Global **Malaria** Programme

Artemisinin and artemisinin-based combination therapy resistance

APRIL 2016

STATUS REPORT

KEY MESSAGES

1. Artemisinin resistance[1] is defined as delayed parasite clearance following treatment with an artesunate monotherapy or with an artemisinin-based combination therapy (ACT). This represents partial resistance.

2. Delayed parasite clearance will not necessarily lead to treatment failure. In the Greater Mekong Subregion (GMS), high treatment failure rates following treatment with an ACT have mostly (but not always) been observed where concomitant resistance to the partner drug exists. Outside the GMS, treatment failure with ACTs (AL, ASAQ, ASSP) occur in the absence of artemisinin resistance and are mainly due to partner drug resistance.

3. A molecular marker for artemisinin resistance has recently been identified and will help to improve the global surveillance of artemisinin resistance.

4. There is no evidence for recent emergence of higher levels of artemisinin resistance (full resistance). However, artemisinin (partial) resistance could facilitate the selection of partner drug resistance.

5. Piperaquine resistance has emerged in Western Cambodia and has in just a few years' time expanded considerably in proportion of strains affected as well as in geographical area.

6. Emergence of multidrug resistance including artemisinin and partner drug resistance causing ACT failures, and independent emergence of artemisinin resistance in the GMS have led to the WHO recommendation of elimination malaria in this region.

World Health Organization

WHO/HTM/GMP/2016.5

BACKGROUND ON ARTEMISININ RESISTANCE

Artemisinin resistance is defined as delayed parasite clearance, which represents a partial resistance affecting thus far only ring stage parasites. Delayed parasite clearance after treatment with an ACT is of paramount concern to WHO. Nevertheless, the majority of patients who have delayed parasite clearance following treatment with an ACT still clear their infections, except in the presence of concomitant resistance to the partner drugs. Further research is needed to evaluate the exact role of artemisinin resistance in the development or selection of drug resistance to partner drugs.

Recently, a molecular marker of artemisinin resistance was identified. Mutations in the Kelch 13 (K13)-propeller domain were shown to be associated with delayed parasite clearance in vitro and in vivo. The identification of the K13 mutations as a marker for artemisinin resistance has allowed for a more refined definition, inclusive of information on the genotype. However, the list of mutations associated with artemisinin resistance is still evolving, and as a result the definition of artemisinin resistance will also evolve, based on new findings. The current definition of artemisinin resistance is divided into suspected artemisinin resistance (defined as a high prevalence of the delayed parasite clearance phenotype or high prevalence of K13 mutants) and confirmed artemisinin resistance (defined as a combination of both delayed parasite clearance and K13 resistance-validated mutations for the same patient) (see annex). Confounding factors in these definitions include the effect of partner drugs, immunity, insufficient levels of drug in the blood, and non-validated K13 mutations.

A total of 186 K13 alleles, including 108 non-synonymous mutations were reported so far. In South-East Asia, distinct alleles originating from multiple independent events of emergence were observed. In the eastern Greater Mekong Subregion (Cambodia, Lao PDR and Viet Nam), C580Y, R539T, Y493H, I543T mutations were frequent. In the western Greater Mekong Subregion (China, Myanmar and Thailand) F446L, N458Y, P574L and R561H mutations were common. In Africa, non-synonymous mutations are still rare and highly diverse. Non-synonymous K13 mutations have been reported in Cameroon, Central African Republic, Chad, Comoros, Democratic Republic of the Congo, Gabon, Gambia, Kenya, Madagascar, Malawi, Mali, Rwanda, Togo, Uganda and Zambia. The most frequent allele observed in Africa is A578S.

