TES challenges and opportunities

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Why monitor the efficacy of antimalarial drugs?

- Antimalarial drug resistance in malaria parasites poses one of the greatest threats to malaria control.
- Surveillance of therapeutic efficacy (in vivo) over time is a critical component of effective malaria case management and generates:
    - critical information for determining whether drugs recommended in the national treatment policy are still effective,
    - evidence-base to inform introduction of new treatment into national malaria treatment policies, and
    - make a timely response to spread of drug resistance possible.

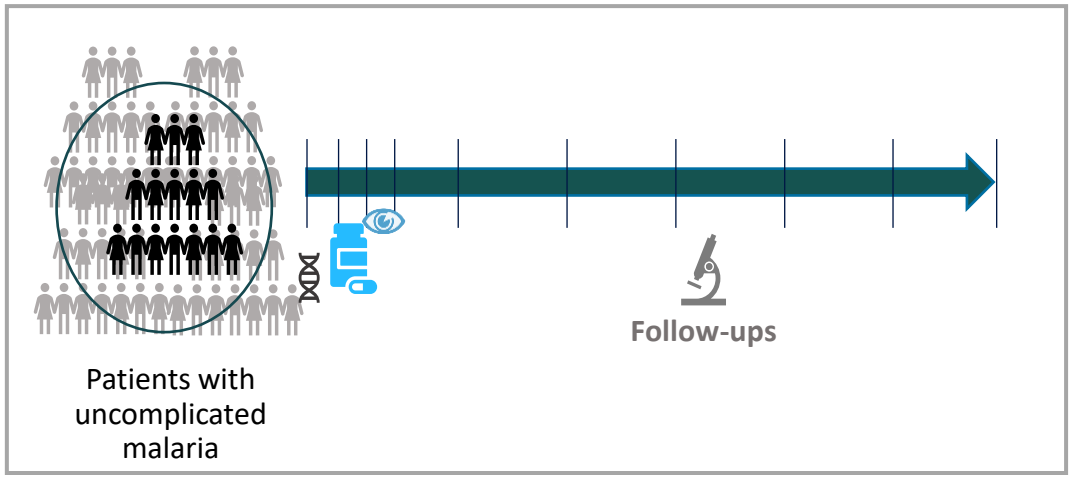
WHO guideline for malaria

"An antimalarial medicine that is recommended in the national malaria treatment policy should be changed if the total treatment failure proportion is ≥ 10%, as assessed in vivo by monitoring therapeutic efficacy. A significantly declining trend in treatment efficacy over time, even if failure rates have not yet fallen to the ≥ 10% cut-off, should alert programmes to undertake more frequent monitoring and to prepare for a potential policy change."

Sources of data: efficacy studies

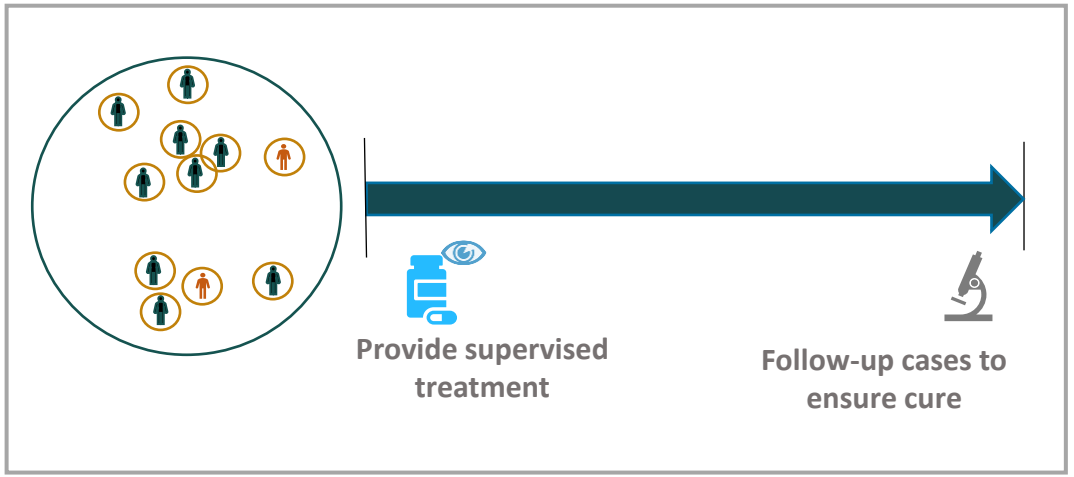
Therapeutic Efficacy Studies (TES)

- Gold standard for monitoring drug efficacy to inform treatment policy. Follow-up and procedures in accordance with standard WHO protocol
- Patients enrolled and followed up on set days to check symptoms and parasitaemia.
- Inclusion criteria varies depending on setting
- WHO recommends sentinel sites are established in different transmission areas and that TES are done at these sentinel sites at least once every 2 years

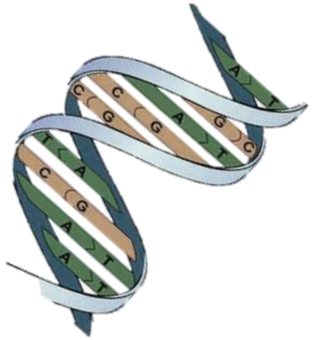


Integrated Drug Efficacy Studies (iDES)

- Done in very low endemic areas implementing elimination activities
- Aim to include all patients in an area
- Methods and data collected in iDES vary between countries depending on the systems in place and the resources available.
- Crucial to ensure that treatment is taken, and cases followed on to ensure cure.

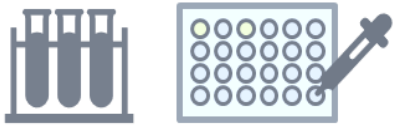


Sources of data: Supplementary information and surveys



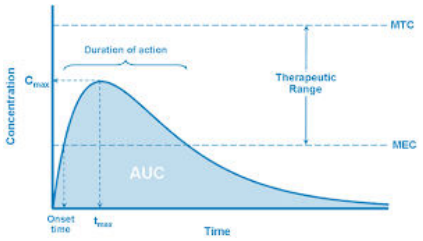
Molecular markers

- For drugs with molecular markers identified, **drug resistance can be confirmed, and trends monitored** with molecular techniques.
- Samples collected in surveys or TES.



