Slope versus day-3 positivity rate

Presentation

The log-linear section of the parasite clearance slope is the most robust part of the curve for measuring changes in the pharmacodynamic properties of the artemisinins. However, there is significant variation and confounders, such as immunity and splenic clearance rates for infected red blood cells (iRBCs). The half-life cut-off of at least 5 hours performs well for SEA, but it is somewhat arbitrary and depends on the underlying proportions of resistant versus sensitive parasite strains. Immunity can affect the half-life of the parasite clearance slope (by 0.3–1.0 hour).

It was reiterated that the parasite positivity rate at day 3 is a useful screening tool, although it is dependent upon the initial parasitaemia. Hence, a better phenotype for artemisinin resistance (or partial resistance) is needed.

Discussion

In areas of high transmission, demonstrating phenotypic artemisinin resistance using a day-3 positivity rate or the parasite clearance slope may be confounded by high levels of immunity, justifying screening in nonimmune populations, as with the therapeutic efficacy assessments. Also, the impact of artemisinin resistance on the dynamics of parasite clearance outside SEA is unknown. Thus, thresholds for defining the in vivo phenotype for artemisinin resistance may require amendment for other regions.

It is important to include a measure describing residual parasitaemia (the tail end of the parasite clearance curve), because this is relevant for the development and spread of resistance. The day-3 parasite positivity is a pragmatic approach to this. In addition, the log-linear portion of the parasite clearance slope provides robust information for evaluating the delay in parasite clearance caused by artemisinin resistance. Thus, the two measures are complementary.

5.4 Slope and artemisinin resistance

Presentation

The presented model of parasite clearance rates suggests that host immunity dominates the dynamics of parasite clearance unless drug resistance is high. Hence, the model indicates that parasite clearance rates have a poor sensitivity for detecting decreases in drug efficacy. Declining immunity is also predicted to increase parasite clearance times in the absence of resistance. The same model indicates that twice-daily dosing of artemisinin may be more appropriate than once-daily dosing, and has the potential to increase drug efficacy.

Discussion

There is no straightforward method in clinical studies for differentiating between dead and live parasites in iRBCs. Thus, the parasite clearance rate is a "noisy" measure of parasite killing. The log-linear section of the parasite clearance slope is the most robust, and has been shown to correlate closely with the in vitro artemisinin resistant phenotype ($RSA_{0–3h}$), and the *K13* genetically defined resistant parasites. Quantification of the effect of immunity (measured as the presence of *P. falciparum* antibodies) shows that the effect on parasite half-life is about 0.5 hours (up to 1 hour).

The fact that a flattening of the parasite clearance slope can be observed for parasites harbouring *K13* propeller mutations indicates that these mutations have a highly significant effect on the survival of ring-stage parasites following artemisinin therapy. However, this may not result in treatment failure if efficacy of the partner drug is maintained.

Confirmation of artemisinin resistance requires an artesunate monotherapy study. Artesunate for 3 days followed by ACT will provide the parasite clearance slope and day-3 positivity rate, but is no longer recommended as a standard design. A 7-day artesunate monotherapy study will provide additional information on changes in treatment efficacy (recrudescence); this is better aligned with the conventional definitions of antimalarial drug resistance and will detect emergence of higher level of artemisinin resistance.

On its own, the parasite clearance slope is not an appropriate metric for the development of new drugs. Efficacy at day 28 or day 42 remains the gold standard for evaluating the efficacy of new therapies. New combinations should contain active ingredients at dosage regimens that provide high efficacy for each compound if given alone.

Recommendations: Session 1

Definitions of *confirmed* and *associated K13* mutations are required. The criteria for determining whether a *K13* propeller mutation is confirmed or associated still follow the criteria suggested by the ERG on *K13* 2014; that is, one of the following:

- a statistically significant association ($p < 0.05$) between a *K13* mutation and either a half-life of the parasite clearance slope of ≥5 hours or positive parasitaemia at 72 hours (±2 hours) via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases; or
- >1% survival using the $RSA_{0–3h}$ (or >2 standard deviations above the mean value for *K13* wild-type parasites from the same area) in at least five individual isolates with a given mutation; or a statistically significant difference ($p < 0.05$) in the $RSA_{0–3h}$ assay between culture-adapted recombinant isogenic parasite lines, produced using transfection and gene-editing techniques, which express a variant allele of *K13* as compared to the wild-type allele.

A *K13* mutation is *confirmed* when both of these requirements are met, and *associated* when only one of these requirements is met. However, the $RSA_{0–3h}$ and thresholds for in vivo tests are currently only validated for South-East Asian parasites and patients.

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A578S	Not associated

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- ≥10% of patients with a half-life of the parasite clearance slope ≥5 hours after treatment with ACT or artesunate monotherapy.