TABLE 1
Canditate and validated K13 resistance mutations*

K13 MUTATION	CLASSIFICATION
E252Q	*Not associated*
P441L	Candidate
F446I	Candidate
G449A	Candidate
N458Y	Candidate
Y493H	Validated
R539T	Validated
I543T	Validated
P553L	Candidate
R561H	Validated
V568G	Candidate
P574L	Candidate
A578S	*Not associated*
C580Y	Validated
A675V	Candidate

*Other less frequent variants were reported associated with in vivo or in vitro tests, or both: M476I; C469Y; A481V; S522C; N537I; N537D; G538V; M579I; D584V; H719N

Not all non-synonymous propeller region K13 mutants reported are necessarily indicating emerging artemisinin resistance, as these can represent 'passer-by' genotypes when there is no evidence of selection of the mutant K13 genotype. In addition, different K13 mutations have varying effects on the clearance phenotype. Validation of the K13 mutant as resistance marker will require a correlation with slow clearance in

clinical studies, reduced drug sensitivity in ex-vivo assays or in vitro assays (e.g. ring-stage assay - RSA_{0-3h}), or transfection studies where insertion of the mutant K13 results in reduced in vitro sensitivity (see annex). A current list (which will have to be updated regularly) of candidate (correlated with delayed parasite clearance) and validated K13 propeller mutations (by in vivo and in vitro data) can be found in Table 1.

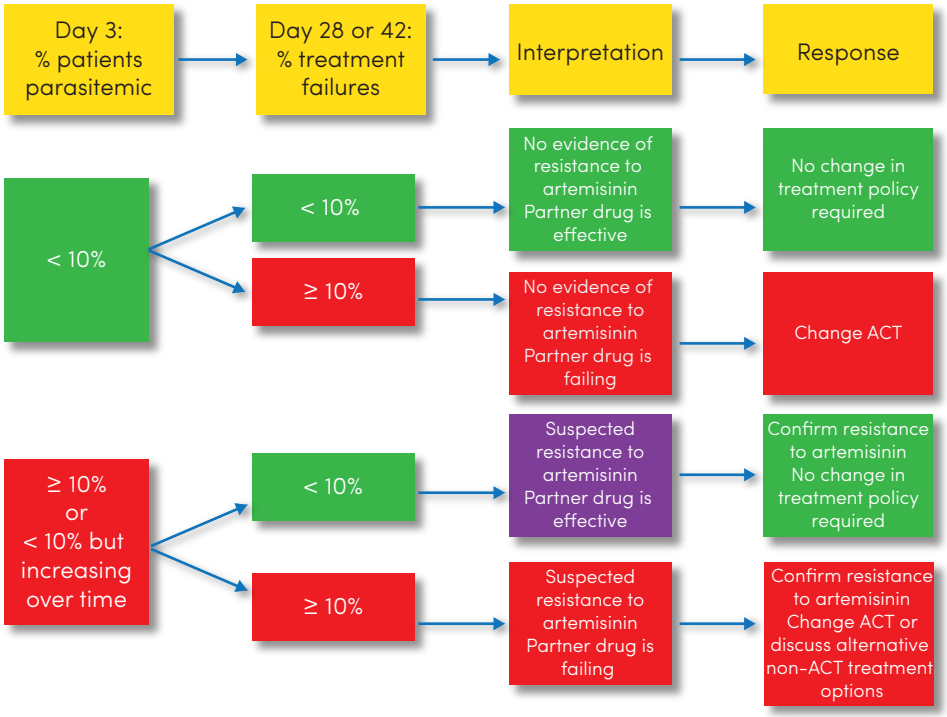
MONITORING THERAPEUTIC EFFICACY OF ACTS

Routine monitoring of the therapeutic efficacy of ACTs is essential for timely changes in treatment policy and can help to detect early changes in *P. falciparum* susceptibility to antimalarial drugs. WHO currently recommends monitoring the efficacy of first-line and second-line ACTs every two years in all falciparum endemic countries. The results of the therapeutic efficacy studies (TES) allow for the determination of:

- the **proportion of patients who are parasitemic on day 3**, which is currently the indicator of choice for routine monitoring to identify suspected artemisinin resistance in *P. falciparum*;
- the **proportion of treatment failure by 28- or 42-day follow-up** (depending on the partner drug half-life in the specific ACT). A treatment failure rate equal to or exceeding 10% should prompt a change in the national antimalarial treatment policy.