In-vitro and ex-vivo studies

- Testing the **sensitivity of parasites** to precise concentration of antimalarial drugs. Typical measures:
  - IC50: drug concentration that inhibits 50% of parasite growth
  - RSA survival: In-vitro measure for response of early ring-stages to dihydroartemisinin



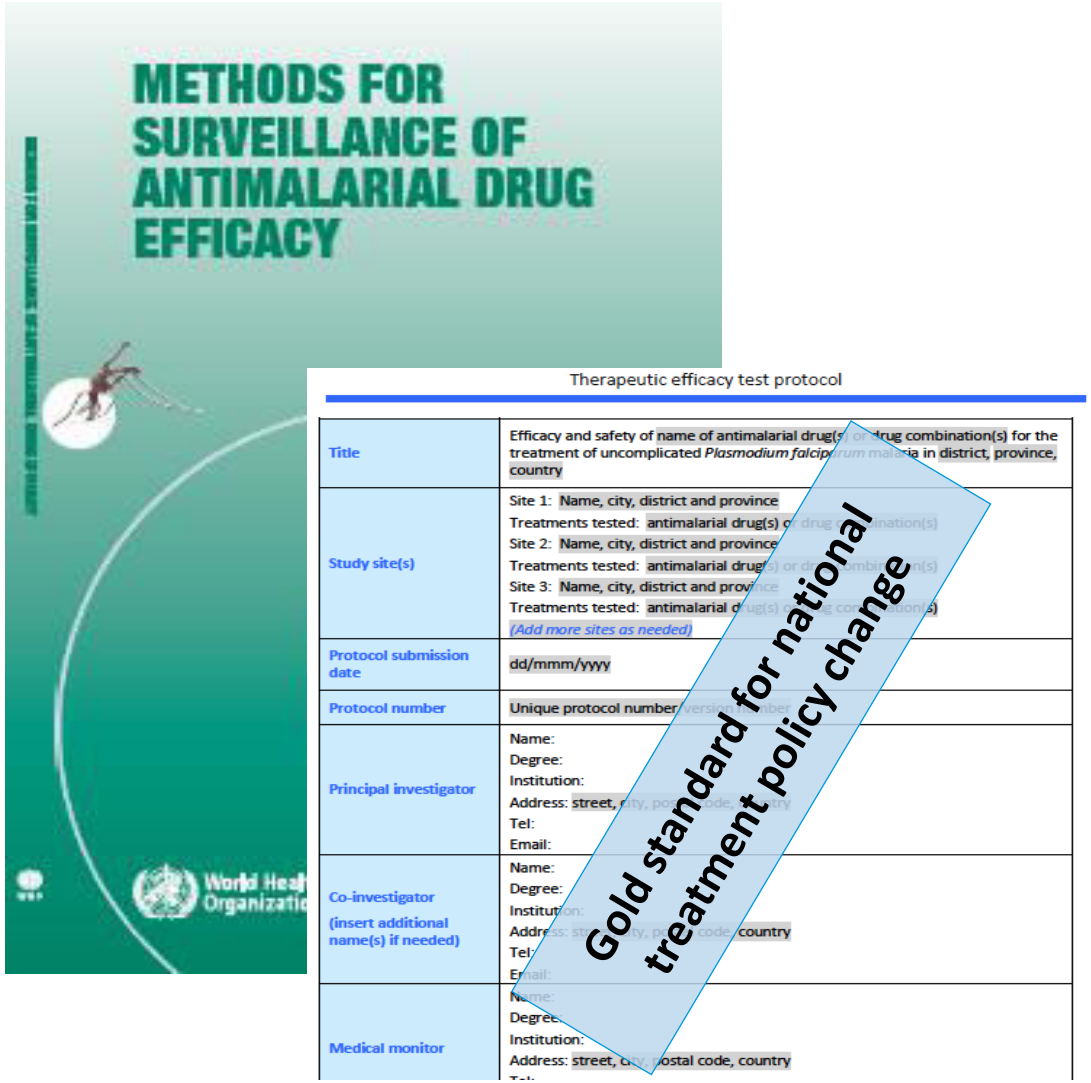
Pharmacokinetics

- Blood level at day 7 and/or day of failure to confirm adequate blood level after treatment.

Therapeutic Efficacy Study (TES): Standardized protocol to monitor drug efficacy

- Designed for efficacy monitoring for:
  - both *P. falciparum* and *P. vivax,*
  - recommended first- and second-line drugs,
  - any drug that needs to be monitored prior to possible introduction into the treatment policy.
- TES are conducted in sentinel sites.
  - A sentinel surveillance system is used when high-quality data are needed that cannot be obtained through an existing routine surveillance system of data collection.
- Repeated TES in a limited number of sites is adequate to collect consistent longitudinal data and document trends.
- Following a standardized protocol allow for the generation of standardized, comparable data.

Protocol 2009



Components of the standardized TES protocol

- **Study design:**
  - Objectives and classification of treatment outcomes

The primary objectives are to measure the clinical and parasitological efficacy of antimalarial drug(s) in patients in a given age group, suffering from uncomplicated malaria, by determining the proportion with:

- early treatment failure,
- late clinical failure, late parasitological failure or
- an adequate clinical and parasitological response.

Secondary objectives are to evaluate the incidence of adverse events; and to determine the prevalence of molecular markers

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Classification of responses to treatment

EARLY TREATMENT FAILURE

- danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia;
- higher parasitaemia on day 2 than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature ≥ 37.5 °C; and
- parasitaemia on day 3 ≥ 25% of count on day 0

LATE CLINICAL FAILURE

- danger signs or severe malaria in the presence of parasitaemia on any day between 4 and 28 (or day 42) in patients who did not previously meet any of the criteria of early treatment failure; and
- presence of parasitaemia on any day between 4 and 28 (or day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure.

LATE PARASITOLOGICAL FAILURE

- presence of parasitaemia on any day between 7 and 28 (or day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.