Confirmed endemic artemisinin resistance is defined as:

- ≥ 5% of patients carrying K13 resistance-confirmed mutations, all of whom have been found, after treatment with ACT or artesunate monotherapy, to have either persistent parasitaemia by microscopy on day 3, or a half-life of the parasite clearance slope ≥ 5 hours.

The detection of artemisinin resistance signifies an epidemiological threat, but does not necessarily signify reduced ACT efficacy as a manifest public health problem. The immediate consequences should be the investigation of possible causes, such as irrational drug use, substandard antimalarial drugs or the importation of resistant genotypes. Detection of resistance must also prompt surveillance and evaluation of ACT efficacy, to assess potential concomitant partner drug resistance (for some partner drugs, molecular markers are available). The priority in such areas is to ensure that antimalarial treatment is based on a definitive diagnosis, that drugs are of good quality, and that there is a good clinical provider and patient adherence. Based on the local epidemiological situation, capacity for intensifying vector-control efforts to interrupt transmission should be investigated, including the potential for malaria elimination. In countries where targeting of malaria

control and treatment interventions is directed by risk stratification, the presence of artemisinin resistance is clearly a criterion for upgrading risk.

6 Session 2: Monitoring efficacy and effectiveness of preventive treatment

6.1 Intermittent preventive treatment in pregnancy

6.1.1 IPTp-SP efficacy and molecular marker for SP resistance

The quadruple mutant (*Pfdhfr* S108N, C59R, N51I; *Pfdhps* A437G) is present in >50% of isolates in central and west Africa. The quintuple mutant (+ *Pfdhps* K540E) is present in >50% of isolates from east Africa. The sextuple mutant (+ *Pfdhps* A581G) is mainly confined to a few areas of east Africa and does not appear to be accelerating towards fixation. Although the impact of SP resistance on the efficacy of intermittent preventive treatment in pregnancy (IPTp)-SP is of concern, there are currently no approved alternatives in Africa.

Low birth weight (LBW) is influenced by many factors. Hence, determining the effect of molecular resistance markers on this outcome in small clinical studies is problematic.

A meta-analysis of individual-level participant data derived from observational studies, including >200 000 pregnancies, indicated that even in areas with >95% prevalence of *Pfdhps* K540E, there was a statistically significant dose–response for a lower risk for LBW associated with each incremental dose of SP, provided the additional (sextuple) *Pfdhps* A581G mutation was still rare (<5% prevalence). There was no association between the administration of SP and a lower risk of LBW in areas where *Pfdhps* A581G was prevalent at >35%, suggesting that the effectiveness of IPTp-SP to reduce LBW is nearly fully compromised in these areas. However, data are lacking for birth outcomes associated with *Pfdhps* A581G sextuple mutation prevalence of 5–35%. Efficacy is probably not compromised by the presence of the A581G mutation where K540E is absent.

Although one study suggested that IPTp-SP in women with the sextuple mutant was harmful, this has not been confirmed in five additional studies (two of quintuple, three of sextuple mutants). However, *Pfdhps* A581G does appear to be associated with higher parasite densities and malaria-associated reduction in birth weights, possibly reflecting the lack of protective efficacy in women infected with these highly resistant parasites.

Discussion

In the individual-level meta-analysis, the prevalence of *Pfdhps* A581G as sextuple mutation was either <5% or >35%. Although data are lacking and more information is needed to understand the impact at prevalence of *Pfdhps* A581G sextuple mutations between 5% and 35%, the current analysis indicates that at >35% prevalence the impact of IPTp-SP on reduction of LBW is lost.

The positive effect of IPTp-SP on birth weight, despite failure to prevent parasitaemia and reduced efficacy at clearing antenatal parasites, could be due to a combination of its antimicrobial effects,

the provision of partial antimalarial protection through suppression of parasite densities in the placenta (rather than clearance) reducing placental pathology, or other off-target effects of SP.

6.1.2 Draft protocol to monitor efficacy of IPTp-SP

Presentation

Three observational protocol modules were considered:

- *Molecular module* – this is a temporal and spatial distribution of molecular markers of SP resistance. In a meta-analysis of aggregated data from 48 published studies reporting more than 54 000 births, the relative risk reduction of LBW associated with IPTp-SP was stratified based on molecular markers in *Pfdhps* with:
    - *low* defined as A437G <50%;
    - *moderate* defined as A437G ≥50% plus K540E <10%, or K540E ≥10% plus <1% A581G; and
    - *high* defined as K540E ≥10% plus A581G ≥1%.