The flow chart (Figure 1) outlines the recommended steps required for the decision making process for the interpretation and response relative to TES findings.

FIGURE 1
Decision-making process based on TES results



If artemisinin resistance is suspected by the observation of slow clearance in a clinical trial or TES, K13 marker analysis should be prioritized, e.g. from filter paper blood spots. If suspected resistance is observed through a survey with molecular data only, resistance should be confirmed by studies obtaining information on both the clinical phenotype (delayed parasite clearance) and the K13 genotype from the same parasite strain.

Possible implications of delayed parasite clearance

Artemisinin is used in combination with other medicines to quickly reduce the parasite biomass. Consequences of partial resistance could include: 1) development of total artemisinin resistance; 2) failure to rapidly clear parasites could compromise the use of artemisinin for the treatment of severe malaria; 3) slow parasite clearance in patients treated with an ACT causes more parasites to be exposed to the partner medicine alone after the artemisinin component is rapidly cleared after the three-day treatment course, increasing the risk of de novo resistance to the partner drug in particular in patients with high parasitaemia at admission.

There is no evidence for recent emergence of higher levels of artemisinin resistance (full resistance). However, artemisinin (partial) resistance could facilitate the selection of partner drug resistance.

Further selection of partner drug resistance is correlated with its half-life. In case of resistance to partner drugs, treatment failures are likely to increase. Currently the majority of patients with a delayed parasite clearance response are still cured by ACTs, provided that the partner drug remains effective.

RESPONSE TO ARTEMISININ RESISTANCE AND ELIMINATING MALARIA IN THE GMS

Emergency response to artemisinin resistance in the GMS

In April 2013, WHO launched the *Emergency response to artemisinin resistance in the Greater Mekng subregion. Regional framework for action 2013-2015.*[2] The framework urges malaria partners to work in a coordinated manner to provide malaria interventions to all at-risk risk groups; to achieve tighter coordination and management of field operations; to obtain better information for artemisinin resistance containment; and to strengthen regional oversight and support.

WHO has received support from the Australian Department of Foreign Affairs and Trade and the Bill & Melinda Gates Foundation to strengthen the coordination and technical support for artemisinin resistance containment activities in the GSM. The project is implemented by the WHO Global Malaria Programme, the WHO Regional Office for South-East Asia, the WHO Regional office for the Western Pacific and WHO country offices. A regional hub has been established in Phnom Penh, Cambodia to support and help coordination of activities.

In line with the call to action and recommendations contained in the ERAR, the Global Fund to Fight AIDS, Tuberculosis and Malaria has allocated US$ 100 million to a regional artemisinin initiative, funding activities to contain and eliminate artemisinin

resistance in Cambodia, Lao People's Democratic Republic, Myanmar, Thailand, and Viet Nam. The regional artemisinin initiative includes a regional component to support cross border activities.

Malaria elimination in the GMS

The incidence of malaria has been greatly reduced over the last 10-20 years. However, there is concern that falciparum malaria in the GMS is becoming increasingly resistant to antimalarial medicines; at the border between Cambodia and Thailand, it could become untreatable within a few years. In addition, molecular studies have confirmed that artemsinin resistance has emerged independently in many areas of the GMS. On this background, the Malaria Policy Advisory Committee of WHO recommended in September 2014 the adoption of the goal of elimination of *P. falciparum* in the GMS by 2030. Subsequently, at the World Health Assembly in May 2015, WHO launched a *Strategy for Malaria Elimination in the GMS (2015-2030)*,[3] which was endorsed by all the GMS countries.

COUNTRY UPDATES ON ACT EFFICACY[4]

South-East Asia

Cambodia

Background

- Artemisinin resistance was first identified in clinical studies in 2006; however retrospective analysis of molecular markers indicates that artemisinin resistance likely emerged in 2001, before the widespread deployment of ACTs in Cambodia.