ADEQUATE CLINICAL AND PARASITOLOGICAL RESPONSE

- absence of parasitaemia on day 28 (or day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

Components of the standardized TES protocol

- **Study populations** (inclusion and exclusion criteria and same age group in similar transmission levels)

High transmission: Patients with fever, **ages 6-59 months** with an asexual parasitaemia ranging between 2000-200,000 parasites/ml.
Moderate transmission: **Modified inclusion criteria to also include older children**, and patients with an asexual parasitaemia ranging between 1000-100,000 parasites/ml.
Low transmission: **Modified to also include adults and patients** with an asexual parasitaemia of more than 250-500 parasites/ml.
Very low transmission: To reduce the required sample size per site, data from different sites can be combined (country aggregated data). The studies are conducted less frequently and where possible, molecular markers of resistance can be used as an early warning system and additional source of data.

Inclusion criteria

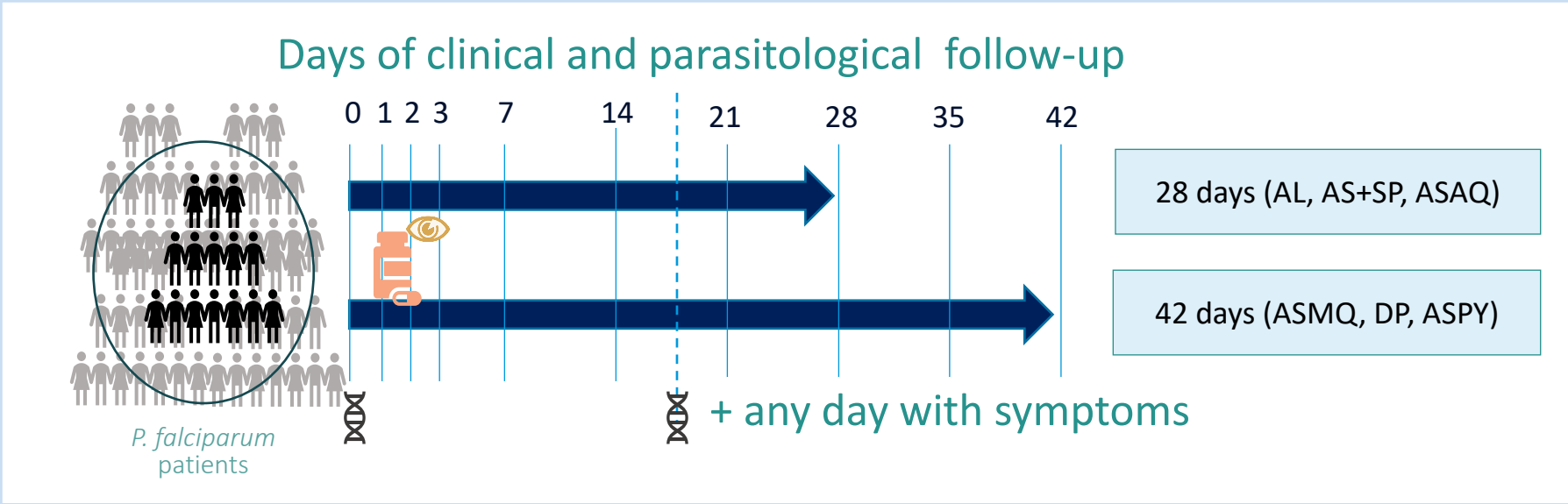
- patients aged 6-59 months (or >6 months);
- mono-infection with *P. falciparum* detected by microscopy;
- parasitaemia between 2000 (250-1000) and 200000 (100000) /µl asexual forms;
- presence of axillary temperature ≥ 37.5 °C or history of fever during the past 24 h;
- ability to swallow oral medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule;
- **informed consent from the patient or from a parent/guardian in the case of children;**
- **written informed consent and/or assent**.

Exclusion criteria

- general danger signs in children aged under 12 years or signs of severe malaria according to f WHO;
- weight under 5 kg;
- mixed or mono-infection with another Plasmodium species detected by microscopy;
- severe malnutrition (defined as a child aged between 6-60 months whose weight-for-high is below –3 z-score, or has symmetrical oedema involving at least the feet or has a mid-upper arm circumference < 115 mm);
- presence of febrile conditions due to diseases other than malaria;
- regular medication, which may interfere with antimalarial pharmacokinetics;
- history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s);
- a positive pregnancy test or breastfeeding; and
- unable to or unwilling to take pregnancy test or to use contraception for women of child-bearing age (defined as age > 12 years and sexually active).

Components of the standardized TES protocol

- **Supervised treatment of <u>all doses</u>**
- **Set follow-up period and times**



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Components of the standardized TES protocol

- Standard procedure for slide reading to ensure **comparable results from quality microscopic blood examination**
  - ➢ All blood slides should be read independently by two qualified microscopists
  - ➢ Defined protocol for discordant/concordant results:
    - ○ Blood smears without discordant results: calculate parasite density by averaging the two counts.
    - ○ Blood smears with discordant results between the two microscopists in (i) species, (ii) the presence of parasites or (iii) parasite density of >50% will be **re-examined by a third independent microscopist**:
      - ○ For species & positivity: the two concordant readings will be used,
      - ○ For parasite density: average of the two closest counts

Aspects of microscopic blood examination

- Parasite count: # asexual parasites/200 white blood cells (complete reading the last field);
- Parasite density: # asexual parasites/µl (assumed WBC count 6000 or 8000/µl);
- If >500 parasites have been counted before 200 WBCs have been reached, the count will be stopped (complete reading the last field);
- When # asexual parasites <100/200 WBC in follow-up smears, count against 500 WBCs (complete reading the last field).
- 100 fields of the thick film at day 0 will be examined to exclude mixed infections;
- The presence of gametocytes on Day 0 or follow-up days can be noted;
- A blood slide is declared negative (asexual parasites) after examining 1000 WBCs