  The relative risk reduction of LBW associated with the receipt of antenatal SP was 28% in low resistance settings, 20% in moderate settings, and 9% in high settings.
- *In vivo module* – this includes the efficacy of SP to clear peripheral parasitaemia in asymptomatic pregnant women and the assessment of post-treatment prophylaxis. Although there is a strong correlation at population level between SP parasite resistance and the efficacy of IPTp with SP to clear existing infections or prevent new infections from occurring, the correlation between the parasite clearance and maternal anaemia, birth weight or other outcomes is weak. These studies are also labour intensive and difficult to conduct, and follow-up is limited to 28 days.
- *Delivery module* – determination of the effects of varying doses of IPTp-SP on malaria infection, maternal anaemia, placental malaria and birth outcomes (LBW). These studies require large sample sizes to detect effects on birth outcomes. They are subject to selection bias because the participant, not the investigator, determines exposure to SP, and pregnant women taking multiple doses of IPTp-SP are different from those taking one or a few doses. Previous studies have shown only a weak correlation between the level of SP parasite resistance in the population and pregnancy outcomes, and there is considerable variation in the SP protective efficacy in areas of high resistance.

Discussion

Clinical studies are difficult to conduct and interpret, and in vivo studies may lead to premature withdrawing of IPTp-SP, because IPTp-SP continues to be associated with a reduced prevalence of LBW, despite poor efficacy in clearing antenatal parasites.

There is now evidence that molecular markers for SP resistance can be used to evaluate the effectiveness of IPTp-SP.

In the consideration of potential alternative drugs for IPTp, such as DP, the risk of jeopardizing first-line antimalarial drug treatment should also be taken into account.

Recommendations: Session 2 IPTp-SP

At a population level, IPTp-SP is associated with improved birth outcomes (fewer LBW), irrespective of SP's failure to clear or prevent parasitaemia, in all settings where the prevalence of sextuple mutant haplotype containing *Pfdhps* A581G is below 5%. The presence of parasites bearing the sextuple mutant haplotype containing *Pfdhps* A581G at a prevalence of >35% appears to negate the benefits of IPTp-SP on birth outcomes. Overall, the evidence suggests that IPTp-SP given to women with the sextuple mutant is not harmful. This concern was suggested in a single study, but was not confirmed by later studies. There are no data at present on the effectiveness of IPTp-SP at the prevalence of sextuple mutant haplotype containing *Pfdhps* A581G of 5–35%.

For national malaria control programme (NMCP) settings, molecular surveillance should be used to guide routine assessment of IPTp-SP effectiveness. IPTp-SP should be continued or implemented in areas of unknown, low or moderate SP resistance. In areas of high SP resistance, IPTp-SP may be of more limited benefit, and this benefit is primarily associated with the specific prevalence of the *Pfdhps* A581G sextuple mutation. The threshold of A581G prevalence at which IPTp-SP is no longer of benefit is unclear, but the evidence suggests that there will be no benefit of IPTp-SP at >35% A581G prevalence. However, IPTp in areas with a high prevalence of A581G is not thought to cause harm. Therefore, even in settings of high SP resistance, molecular monitoring of the prevalence of A581G mutations can be used as a proxy for IPTp-SP effectiveness. Molecular surveillance should focus on the *Pfdhps* gene, and in particular on the mutations occurring at codons S436A/F, A437G, K540E, A581G and A613S/T. Methods include aggregate genotyping by sequencing of pooled samples (frequency) or individual-level genotyping by polymerase chain reaction (PCR) and sequencing, or through allele-specific assays such as PCR-RFLP (restriction fragment length polymorphism), PCR-SSOP (sequence specific oligonucleotide probe) and real-time PCR (prevalence). Genotyping may be carried out on parasite samples if collected from a population that has not recently (i.e. in the previous 6 weeks) been treated with antifolates. Sampling should take place every 3 years in areas of low SP resistance, every 2 years in moderate areas, and every year in high areas. Quality control of genotyping should be implemented whether molecular data are generated in-country, in regional laboratories or with international partners.

In research settings, additional considerations are mutations in *Pfdhfr* and the sequencing of each locus, which may identify new mutations in the gene targets of interest. The impact of I431V, which is emerging in Nigeria, needs to be investigated. Although there are sufficient data from areas with low, moderate and high SP resistance, more information is needed from areas with the highest levels of SP resistance, defined by the presence of *Pfdhps* A581G mutants; when these data become available, they will help to define the thresholds at which alternative strategies are needed. In these areas with the highest levels of SP resistance, priority research themes include:

- ecological studies of the impact of IPTp-SP on birth outcome (birth weight), maternal anaemia, and maternal and placental malaria measured at the time of delivery;
- individual-level studies of the impact of *Pfdhps* A581G-bearing parasites on birth outcomes, and of whether this relationship is modified by IPT-SP; and
- effective, well-tolerated, feasible alternatives for prevention.

