



Artemisinin and artemisinin-based combination therapy resistance

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STATUS REPORT

KEY MESSAGES

1. Artemisinin resistance¹ 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 does not necessarily lead to treatment failure. In the Greater Mekong subregion (GMS), high treatment failure rates following treatment with an ACT have almost always been observed in areas where there is concomitant resistance to artemisinin and the partner drug. Outside the GMS, treatment failure with ACTs (artemether-lumefantrine, artesunate-amodiaquine and artesunate-sulfadoxine-pyrimethamine) has occurred in the absence of artemisinin resistance mainly due to partner drug resistance.
3. A molecular marker for artemisinin resistance has been identified and is helping to improve the global surveillance of artemisinin resistance.
4. There is no evidence that higher levels of artemisinin resistance (full resistance) have emerged. Nevertheless, artemisinin (partial) resistance could facilitate the selection of partner drug resistance.
5. Piperaquine resistance has emerged in Western Cambodia and, in just a few years, expanded considerably in terms of the proportion of strains and the geographical area affected.
6. The emergence of multidrug resistance, including artemisinin and partner drug resistance causing ACT failure, and the independent emergence of artemisinin resistance in multiple locations in the GMS have led WHO to recommend the elimination of malaria in this region.

BACKGROUND ON ARTEMISININ RESISTANCE

Artemisinin resistance is defined as delayed parasite clearance; it represents a partial resistance, which has affected only ring-stage parasites thus far. 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 are still able to clear their infections, except in the presence of concomitant resistance to partner drugs. Further research is needed to evaluate the exact role of artemisinin resistance in the development and/or selection of drug resistance to partner drugs.

A molecular marker of artemisinin resistance was identified: several mutations in the Kelch 13 (K13)-propeller domain were found 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 that includes information on the genotype. However, since the list of mutations associated with artemisinin resistance is still evolving, the definition of artemisinin resistance will also continue to evolve based on new findings. The current definition of artemisinin resistance is divided into suspected artemisinin resistance (defined as the high prevalence of the delayed parasite clearance phenotype or high prevalence of K13 mutants) and confirmed artemisinin resistance (defined as the combination of delayed parasite clearance and K13 resistance-validated mutations for the same patient) (see Annex). Confounding factors in these definitions include the effects of partner drugs, immunity, insufficient levels of drug in the blood, and non-validated K13 mutations.

To date, more than 200 non-synonymous mutations in the K13 gene were reported. Distinct alleles originating from multiple independent events of emergence were observed in South-East Asia. The KARMA project reported that in the eastern GMS (Cambodia, Lao People's Democratic Republic (PDR) and Viet Nam), C580Y, R539T, Y493H, and I543T mutations were frequent or specific. In the western GMS (China, Myanmar and Thailand), F446L, N458Y, P574L and R561H mutations were specific. The P553L allele was distributed in the two areas. The results of the TES conducted in 2015 in Cambodia, Lao PDR and Viet Nam and for which K13 sequencing is available have shown that C580Y has become the dominant mutation ranging from 48.8% in Lao PDR to 92.6% in Cambodia.

In Africa, non-synonymous mutations are still rare and highly diverse. Non-synonymous K13 mutations have been reported in Angola, Burkina Faso, Cameroon, Central African Republic, Comoros, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Ethiopia, Gabon, Gambia, Kenya, Liberia, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Togo, Uganda and Zambia.

Not all non-synonymous propeller-region K13 mutants reported indicate the emergence of artemisinin resistance; rather, such mutants can represent 'passer-by' genotypes in the absence of evidence for the selection of the mutant K13 genotype. In addition, different K13 mutations have varying effects on the clearance phenotype. The validation of the K13 mutant as a resistance marker will require the 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 reduced *in vitro* sensitivity resulting from the insertion of the K13 mutant in transfection studies (see Annex). A current list 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 below (this list will be updated regularly):