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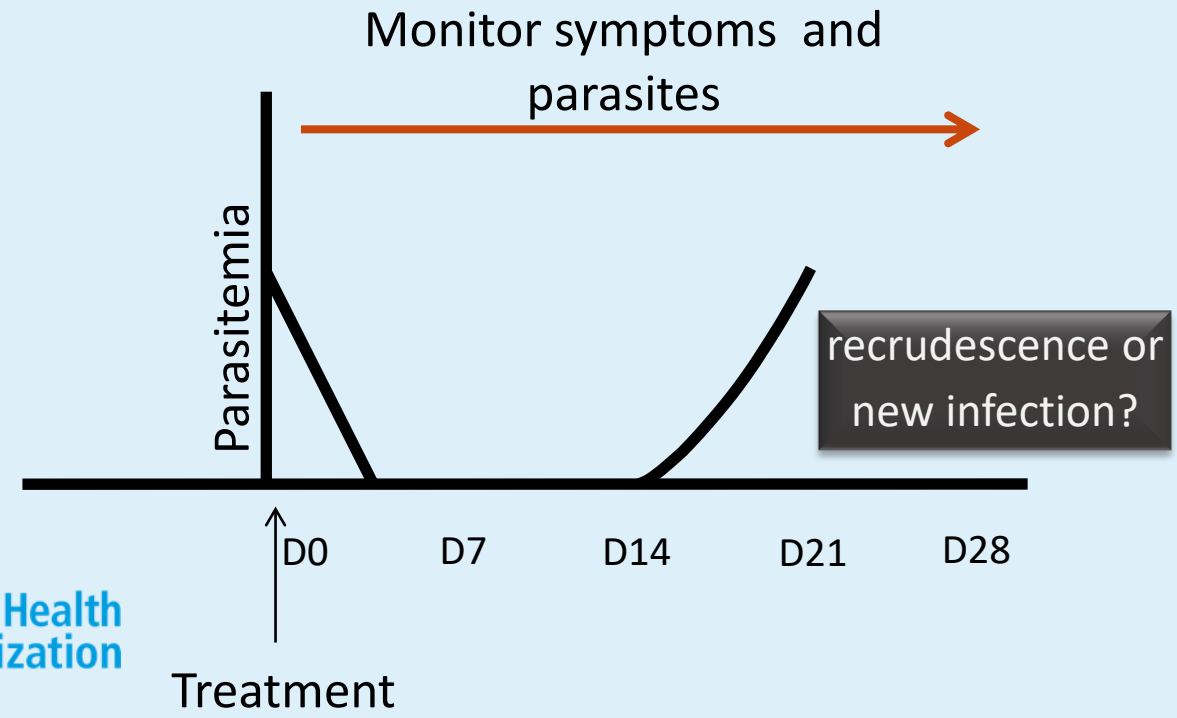
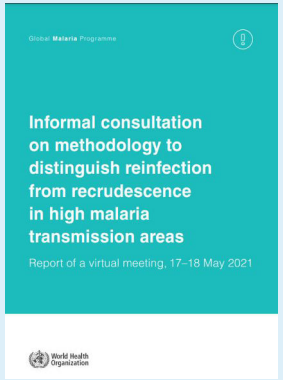
Components of the standardized TES protocol

- Recurrent malaria could be recrudescence or reinfection
- Filter-paper blood sampling for **parasite genotyping to classify recurrent parasitaemia as recrudescence or new infection:**
  - Genotyping only D0 and D failure



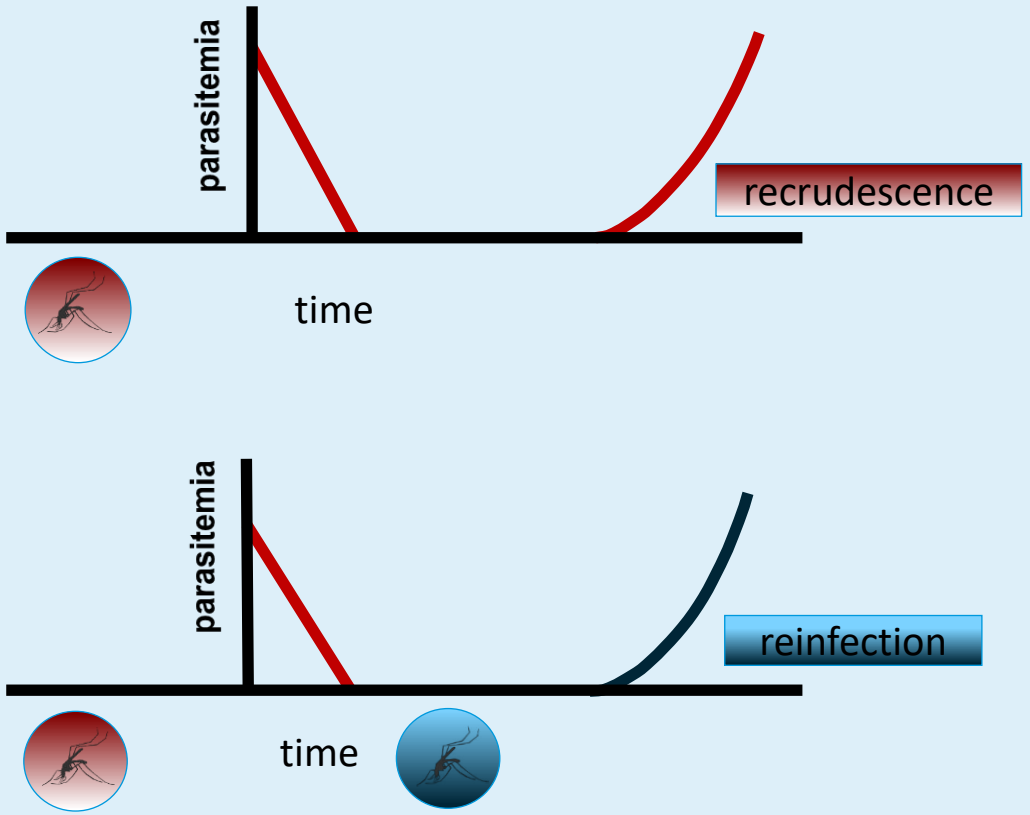
Components of the standardized TES protocol

- **Genotyping to classify cases as recrudescence or reinfections**
  - Since 2008, the recommendation was to use the markers msp1, msp2 and glurp



Recommendation in 2021 on markers and methods

- msp1 and msp2 continued to be used but glurp should be replaced with one microsatellite from the following: Poly-α, Pfpk2 and TA1.
- Match-counting (3/3) should be maintained as the primary analysis methodology for reporting and policy change. Bayesian and 2/3 algorithms may be applied for evaluation and comparison, but not for primary reporting.
- These methods should be applied in both low to moderate and high transmission settings in Africa. Outside Africa, the current method (msp1/msp2/glurp) should still be applied.