6.2 Seasonal malaria chemoprevention

6.2.1 Monitoring efficacy of SMC

Presentation

The ACCESS-SMC project aims to provide seasonal malaria chemoprevention (SMC) for 8 million children in seven countries in the Sahel over 2 years. The London School of Tropical Medicine and Hygiene is working with research groups in each country to measure SMC coverage and the impact of SMC on malaria, to support pharmacovigilance and to monitor the efficacy of SMC drugs (AQ+SP). Children aged under 5 years are included, except in Senegal where children aged under 10 years are also treated. The study includes 30 sentinel sites, but is limited to 2 years. A system is needed for collecting surveillance and efficacy data in the future.

Monitoring will provide reassurance about efficacy after 2 years, establish a baseline for future monitoring and indicate factors that may limit the selection of resistance.

The objectives of the protocols are to:

- measure the prevalence of molecular markers associated with resistance to SP and amodiaquine before SMC in the community in children aged under 5 years and in age groups that are too old for SMC;
- measure the change in the prevalence of these markers after 2 years of SMC;
- measure the prevalence of markers in samples from clinical cases (children and adults) in selected clinics before and after SMC;
- monitor the prevalence of markers in cases in adjacent non-SMC areas;
- measure the protective efficacy of SMC treatments (using case–control studies);
- assess the utility of the screening method for monitoring efficacy (from dates of any SMC doses in malaria cases in children in sentinel surveillance clinics);
- monitor coverage and adherence through surveys at the end of each cycle and at the end of each season; and
- if possible, measure clearance of parasitaemia after AQ+SP treatment.

There is still an opportunity to make limited amendments to the protocol.

Discussion

Based on the ACCESS-SMC protocol, recommendations are needed as to what studies should be continued following the 2-year scope of the study. The studies need to be practical, affordable and sustainable for implementation within NMCPs. SMC is a new tool, and data upon which to base recommendations are sparse. However, there is a need for systems that can be activated at the end of the 2-year ACCESS-SMC study. Thus, general recommendations should be made at this time and re-examined once data are available.

Resources for high-throughput molecular evaluation are currently absent in the region. Coordination and standardization between laboratories needs to be established. It may be possible to leverage existing networks in the region to enhance capacity.

Signals for an effect on transmission should be examined, particularly in Senegal where children aged up to 10 years are being treated.

Reserving amodiaquine for SMC may deter the use of ASAQ and drive the increased use of AL in Africa. AL is already heavily used in Africa, whereas greater ACT diversity should be encouraged.

The use of SP in SMC may influence how it is used in IPTp, for example. In turn, this may affect the type of monitoring necessary.

6.2.2 Modelling prophylactic effect of antimalarial medicines

Presentation

Pharmacokinetic and pharmacodynamic modelling can provide information on the prophylactic potential of antimalarial drugs. As parasite susceptibility decreases, the duration of prophylaxis decreases. Models for AL, ASMQ and DP are well developed and published. Models for ASAQ and IPTp-SP require further development.

Modelling indicates that DP efficacy is extremely vulnerable to decreases in parasite susceptibility to piperaquine, because of its long flat terminal elimination. A small increase in piperaquine resistance can also substantially reduce the period of prophylaxis, which could jeopardize its use in MDA.

Discussion

The drug concentration-parasite clearance relationship is poorly defined, particularly for the artemisinins. It is not clear how the period of prophylaxis for long-acting medicines relates to the window of selection of resistance. However, the models can be used to look at this.

Recommendations: Session 2 SMC

The ongoing ACCESS-SMC study will provide more robust information about which SMC measures will be most appropriate – an issue that will need to be revisited by the TEG once the data are available. Both molecular markers and efficacy evaluation are required and, ideally, some measure of transmission intensity. In addition to the protocol for monitoring drug resistance of the ACCESS-SMC study presented at the TEG meeting, the following parameters should be explored:

- Efficacy evaluation – the ratio of malaria cases in children aged under 5 years versus those aged over 10 years; the occurrence of clinical malaria relative to the time of the previous SMC dose; the incidence of severe malaria at sentinel sites; case–control sampling before each dose for microscopy, gametocytaemia and PCR positivity relative to the time of previous SMC dose.
- Molecular markers – at least Pfcrt K76T and Pfmdr1 N86Y, Y184F and D1246Y (still rare in west Africa) should be determined routinely to track any changes in their prevalence, as an indicator of changes in amodiaquine efficacy. In particular, the prevalence of the Pfcrt codon 72 to 76 haplotype SVMNT (Ser-Val-Met-Asn-Thr) should be determined. Also Pfdhfr and Pfdhps should be included in the molecular markers to be tested.
- Capacity-building – local capacity-building for the monitoring of molecular markers is needed.