TABLE 1
Candidate and validated K13 resistance mutations*

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

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

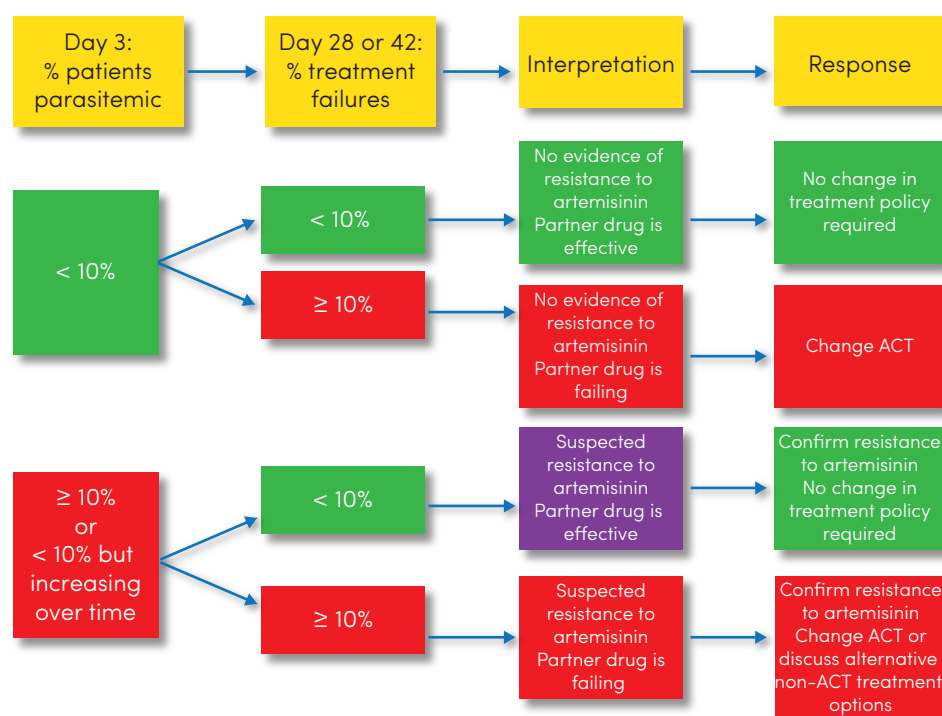
MONITORING THERAPEUTIC EFFICACY OF ACTs

Routine monitoring of the therapeutic efficacy of ACTs is essential to ensure timely changes in treatment policy and to help detect early changes in *P. falciparum* susceptibility to antimalarial drugs. WHO currently recommends monitoring the efficacy of first-line and second-line ACTs every 2 years in all falciparum-endemic countries. The results of therapeutic efficacy studies (TES) enable the determination of:

- the **proportion of patients who are parasitaemic on day 3**; this is currently the indicator of choice for the routine monitoring of 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 in Figure 1 outlines the recommended steps in the decision-making process for interpreting and responding to TES findings.

FIGURE 1
Decision-making process based on TES results



If artemisinin resistance is suspected due to 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 resistance is suspected based on a survey with molecular data only, resistance should be confirmed by 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. The consequences of partial resistance could include: 1) the development of total artemisinin resistance; 2) failure to rapidly clear parasites, which could compromise the use of artemisinin for the treatment of severe malaria; and 3) slow parasite clearance in patients treated with an ACT, which could cause more parasites to be exposed to the partner medicine alone once the artemisinin component is rapidly cleared following the 3-day treatment course; this increases the risk of de novo resistance to the partner drug, particularly in patients with high parasitaemia at admission, and facilitates the selection of partner drug resistance. Treatment failures are likely to increase with the resistance to partner drugs.

There is no evidence that higher levels of artemisinin resistance (full resistance) have emerged. Currently, the majority of patients with a delayed parasite clearance response can still be cured with ACTs, as long as the partner drug remains effective.

RESPONSE TO ARTEMISININ RESISTANCE AND ELIMINATING MALARIA IN THE GREATER MEKONG SUBREGION (GMS)

Emergency response to artemisinin resistance in the GMS

In April 2013, WHO launched the “Emergency response to artemisinin resistance in the GMS: Regional framework for action 2013–2015” (ERAR) (1). The ERAR framework urges malaria partners to work in a coordinated manner to provide malaria interventions to all at-risk groups; to achieve the tighter coordination and management of field operations; to obtain better information for the containment of artemisinin resistance; 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 coordinate the activities.

In line with the ERAR’s call to action and recommendations, 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 PDR, Myanmar, Thailand and Viet Nam. The regional artemisinin initiative includes a component to support cross-border activities.