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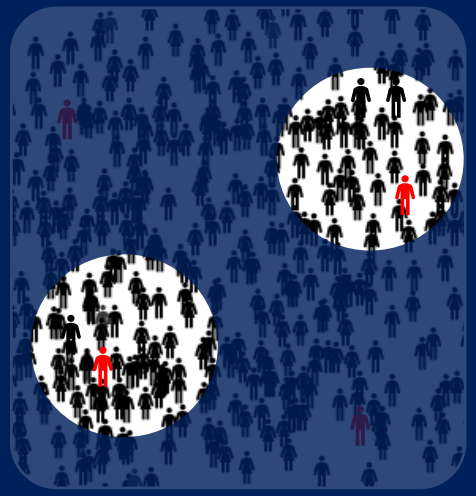
Supervising and monitoring TES

- **TES should be supervised and monitored to ensure that:**
  - the rights and well-being of patients are protected;
  - the reported TES data is correct, complete and verifiable from the source documents;
  - the study is conducted in accordance with the approved protocol
- **Study monitor should verify that:**
  - investigators follow the protocol and have the necessary resources to conduct the study correctly and safely;
  - all consents are obtained before the inclusion of patients;
  - source documents and all records are correct and complete, and necessary data are in the patient's file;
  - doses of the drugs are well indicated, and side effects of the medications administered are well noted;
  - reason for withdrawal and lost to follow up of the patients included is well indicated and explained
- **Monitors: locally or external expert.**

<u>Tools for monitoring:</u>

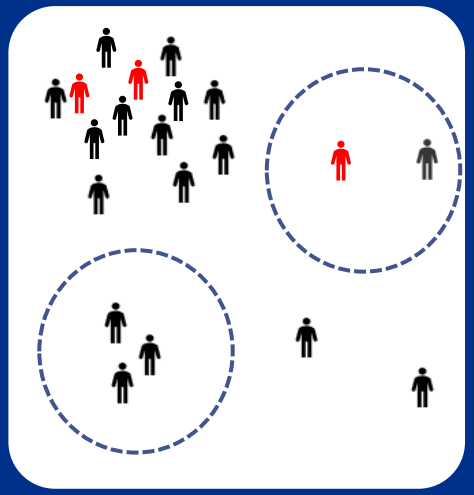
- **Study protocol**
- **Monitoring checklists**
- **Agreements of the ethics committee; laboratory procedure; procedure for entering data.**

Shifting to integrated Drug Efficacy Studies (iDES)



- TES = Gold standard for monitoring drug efficacy
- Aim to estimate %treatment failure (%TF) in all cases to be able to inform treatment policy
- TES are conducted in sentinel sites
- In TES, sample size is determined so in the area:

%TF in sample ≈ %TF in all malaria cases



- In very low transmission area, sample size is difficult to achieve
- In countries with enough total cases, the changing epidemiology can make planning studies difficult
- %TF in cases cannot be estimated using TES
- If elimination activities are implemented, data from the routine system may be used looking at <u>all</u> patients in the area

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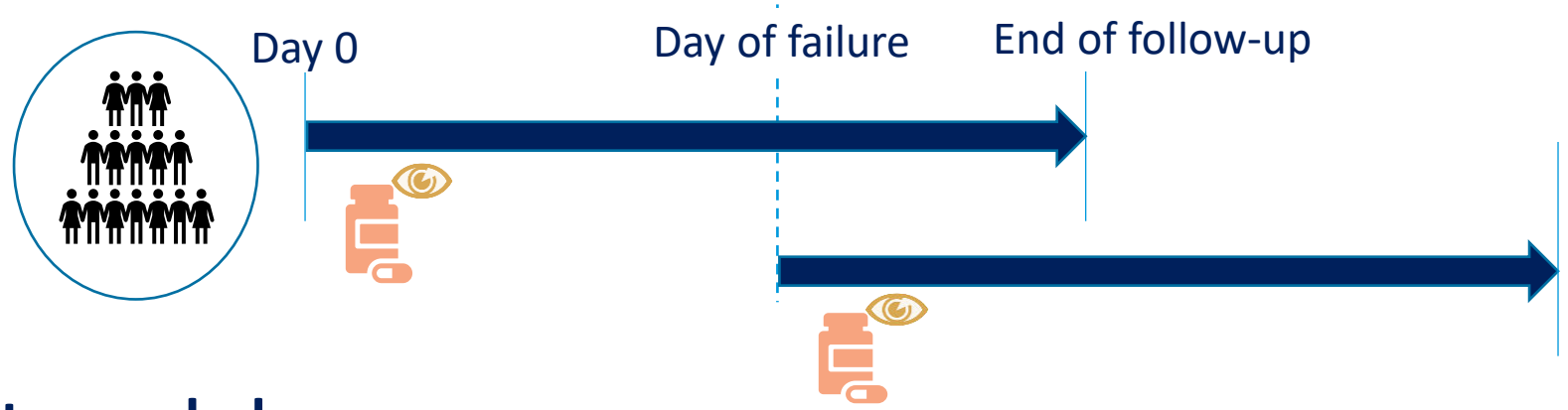
Minimum data in iDES

- Methods and data collected in iDES vary between countries depending on the systems in place and the resources available.