- Impact on transmission – assessed through standard membrane feeding assay, if feasible, and parasite genetic indicators of complexity of infection and overall changes in parasite diversity levels, where possible.
- Drug policy effects – the impact of SMC on first-line ACT diversity (and thus drug pressure) should be monitored.

7 Session 3: Prevention and treatment of multidrug resistant malaria

7.1 Definition of multidrug resistant malaria and rotational first line: the example of Cambodia

Presentation

In Cambodia, resistance to DP has spread rapidly. In areas of DP resistance, efficacy of ASMQ was restored to 100%, leading to the recommendation that ASMQ be reintroduced as the first-line ACT in these areas. However, switching to ASMQ has been delayed by complications in procuring the medicine and by issues in the supply chain. The efficacy of ASAQ in Cambodia is unknown, but data from Myanmar and Viet Nam suggest that this drug may have efficacy in the region.

Discussion

Artemisinin delayed clearance does not meet the current conventional WHO 1973 definition of antimalarial drug resistance[3], though a limited number of cases were described as potentially fully resistant to artemisinin.

The definition of multidrug resistance (MDR), which is still valid, requires resistance to more than two operational antimalarial compounds of different chemical classes.

In reporting the findings of therapeutic efficacy tests, ACT resistance is imprecise. ACT treatment failure is a more appropriate term that notes the specific ACT and the nature of the resistance (i.e. artemisinin partial resistance or partner drug resistance, or both).

The restoration of ASMQ efficacy in areas of DP resistance is probably a combination of competing resistance mechanisms and the removal of mefloquine drug pressure.

ASAQ may have a role to play in Cambodia. There is no evidence of cross-resistance between piperaquine and amodiaquine in South-East Asian parasites. Although ASPY does not meet WHO criteria to be introduced as a first-line treatment in western Cambodia, it may be an option in other regions of Cambodia and in the GMS. Cross-resistance between piperaquine and pyronaridine needs to be urgently explored.

[3] Ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject.

Until trials on alternative regimes provide results, there is no alternative in Cambodia other than to be flexible and rotate the first-line ACT based on surveillance data. However, the operational issues involved in changing drug regimens are challenging.

7.2 Prolonged duration of treatment

Presentation

In a study conducted in the Myanmar–Thailand border area comparing 3-day and 5-day AL therapy, efficacy was 100% and 97%, respectively. There was no difference in quantitative PCR (qPCR) positivity between arms; about 40% of patients were qPCR positive at day 21. Both regimens were well tolerated.

Discussion

There is no efficacy argument to extend AL therapy from 3 days to 5 days in Myanmar, where AL is still highly efficacious. However, extended therapy could theoretically reduce the potential for resistance to develop. Extension of the artemisinin dose to 5 days is closer to the fully effective dose of artemisinin (as a single agent).

7.3 Triple combination

Presentation: TRAC II

TRAC II is testing several different drug regimens in regions with failing ACTs:

- triple ACTs: AL versus AL+AQ, and DP versus DP+mefloquine (currently recruiting);
- arterolane–piperaquine;
- extended course AL (see above); and
- extended course DP (planned, no funding).

Presentation: combined ACTs

An additional short-to-medium solution to a decline in ACT efficacy may be to administer sequential courses of two different ACT regimens, giving a full 6 days of artemisinin. A protocol has been developed using AL followed by DP in Kenya and Tanzania, and ASAQ followed by AL in Burkina Faso. The primary outcomes are parasitological cure, incidence of severe adverse events, laboratory assessment, gametocyte clearance, selection of marker of drug resistance, and acceptability of and adherence to the proposed treatment schemes. This study is powered to detect the effect of submicroscopic residual parasitaemia on late recrudescence.

Discussion

Implementing triple combination therapy or a sequential ACT regimen requires drug interaction data and rigorous safety monitoring. Cardiotoxicity must be assessed particularly, but not only, for piperaquine and mefloquine. A study in Cambodia reported that 30% of care-seeking patients have residual levels of piperaquine in their blood. Higher cumulative artemisinin doses can cause bone marrow suppression at 10–14 days post-therapy; thus, monitoring should be included in any protocol evaluating increased dose or duration of artemisinin.

For triple therapy, it is unlikely that pharmaceutical partners will develop a co-formulated triple combination therapy quickly; hence, a co-blister pack would be necessary. However, the 3-day therapy duration is retained, aiding adherence. The TRAC II protocol was developed before the rapid spread of piperaquine resistance in Cambodia. The efficacy of the triple combination thus requires fast and careful evaluation, because all possible options need to be considered in this region.

For sequential ACT regimens, the drug formulations concerned are already available. In Africa, where standard ACTs are still working, there is the benefit of time, meaning that safety can be assessed rigorously in clinical trials including roll-out to large community trials. Adherence may be problematic with 6-day therapy, although education may improve this situation. A 6-day regimen may be more acceptable in areas with high ACT failure rates. If AL is a component, the switch between once-daily to twice-daily ACT is perhaps a more challenging adherence issue. Ensuring adequate stocks of both components may also be problematic.