Malaria elimination in the GMS

Over the last 10 years, the incidence of malaria has been greatly reduced. However, there is concern that falciparum malaria in the GMS is becoming increasingly resistant to antimalarial medicines including ACTs (Figure 2). In addition, molecular studies have confirmed that artemisinin resistance has emerged independently in many areas of the GMS. In light of these developments, in September 2014, the Malaria Policy Advisory Committee of WHO recommended the adoption of the goal to eliminate *P. falciparum* in the GMS by 2030. Subsequently, during the World Health Assembly in May 2015, WHO launched a *Strategy for malaria elimination in the GMS (2015–2030)* (2), which was endorsed by all the GMS countries.

COUNTRY UPDATES ON ACT EFFICACY (3)

South-East Asia

Cambodia

Background

- Artemisinin resistance was first identified in clinical studies in 2006; however, the retrospective analysis of molecular markers has indicated that artemisinin resistance likely emerged prior to 2001 and the widespread deployment of ACTs in Cambodia;

- Due to the high failure rates associated with artesunate-mefloquine, the first-line treatment for 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 through TES using dihydroartemisinin-piperaquine in Pailin. Between 2008 and 2015, similar trends were observed in nine provinces, mainly in the western and northern parts of the country. The high treatment failure rates observed with dihydroartemisinin-piperaquine are related to the presence of piperaquine resistance, which was present in Cambodia since 2002 and has spread geographically from western to north-eastern Cambodia.

Update

- A consensus meeting on the national treatment policy for *P. falciparum* was held in January 2014. Artesunate-mefloquine was reintroduced 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 7 days has been adopted as rescue therapy;
- Studies with artesunate-mefloquine conducted between 2014 and 2016 have reported 100% efficacy, even though 94.2% (278/295) of parasites were found to carry the K13 C580Y mutation.

Lao PDR

Background

- In 2013, a trial conducted in Champasack province found that 22.2% of the patients treated with artemether-lumefantrine were still parasitaemic on day 3 after treatment, with 10% treatment failures; by contrast, in Saravanh province, the positivity rate on 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.

Update

- The therapeutic efficacy of artemether-lumefantrine has begun to be affected; TES were conducted in 2015 in Atteupeu, Sekong and Champasack provinces. Positivity rates on day 3 ranged between 9.7% and 20%, whereas treatment failure rates ranged between 0% and 14%. In 2016, dihydroartemisinin-piperaquine is being monitored for potential use in the country.

Myanmar

Background

- Artemisinin resistance likely emerged at the border between Thailand and Myanmar in 2001, but was only clearly identified in 2008;
- Since 2009, available data have shown that parasite clearance times are consistently delayed among a significant proportion of patients treated with ACTs, and have been observed with all 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)* (4);
- 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 that there is a high prevalence of the K13 F446I mutation along the China–Myanmar and India–Myanmar borders. This new mutant appears to be associated with an intermediate rate of delayed clearance, and additional research is ongoing to validate its role in artemisinin resistance;
- ACT efficacy remains high on both sides of the border between India and Myanmar.

Thailand

Background

- Previously, Thailand had used a regimen of 2-day artesunate–mefloquine as first-line treatment. Despite the change to a 3-day regimen in 2009, treatment failures with artesunate–mefloquine increased in Kanchanaburi, Ranong, Tak and Ubonratchathani, reaching a treatment failure rate $\geq 10\%$;
- The high numbers of treatment failures observed in Thailand following 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 past few 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 rates were close to or greater than 10%;
- During a consensus meeting held in 2015, dihydroartemisinin–piperaquine was chosen as the first-line treatment, and its efficacy is currently being evaluated;
- In 2014–2015 in Kanchanaburi province, the efficacy of dihydroartemisinin–piperaquine was 94%.