Minimum data needed:

1) Confirmation (as good as possible) that the patient took the drugs
2) Data for two points: day 0 and end-day

   End-day: End of follow-up with confirmed cure or day of failure

- ➢ In case of failure another full follow-up period is needed
- ➢ Additional data can be collected depending on need and resources

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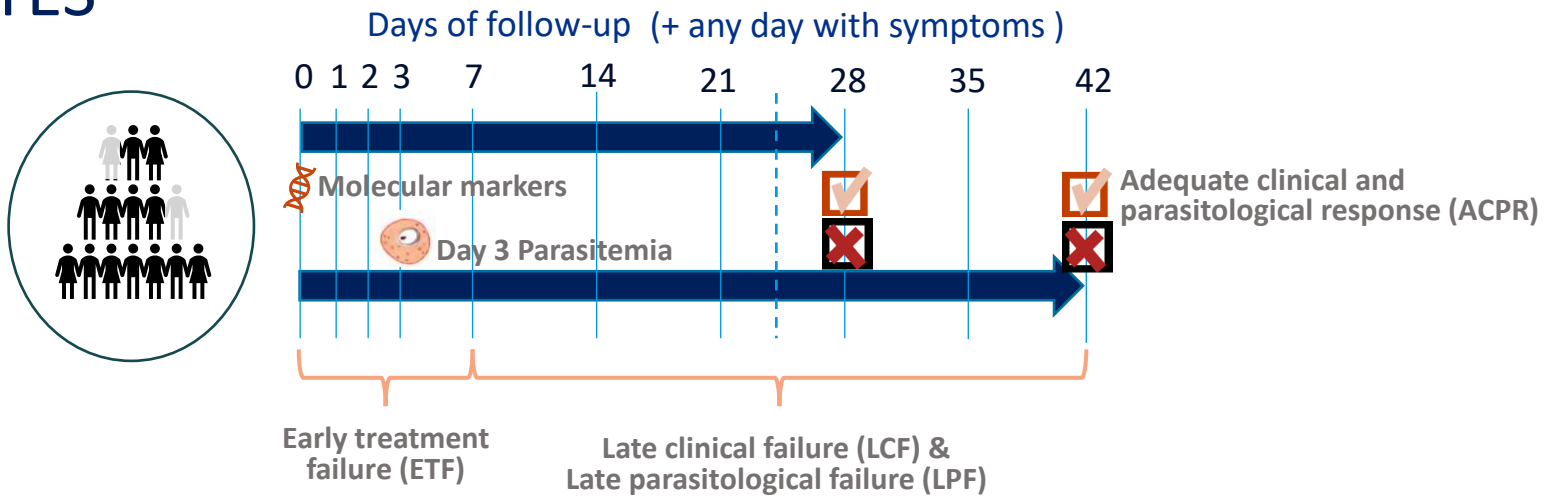
Information from TES



Information

% Treatment Failure (TF)	% of patients without ACPR	*Example: 10% with failure*
% Day 3 parasitemia	% patients on day 3 that are found to have parasites (Day 3: 72 hours after start of treatment)	*Example: 25% with day 3 parasitemia*
Molecular markers	Molecular markers indentified on day 0	*Example: 80% with increased copy number of Pfmdr1* *25% with K13 mutations*

Most used to inform treatment policy

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Molecular markers of *P. falciparum* resistance

Drug	Molecular markers	
	Gene	Mutation
4-aminoquinolines		
Chloroquine	*Pfcrt*	K76T + different sets of mutations at other codons (including C72S, M74I, N75E, A220S, Q271E, N326S, I356T, R371I)
	Pfmdr1 (in combination with Pfcrt mutations only)	N86Y, Y184F, S1034C, N1042D, D1246Y
Amodiaquine	Yet to be validated	Studies show that amodiaquine selects for *Pfmdr1* mutations
Piperaquine	*Pfpm2-3*	Pfpm2-3 increased copy number
	Pfcrt	Detected in vivo: T93S, H97Y, F145I, I218F C350R
		Detected in vitro: T93S, H97Y, F145I, I218F M343L, G353V
Antifolates		
Pyrimethamine	Pfdhfr	N51I, C59R, S108N, I164L
Sulfadoxine	Pfdhps	S436A/F, A437G, K540E, A581G, A613T/S
Proguanil	Pfdhfr	A16V, N51I, C59R, S108N, I164L
Amino-alcohols		
Lumefantrine	Yet to be validated	Studies show that lumefantrine selects for *Pfmdr1* N86.
Mefloquine	*Pfmdr1*	*Pfmdr1* increased copy number
Quinine	Yet to be validated	
Mannich base		
Pyronaridine	Yet to be validated	
Naphthoquinone		
Atovaquone	*Pfcytb*	Y268N/S/C
Sesquiterpene lactones		
Artemisinin and its derivatives	*PfK13*	List of candidate and validated markers developed

PfK13 markers of artemisinin partial resistance

Validated markers

- F446I
- N458Y
- C469Y
- M476I
- Y493H
- R539T
- I543T
- P553L
- R561H
- P574L
- C580Y
- R622I
- A675V

Candidate markers

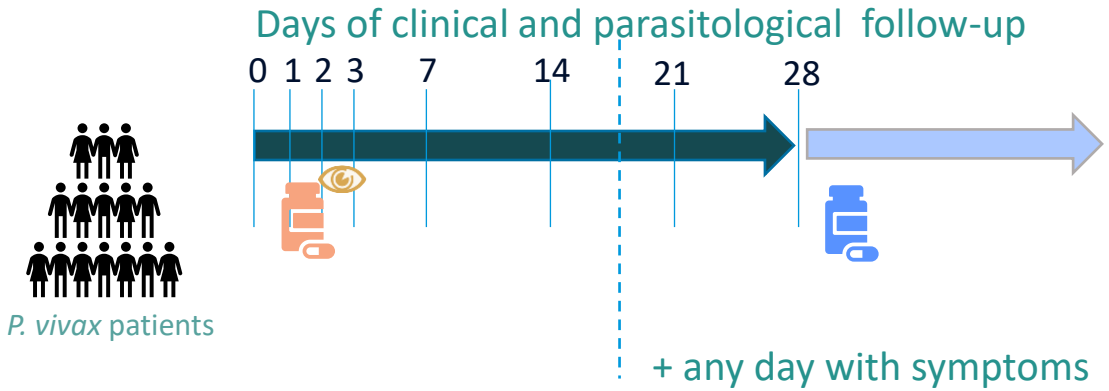
- P441L
- G449A
- C469F
- A481V
- R515K
- P527H
- N537I/D
- G538V
- V568G

Candidate: significantly associated with delayed parasite clearance in vivo or identified as having reduced susceptibility using Ring Stage Assay

Validated: significantly associated with delayed parasite clearance in vivo and identified as having reduced susceptibility using Ring Stage Assay

TES: Vivax specific challenges

- Routine TES for vivax infections looks at efficacy and resistance to the treatment of the **blood stages parasites**
- Concomitant treatment against liver stage parasites can increase efficacy of treatment against blood stage parasites.
- Therefore, radical treatment is moved to day 28 if locally acceptable