In addition to addressing the issue of ACT failures, enhanced ACT regimens (e.g. triple, sequential and extended) should be evaluated for their potential to prevent the emergence and spread of resistance; for example, by computer modelling or the effect on submicroscopic residual parasitaemia. Cost implications for using enhanced ACT regimens should also be taken into consideration.

7.4 Multiple first-line treatments

Presentation

Multiple first-line treatments (MFLT) is the simultaneous distribution of multiple, different first-line treatments against uncomplicated malaria. Thus, with MFLT, different patients receive different treatments. The effect of this approach on delaying resistance development cannot be evaluated in clinical trials because resistance may not emerge for many years. Thus, computer modelling is the only method available to predict whether MFLT is a better approach than the alternative (i.e. cycling of first-line therapies). Two modelling groups had previously presented data to address this question but results differed between the two groups.

The results of a revised model from Oxford University Clinical Research Unit (OUCRU) were presented, indicating that MFLT delays the emergence of resistance to a greater extent than cycling first-line therapies. It also indicated that including a non-ACT drug with >85% efficacy plus two ACTs may be preferable to the deployment of three ACTs.

Implementation of MFLT requires consideration. For example, can therapy distribution be achieved by sector, location, age, or type of clinic or pharmacy? Day-of-the-week randomization requires a lot of infrastructure and education, but allows long-term flexibility.

Discussion

MFLT is not analogous to combination therapy at a population level because simultaneous resistance development to all deployed regimens is not required for parasite survival, only resistance to each individual treatment regimen.

The two published models diverge mainly at high MFLT drug coverage levels (>60%), but not greatly, with one showing a 10% increase and the other a 10% decrease in treatment failures. In the OUCRU model presented, this translates into a longer useful therapeutic life, with resistance emerging after 11 years with MFLT versus 7 years with cycling of first-line therapies. The modelling uses conservative assumptions; in particular, it tests scenarios, which are potentially averse to yielding a benefit, and it includes all reasons for treatment failure rather than just drug resistance. Consequently, the benefit of MFLT is probably underestimated in the OUCRU model.

The model does not allow for pre-existing resistance in the population, only de novo mutations. Thus, in areas such as Cambodia, where resistance is already established and drug therapies are limited, MFLT would probably not be appropriate. The model suggests that MFLT is appropriate for areas where resistance mutations are absent or at a prevalence of about 0.1%.

Modelling how drug resistance mutations appear in a population is complex. For some drugs (e.g. chloroquine), the mutations arise rarely and spread, but for others (e.g. mefloquine), resistance may arise much more frequently. Thus, constructing a model based on how mutations arise is contentious.

The current models are not sufficient to make a clear recommendation for MFLT. Multiple models need to be evaluated, and the Malaria Modelling Consortium should be consulted. However, other existing models of drug resistance may be limited and require further development.

An advantage of having MFLT available is that failing therapies can be discontinued without affecting malaria treatment provision. MFLT can also help to limit drug stock-outs.

Recommendations for MFLT should include pragmatic implementation methods (e.g. using paediatric formulations in children and tablets in adults).

In many countries, MFLT is already a reality. Public and private sector prescribing can vary greatly, with data on private sector prescribing being largely ignored, even though it may account for 80% of treatments in some regions.

Recommendations: Session 3

Safe and effective treatment in areas of confirmed MDR malaria

Rotational first-line treatment (where the first-line treatment is changed based on updated surveillance data, which can include molecular markers) is already being implemented in Cambodia. However, operational issues in switching therapies are challenging. At present, there is no alternative in Cambodia other than to be flexible, and use rotational first-line treatment.

Information is limited on the efficacy and safety of prolonged treatment with an ACT, triple combination treatment containing an artemisinin and two partner drugs, or sequential ACTs. Once more data are available, it is recommended that the DER TEG should hold a joint session with the Chemotherapy TEG to evaluate the information emerging from these studies.

ASAQ may have a role to play in Cambodia. As a first step, resistance markers for amodiaquine (single nucleotide polymorphism alleles *Pfcrt* 72-76, *Pfmdr1* N86Y, Y184F and D1246Y) and in vitro

susceptibility should be examined in GMS isolates. This should be followed by a therapeutic efficacy study of a fixed-dose ASAQ combination in Cambodia in 2016, if molecular marker data suggest reasonable amodiaquine efficacy.