- Due to high failure rates with artesunate-mefloquine, the first-line treatment for the treatment of uncomplicated falciparum malaria was changed from co-blistered artesunate-mefloquine to fixed-dose dihydroartemisinin-piperaquine in Pailin in 2008 and then nationwide in 2010.

- After the implementation of this new treatment policy, an increase in treatment failures was quickly identified in therapeutic efficacy studies (TES) using dihydroartemisinin-piperaquine in Pailin. Between 2008 and 2015 similar trends were observed in seven provinces mainly in the western and northern part of the country. The high treatment failure rates observed with dihydroartemisinin-piperaquine is related to the presence of piperaquine resistance which is geographically spreading from western to north-eastern Cambodia.

- A consensus meeting held in November 2011 recommended the use of atovaquone-proguanil delivered as directly-observed therapy for Pailin province as a short-term interim solution, with stringent follow-up for monitoring resistance. Atovaquone-resistance conferring mutations were observed less than a year after the implementation of the drug as first-line therapy, which was reason to change the recommendation.

Update

- A consensus meeting on the national treatment policy for *P. falciparum* was held in January 2014. Artesunate-mefloquine was re-introduced as first-line

treatment, since the proportion of falciparum strains with multiple *Pfmdr1* copy numbers (which confer mefloquine resistance) is currently minimal in the area. Quinine plus doxycycline over seven days has been adopted as rescue therapy.

- Studies with artesunate-mefloquine conducted in 2015 reported 100% efficacy.

Lao PDR

Update

- In 2013, a trial conducted in Champasack province found that 22.2% of the patients treated with artemether-lumefantrine were still parasitemic on day 3 after treatment and 10% treatment failures; conversely in Saravanh province the positivity rate at day 3 was 2% with an overall treatment failure rate of 2.4%.

- The emergence of artemisinin resistance in Southern Lao PDR is supported by the recent (2013) identification of the presence of K13 mutants (mainly C580Y and R539T) in the circulating parasite populations.

- Containment activities started in 2014.

- The therapeutic efficacy of artemether-lumefantrine starts to be affected though cure rates have remained high since 2005; TES were conducted in 2015 in Atteupeu, Sekong and Champasack provinces. Positivity rate at day 3 ranged between 9.7 and 20% whereas and treatment failure rates range between 0% and 14%. In 2016 other ACT will be monitored for potential use in the country.

Myanmar

Background

- Artemisinin resistance likely emerged at the border between Thailand and Myanmar in 2001, but was clearly recognized in 2008.

- Since 2009, available data show consistently delayed parasite clearance times among a significant proportion of patients treated with ACTs, and observed in all the three first-line ACTs (artemether-lumefantrine, artesunate-mefloquine and dihydroartemisinin-piperaquine).

- The results showing delayed parasite clearance rates in several parts of the country led to the initiation of the Myanmar Artemisinin Resistance Containment (MARC) framework, in-line with the recommendations described in the *Global Plan for Artemisinin Resistance Containment* (GPARC).[5]

- The three first-line ACTs used in the country are still effective with high cure rates.

Update

- Studies evaluating the presence of K13 mutants have shown that the predominant K13 mutant found in Myanmar does not appear to have spread from Cambodia but likely arose independently.

- A new K13 propeller polymorphism (F446I) was significantly associated with delayed parasite clearance. Preliminary results indicate a high prevalence of the K13 F446I mutation along the China-Myanmar border and along the India-Myanmar border. Additional research is ongoing to validate the role of this

new mutant in artemisinin resistance which appears to be associated with an intermediate rate of delayed clearance.

- ACT efficacy remains high on both side of the border between India and Myanmar.

Thailand

Background

- Containment activities on the Thailand side of the border between Cambodia and Thailand began simultaneously with Cambodia in 2008.
- Thailand used a regimen of two-day artesunate-mefloquine as first-line treatment. Despite the change to a three-day regimen in 2009, treatment failures with artesunate-mefloquine increased in Kanchanaburi, Ranong, Tak, and Ubonratchathani, reaching treatment failure ≥ 10%.
- High treatment failures observed in Thailand after treatment with artesunate-mefloquine could be explained by the presence of mefloquine resistance (which has been confirmed countrywide) in addition to artemisinin resistance. Mefloquine drug pressure has been considerable over the last decades, since Thailand has been using different regimens of mefloquine (15 to 25 mg/kg) as monotherapy or in combination with artesunate.