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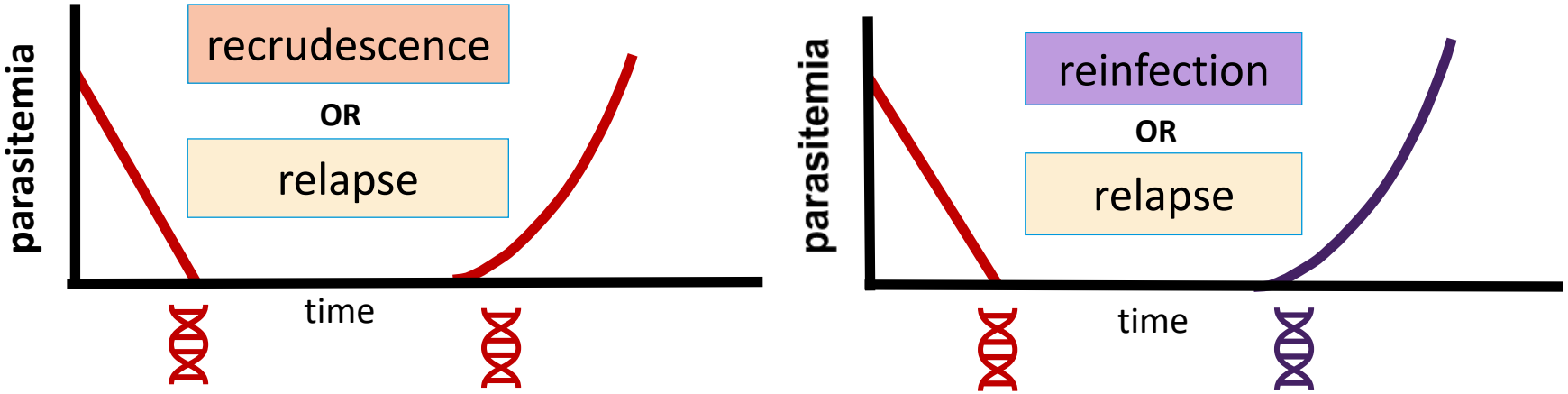
TES: Vivax specific challenges

- Not possible to distinguish between recrudescence, reinfection and relapse
- Sufficient drug blood concentration should prevent both recrudescence and relapse. If drug given has long half-life (and has been absorbed as expected), recurrent parasitaemia would not be expected before day 28
- Treatment failures by day 28 defined as for *P. falciparum*



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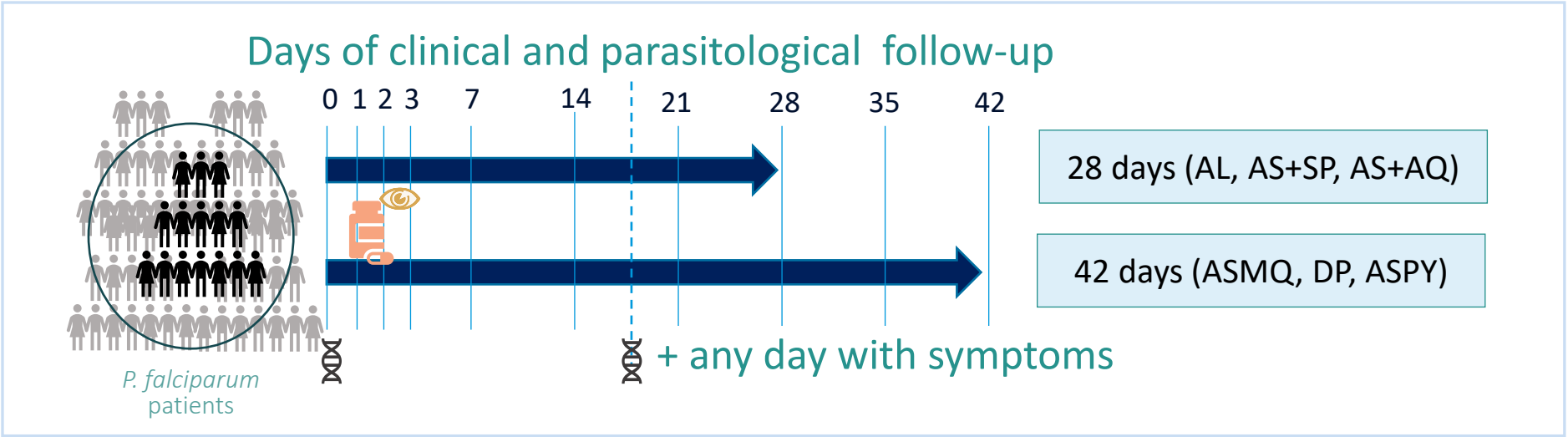
Therapeutic Efficacy Studies: Many factors can affect failure rates. TES aims to account for these



Factors	Mitigating action
Poor quality drugs	→ Quality assured drugs
Poor microscopy	→ Retraining as needed. Independent double reading
Lack of adherence to treatment	→ Supervised treatment
Drug interactions, comorbidities, medical conditions	→ Controlled to extent possible (exclusion criteria) Enrolling subset of patients
Immunity	→ Target special age group based on transmission level
Reinfection	→Genotyping (PCR)