Viet Nam

Background

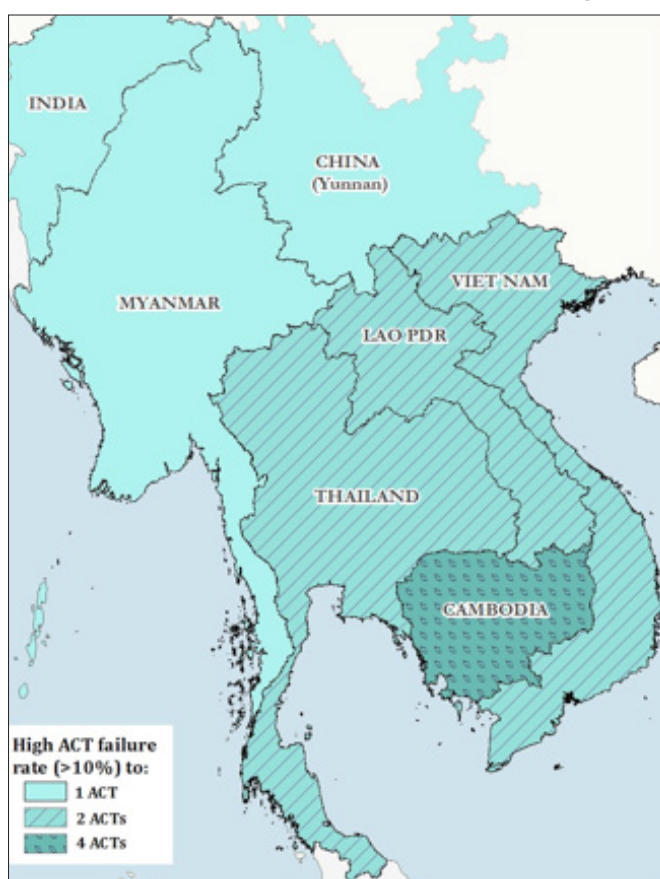
- Delayed parasite clearance was first detected after treatment with dihydroartemisinin–piperaquine in the Bu Dang district of Binh Phuoc province in 2009;

- Routine monitoring with dihydroartemisinin-piperazine also detected other foci of delayed parasite clearance in Gia Lai province (2010), Dak Nong province (2011), Quang Nam province (2012), Khanh Hoa province (2014) and Ninh Thuan (2015).

Update

- TES conducted from 2010–2014 using dihydroartemisinin-piperazine have confirmed a treatment efficacy of > 95%, despite a day-3 positivity rate of up to 36%;
- A recent study in Binh Phuoc province reported a high treatment failure rate (> 10%) after treatment with dihydroartemisinin-piperazine. Investigation has confirmed the emergence of piperazine 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-piperazine. 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 to have a high failure rate in the region because high treatment failure rates with SP and/or quadruple and quintuple Pfdhfr and Pfdhps mutations (which are usually fixed) were reported in the region.

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;
- The most frequent allele observed in Africa is A578S. This allele was not associated with clinical or in vitro resistance to artemisinin;
- A high number of mutations, including some associated with delayed clearance in the GMS in particularly C580Y, were reported in Africa. However many of these mutations reported in Africa appear to be neutral and not undergo clonal expansion.

South America

Suriname

- Routine surveillance of ACT efficacy between 2005–2006 and 2011 among gold miners showed an increase in the 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 the sequencing of the K13 of strains collected during this study revealed only wild-type K13.

Guyana

- A retrospective analysis of blood samples collected in 2010 for a *Pfhrp2* surveillance study detected C580Y. All five C580Y mutant samples detected had nearly identical haplotypes, suggesting a common origin distinct from the South-East Asian C580Y haplotype. A K13 sequencing survey is currently underway in the region where five of the earlier cases originated;
- The last TES evaluating artemether-lumefantrine was conducted from May 2011 to July 2012. 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 began in 2014. The efficacy of artesunate was found to be 100% at day 28, whereas only 2% of the patients had persistent parasitaemia on day 3 after treatment. The 47 strains collected all showed the K13 wild type.

French Guyana

- Between 2009 and 2013, the day-3 positivity rate among patients treated in Cayenne hospital with artemether-lumefantrine was 7.5%; however, the treatment was not systematically supervised. To date, no C580Y mutant has 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 in order to ensure that the recommended ACTs are effective, that timely changes to 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

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

Endnote

1. Artemisinin refers to artemisinin and its derivatives.

REFERENCES

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4. Global plan for artemisinin resistance containment (GPARC). Geneva: World Health Organization; 2011 (<http://www.who.int/malaria/publications/atoz/9789241500838/en/>)

ANNEX

Definition of artemisinin resistance

Definitions of candidate and validated K13 mutations are required, through:

- 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 a 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 TEG's 2014 definition of partial artemisinin resistance has not been amended, except for day 3 being specified as 72 hours (± 2 hours) after the start of a full artemisinin-based treatment course.

Suspected endemic artemisinin resistance is defined as:

- $\geq 5\%$ of patients carrying K13 resistance-confirmed mutations; or
- $\geq 10\%$ of patients with persistent parasitaemia by microscopy at 72 hours (± 2 hours; i.e., day 3) after treatment with ACT or artesunate monotherapy; or
- $\geq 10\%$ 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:

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



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>

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