Update

- The efficacy of artemether-lumefantrine was evaluated in two provinces in 2012 but the treatment failure rate was close to or exceeded 10%.
- During a consensus meeting held in 2015, dihydroartemisinin-piperaquine became the first-line treatment and its efficacy is currently being evaluated.
- Efficacy of dihydroartemisinin-piperaquine monitored in 2014-2015 in Kanchanaburi province was 94%.

Viet Nam

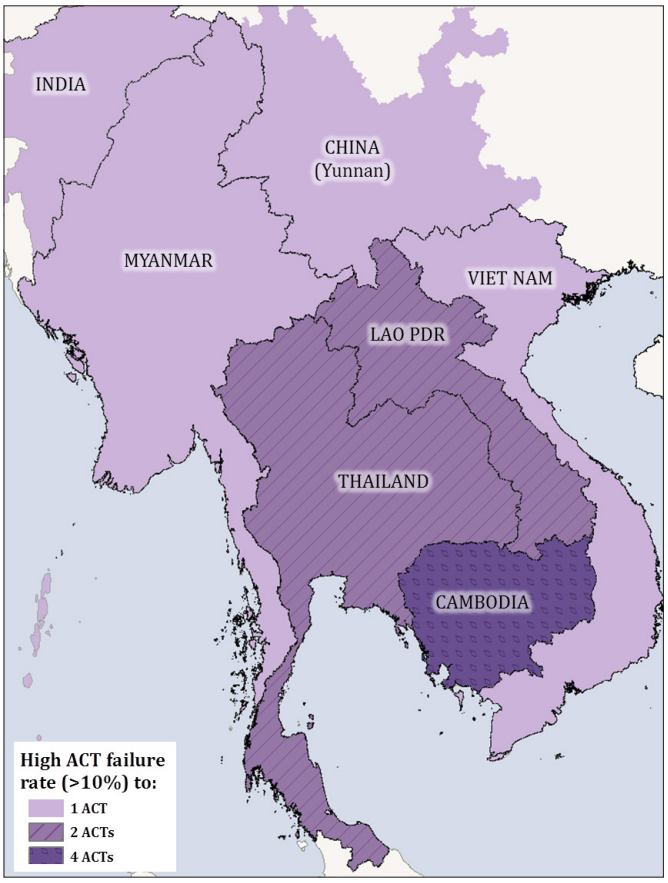
Background

- Delayed parasite clearance was first detected after treatment with dihydroartemisinin-piperaquine in Bu Dang district of Binh Phuoc province in 2009.
- Routine monitoring with dihydroartemisinin-piperaquine also detected other foci of delayed parasite clearance in Gia Lai province (2010), in Dak Nong province (2011) and Quang Nam province (2012), Khanh Hoa provinces (2014) and Ninh Thuan (2015).
- In mid-2011, Viet Nam began containment activities following GPARC recommendations with the support from WHO Western Pacific Regional Office and the WHO country office.

Update

- TES conducted since 2010 using dihydroartemisinin-piperaquine confirmed treatment efficacy > 95% despite a day 3 positivity rate of up to 36%.
- A recent study in Binh Phuoc province reported high treatment failure (> 10%). Investigation is ongoing to confirm emergence of piperaquine resistance.

FIGURE 2
Situation of ACT failures in the Greater Mekong subregion



There are currently five ACTs recommended by WHO: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine (ASSP) and dihydroartemisinin-piperaquine. A sixth ACT, artesunate-pyronaridine, was given a positive scientific opinion by the European Medicines Agency (EMA) under article 58 and is being considered for recommendation by WHO. By default, ASSP is considered having a high failure rate in the region as quadruple and quintuple dhfr and dhps mutations are fixed.