Prevention or delay of MDR where it has not been identified

In areas where there is no established MDR, simultaneous deployment of multiple effective ACT first-line treatments (MFLT) is unlikely to hasten, and may actually delay, the emergence of drug resistance, according to modelling studies. Therefore:

- countries that presently have multiple approved ACT first-line treatments should continue to use them; and
- countries that rely on a single ACT first-line treatment are encouraged to add additional effective ACT treatments to the national treatment guidelines, both to potentially delay the onset of resistance and to be better prepared to respond to failure (or stock-outs) of the current first-line treatment.

Because modelling is the only means of evaluating the impact of MFLT on delaying resistance, the TEG recommends that the Malaria Modelling Consortium be asked to further develop these modelling approaches. Implementation issues should also be considered. The DER TEG is ready to examine outputs from the Malaria Modelling Consortium and any supporting clinical data.

References

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Annex 1: List of participants

Technical experts

Arjen Dondorp, Chair
Mahidol Oxford Research Unit
Bangkok, Thailand

Kevin Baird
Eijkman Oxford Clinical Research Unit
Jakarta, Indonesia

Karen Barnes
University of Cape Town
Cape Town, South Africa

James Eliades
Mailman School of Public Health, Colombia University
New York, United States of America (USA)

Ian Hastings
Liverpool School of Tropical Medicine
Liverpool, United Kingdom of Great Britain and Northern Ireland (United Kingdom)

Sylvia Meek
Malaria Consortium
London, United Kingdom

Didier Ménard
Institut Pasteur in Cambodia
Phnom Penh, Kingdom of Cambodia

Harald Noedl
Medical University of Vienna
Vienna, Austria

Chris Plowe
University of Maryland
Baltimore, USA

Allan Schapira
Independent Consultant
Manila, Philippines

Frank Smithuis
Medical Action Myanmar
Yangon, Myanmar

Siv Sovannaroath
National Center for Parasitology, Entomology and Malaria Control
Phnom Penh, Kingdom of Cambodia

Nguyen Quang Thieu (unable to attend)
National Institute of Malariology, Parasitology and Entomology
Hanoi, Viet Nam

Julie Thwing (unable to attend)
United States Agency for International Development/Centers for Disease Control and Prevention
Dakar, Senegal

Sarah Volkman
Harvard University
Boston, USA

Ad hoc invited speakers

Ter Kuile Feiko
Liverpool School of Tropical Medicine
Nairobi, Kenya

Boni Maciej
Oxford University Clinical Research Unit
Hanoi, Viet Nam

Paul Milligan
London School of Hygiene and Tropical Medicine
London, United Kingdom

Collin Sutherland
London School of Hygiene and Tropical Medicine
London, United Kingdom

Steve Taylor
Duke University Medical School
Durham, USA

Observers

Izaskun Gaviria
The Global Fund to Fight AIDS, Tuberculosis and Malaria
Geneva, Switzerland

Thomas Kanyok
The Bill & Melinda Gates Foundation
Seattle, USA

Malcolm McNeil and Susan Elden
Department for International Development
London, United Kingdom

Meera Venkatesan
United States Agency for International Development
Washington, USA

Adam Aspinall and Stephan Duparc
Medicines for Malaria Venture
Geneva, Switzerland

Rapporteur

Naomi Richardson
Magenta Communications Ltd
Abingdon, United Kingdom

WHO Secretariat

Global Malaria Programme

Pedro Alonso
Director
Global Malaria Programme (GMP)

Amy Barrette
Technical Officer
Drug Efficacy and Response, GMP

Andrea Bosman
Coordinator
Prevention Diagnostics and Treatment, GMP

Karin Elsea
Team Assistant
Drug Efficacy and Response, GMP

Peter Olumese
Medical Officer
Prevention Diagnostics and Treatment, GMP

Charlotte Rasmussen
Technical Officer
Drug Efficacy and Response, GMP

Pascal Ringwald
Coordinator
Drug Efficacy and Response, GMP

Marian Warsame
Medical Officer
Drug Efficacy and Response, GMP

Annex 2: Meeting agenda

Technical Expert Group on Drug Efficacy and Response

10–11 December – Crowe Plaza, Geneva, Switzerland

Thursday 10 December 2015		
09:00–09:15	Welcome **P. Alonso – Director GMP** **A. Dondorp – Chair TEG DER**	
09:15–09:20	Declaration of interest **P. Ringwald**	
09:20–10:00	Minutes and action points of the last TEG meeting and ERG on *K13* **A. Dondorp**	→ For information
10:00–10:30	Update on drug resistance and new WHO policies **P. Ringwald**	→ For information