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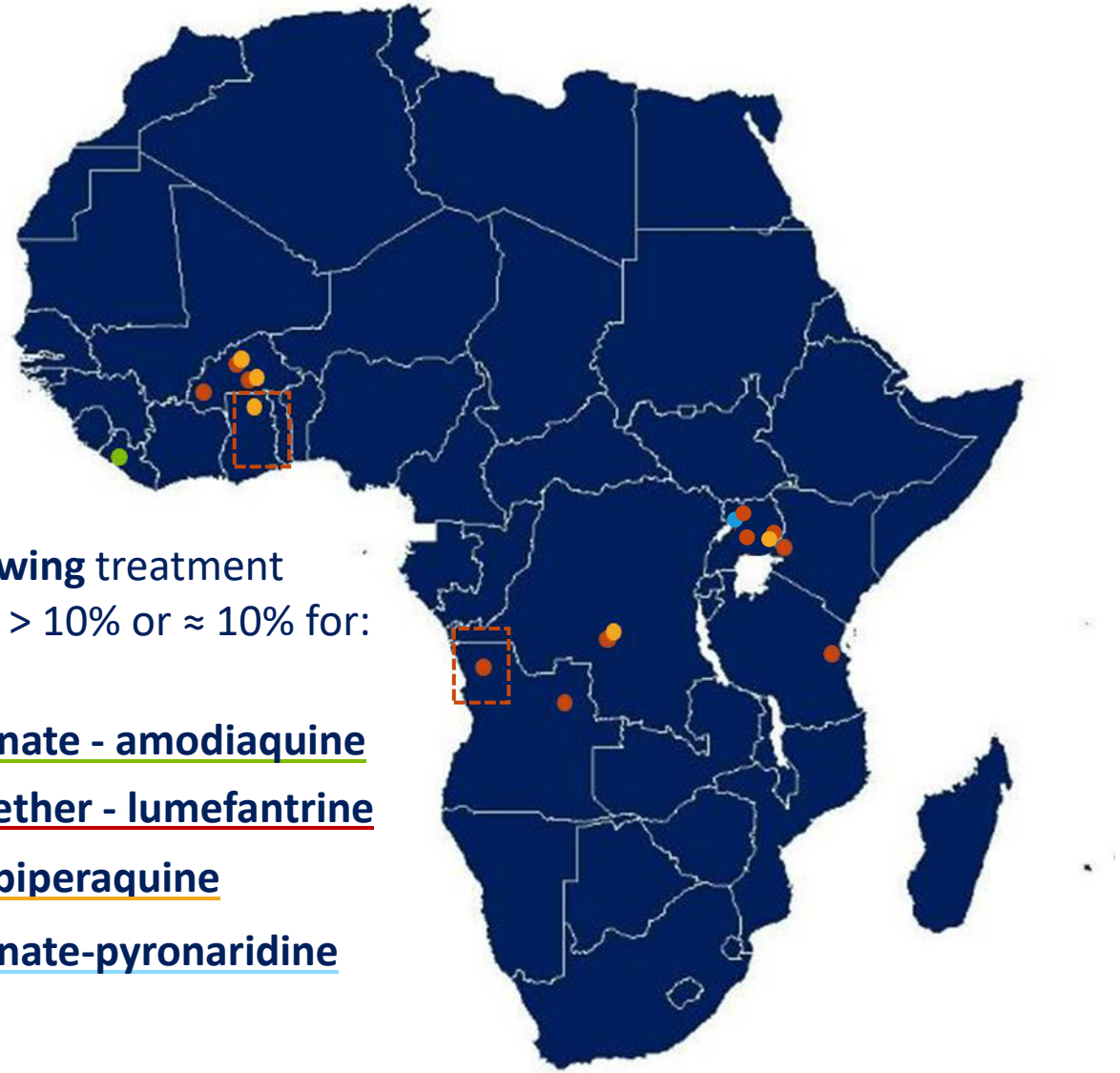
Status and challenges in interpretation of data from therapeutic efficacy studies



Studies showing treatment failure rates > 10% or ≈ 10% for:

- **Artesunate - amodiaquine**
- **Artemether - lumefantrine**
- **DHA - piperaquine**
- **Artesunate-pyronaridine**

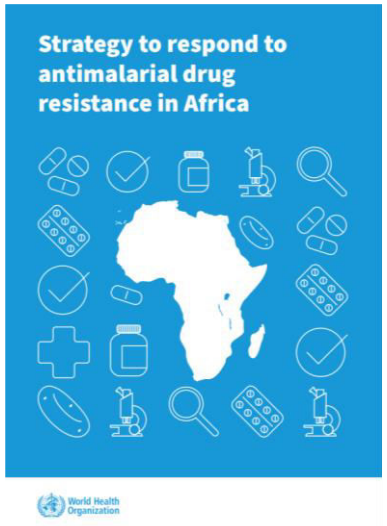
TES with high reported failure rates from 2015 - 2024

Scientific challenges include:

- Molecular markers for resistance missing for key ACT partner drugs. Markers would facilitate confirmation of resistance and monitoring of spread.
- There is a need to have improved methods available to distinguish recrudescence and reinfection.

Challenges related to adherence to standard protocol and quality of implementation:

- Some studies does not follow the standard protocol making comparison difficult.
- Challenges with the quality of the implementation of some studies including the quality of microscopy.
- Reporting using different methods to distinguish recrudescence and reinfection.



Pillar 1 in the Strategy: Strengthen the surveillance of antimalarial drug efficacy and resistance

Challenges

- Data not collected in line with standard protocols causing issues of comparability and quality
- Quality of samples and analysis inconsistent
- Insufficient geographical and longitudinal coverage of surveillance
- Detailed data needed to characterize resistance and track parasite changes
- Delays in communication and dissemination of data hinders timely, coordinated response

Pillar 1 interventions

- 1.1 Enhance capacity and ensure better quality and standardised data on efficacy and resistance
- 1.2 Increase coverage of surveillance on efficacy and resistance
- 1.3 Increase detailed data collection on resistance in selected sites
- 1.4 Improve data dissemination systems to facilitate reactive and coordinated response to resistance data

Opportunities to improve quality of TES

Pillar 1 interventions

1.1 Enhance capacity and ensure better quality and standardised data on efficacy and resistance

1.2 Increase coverage of surveillance on efficacy and resistance

1.3 Increase detailed data collection on resistance in selected sites

1.4 Improve data dissemination systems to facilitate reactive and coordinated response to resistance data

- The implementation of the strategy's interventions requires collective efforts from all involved.
- WHO working to contribute to the solution through:
  - Continual discussion ongoing with partners on how to improve TES quality
  - Review of guidance for drug efficacy and resistance monitoring
  - WHO is establishing a roster of consultants to support training in countries and TES site visits
  - External Quality Assurance (EQA) scheme for molecular markers being established
  - A push for the strengthening or re-establishment of networks for surveillance of drug efficacy and resistance. These networks can serve as platforms for exchange of information and capacity building

! Aim is to collect data that can be used to inform policy

Malaria Threat Maps

https://apps.who.int/malaria/maps/threats/

Thank you

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