Africa

- The efficacy of ACTs is being monitored in most malaria endemic countries. There have been some reports of delayed parasite clearance during routine TES of ACTs conducted in Africa, however these reports have not been consistent over time;
- To date, the K13 mutations observed have not been associated with slow parasite clearance. At this moment, Africa appears free of resistance-associated Asian alleles;
- TES show that in general ACTs remain efficacious.

South America

Suriname

- Routine surveillance of ACT efficacy between 2005-2006 and 2011 in gold miners, reported an increase of day-3 positivity rate (from 2% to more than 20%) with a high cure rate at day 28. In 2013-2014, a study using artesunate and

mefloquine did not confirm the high positivity rate at day 3 and sequencing of K13 of strains collected during this study revealed only wild type K13.

Guyana

- A retrospective analysis of blood sample collected in 2010 for a HRP2 surveillance study, detected C580Y. All five C580Y mutant samples detected had nearly identical haplotype suggesting their common origin which was distinct from the South-East Asian C580Y haplotype. A survey for K13 sequencing is currently ongoing in the region where five of the earlier cases originated.

- The last TES study evaluating artemether-lumefantrine was conducted from May 2011 to July 2012 during which a total of 92 patients were enrolled, with 68 completing the 28 day follow-up. A total of 70.8% of day 3 slides were reported to be positive, but after quality control review, this result was considered to be flawed. A new clinical study evaluating 7-day artesunate for uncomplicated falciparum malaria was started in 2014. Efficacy of artesunate was 100% at day 28 whereas only 2% of the patients had persistent parasitaemia at day 3 after treatment. The 47 strains collected all showed K13 wild type.

French Guyana

- Between 2009 and 2013, the day-3 positivity rate among patients treated in Cayenne hospital after treatment with artemether-lumefantrine was 7.5%, but the treatment was not systematically supervised. So far no K13 mutant parasite strains have been reported from French Guyana.

CONCLUSION

Despite the delayed response to artemisinin in some areas of the GMS, ACTs remain the most effective treatment for uncomplicated falciparum malaria. Most patients with delayed parasite clearance are cured as long as the partner drug remains effective. Routine monitoring must continue to ensure that the recommended ACTs are effective, that timely changes in national treatment policies can be implemented, and that artemisinin resistance can be detected early. Assessment of Kelch-13 propeller region mutants will greatly facilitate the tracking of artemisinin resistance as it emerges. In the context of multidrug resistance including ACT resistance in the GMS, elimination of falciparum malaria has become a high priority. The role played by artemisinin resistance in the development or selection of partner drug resistance needs to be further evaluated.

Further information

For more information, please contact:

Dr Pascal Ringwald
Drug Efficacy and Response, Global Malaria Programme
World Health Organization
Tel: +41 (0) 22 791 3469
Email: ringwaldp@who.int

Please also visit the following WHO website for additional information and data:
http://www.who.int/malaria/areas/drug_resistance/en/index.html

Notes

1. Artemisinin refers to artemisinin and its derivatives.
2. WHO (2013). Emergency response to artemisinin resistance in the Greater Mekong Subregion.
3. http://apps.who.int/iris/bitstream/10665/79940/1/9789241505321_eng.pdf
4. WHO (2015). Strategy for malaria elimination in the Greater Mekong Subregion (2015-2030).
5. http://www.who.int/malaria/areas/greater_mekong/en/
6. WHO (2011). Global report on antimalarial efficacy and drug resistance: 2000-2010.
7. http://whqlibdoc.who.int/publications/2010/9789241500470_eng.pdf
8. WHO (2011). Global Plan for Artemisinin Resistance Containment (GPARC). http://www.who.int/malaria/publications/atoz/artemisinin_resistance_containment_2011.pdf

ANNEX

Definition of artemisinin resistance

Definitions of candidate and validated K13 mutations are required. as 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 validated when both of these requirements are met, and candidate 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 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.

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.

What does this icon represent? Learn more about the Global Malaria Programme's new visual refresh at: http://www.who.int/malaria/visual-refresh/en