10:30–11:00	Coffee/tea break	
Session 1: Update on artemisinin resistance		Purpose of session and expected outcomes
11:00–12:30	i) Current definition of artemisinin resistance and tools to monitor **P. Ringwald 20' + 10'** ii) KARMA project **D. Ménard 20' + 10'** iii) Slope versus day 3 positivity rate **A. Dondorp 20' + 10'**	→ For information and decision
12:30–13:30	Lunch	
13:30–15:30	iv) Slope and artemisinin resistance **I. Hastings 20' + 10'** **Discussion 90'**	
15.30–16.00	Coffee/tea break	
Session 2: Monitoring efficacy/effectiveness of preventive treatment		Purpose of session and expected outcomes
16:00–18:00	i) IPTp-SP efficacy and molecular marker for SP resistance **S. Taylor invited speaker 20' + 10'** ii) Draft protocol to monitor efficacy of IPTp-SP	→ For information and decision

	F. ter Kuile invited speaker 20' + 10' **Discussion 60'**	
18:30–20:00	Reception	
Friday 11 December 2015		
Session 2: Monitoring prophylactic effect of preventive treatment		Purpose of session and expected outcomes
8:00–9:45	iii) Monitoring efficacy of SMC **P. Milligan invited speaker 20' + 10'** iv) Modelling prophylactic effect of antimalarial medicines **I. Hastings 20' + 10'** **Discussion 45'**	→ For information and decision
9:45–10:15	Coffee/tea break	
Session 3: Prevention and treatment of MDR malaria		Purpose of session and expected outcomes
10:15–13:15	i) Definition of MDR malaria and rotational first line: the example of Cambodia **P. Ringwald & S. Sovannaroath 15' + 10'** ii) Prolonged duration of treatment **A. Dondorp & F. Smithuis 15' + 10'**	→ For information and decision

	iii) Triple combination TRAC 2 **A. Dondorp 15'** Combined ACTs **C. Sutherland invited speaker 15' + 10'** iv) Multiple first-line treatments **M. Boni invited speaker 15' + 10'** **Discussion 60'**	
13:15–14:00	Lunch	
14:00–17:00	Adoption of TEG recommendations **A. Dondorp**	Closed session
17:00	Closing remarks **A. Dondorp/P. Ringwald**	Closed session

Annex 3: List of questions

Session 1: Update on artemisinin resistance

K13

1. Are both definitions of *confirmed* and *associated K13* mutation necessary and should the list be updated?

2. Are confirmed *K13* mutations necessary and sufficient to determine the presence of artemisinin resistance?

Tools for detection of artemisinin resistance

3. Are the current tools used for the early detection and monitoring of artemisinin resistance (i.e. percentage of patients positive on day 3, parasite clearance slope, presence of *K13* mutation) adequate?

4. Are there additional or better parameters that could ensure timely detection of AR (e.g. PC90, PCT, …)?

Definition of artemisinin resistance

5. Does the current definition of artemisinin resistance require modification? If so, which ones?

6. Based on the modification of the definition, when is action needed to respond to the presence of artemisinin resistance?

7. What type of programmatic action is needed to respond to confirmed artemisinin resistance?

Session 2: Monitoring efficacy/effectiveness of preventive treatment

Intermittent preventive treatment in pregnancy (IPTp-SP)

8. In asymptomatic pregnant women, is SP failure in clearance and prevention of parasitaemia associated with low birth weight (LBW), maternal anaemia at delivery or placental malaria infection?

9. In pregnant women on IPTp-SP, are infections with *P. falciparum* carrying specific sextuple mutations associated with LBW, increased incidence of maternal parasitaemia, maternal anaemia at delivery or placental malaria compared to falciparum infections not carrying these mutations?

10. Propose the outline protocol of a prospective study to monitor IPTp-SP effectiveness, in particular should the protocol be based on SP parasite clearance, molecular markers of resistance, and/or delivery outcomes (LBW, maternal parasitaemia, maternal anaemia and/or placental malaria)?

Seasonal malarial chemoprevention (SMC)

11. Propose an outline protocol of a prospective study to monitor amodiaquine-SP effectiveness in the context of SMC.

Session 3: Prevention and treatment of multidrug resistant (MDR) malaria

Safe and effective treatment in areas of confirmed MDR malaria

12. In areas with high treatment failures to more than one recommended ACTs, should rotational first-line treatment (where the first-line treatment is changed based on updated surveillance data) be implemented and how?

13. Compared to currently recommended 3-day treatment, will patients diagnosed with falciparum malaria in areas with confirmed MDR, be provided with a more efficacious and safe cure?

a. when given a prolonged treatment with an ACT?

b. when given a triple combination treatment containing an artemisinin and two partner drugs?

14. Is there a role for artesunate–amodiaquine in the GMS?

Prevention/delay of multidrug resistance where it has not been identified

15. What is the evidence that multiple first-line treatment (MFLT) will delay or prevent the development of MDR?

Annex 4: Minutes of the ERG on *K13*

Final Report_Expert Review Group (ERG)

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