The PMI-supported Antimalarial Resistance Monitoring in Africa (**PARMA**) Network

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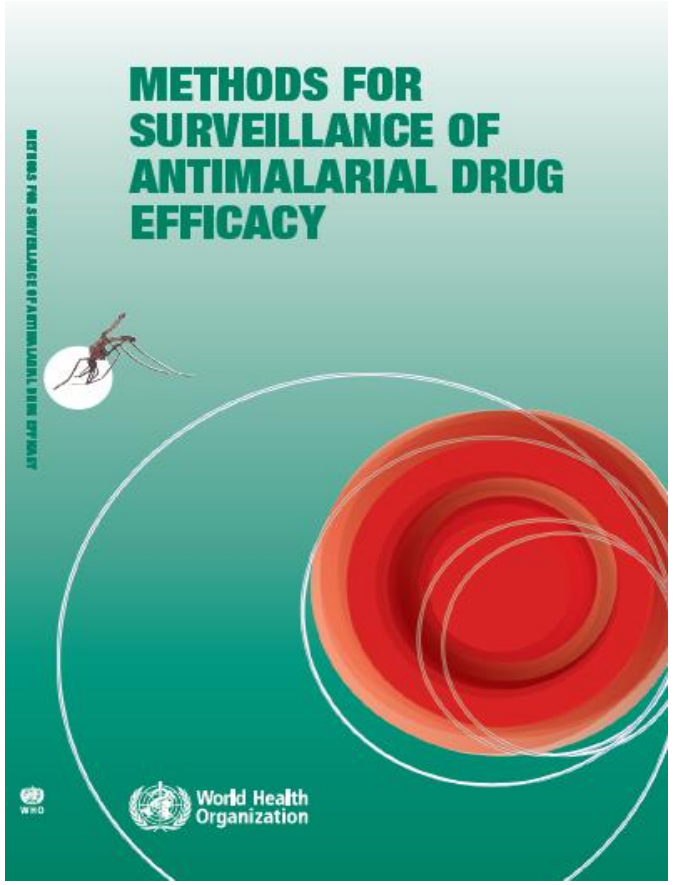
Presentation outline

- Therapeutic efficacy study background
- PARMA background
- Molecular markers background and PARMA results (preliminary)
- Moving forward
- Discussion

Therapeutic Efficacy Studies (TESs)



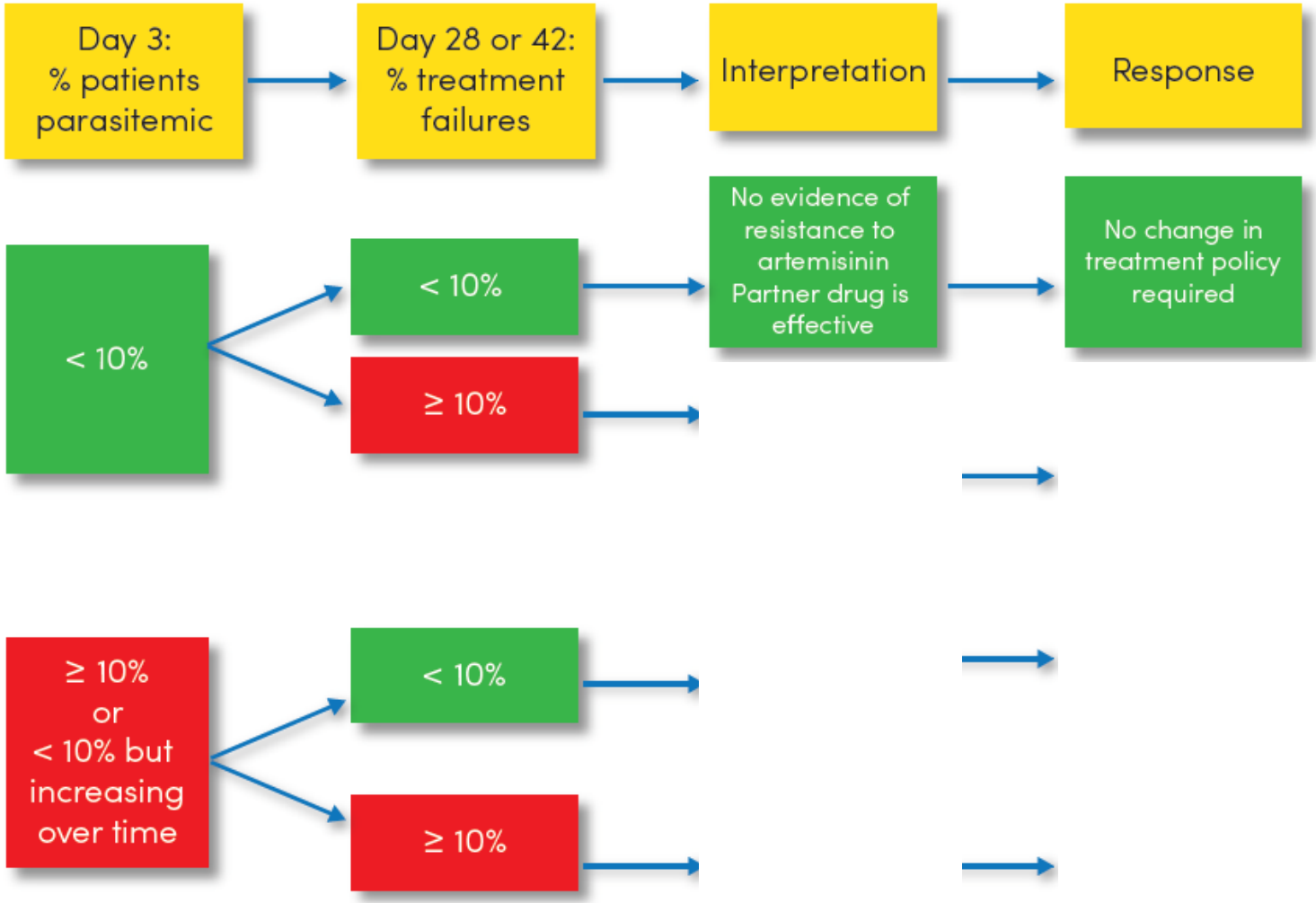
- **Routine monitoring of antimalarials**
  - All PMI countries should have ongoing or recent TES
  - WHO recommends testing every 2 years
  - Number of sites depends upon country-specific epidemiology
  - Often test 1st and 2nd line drug

TES schedule of follow-up activities

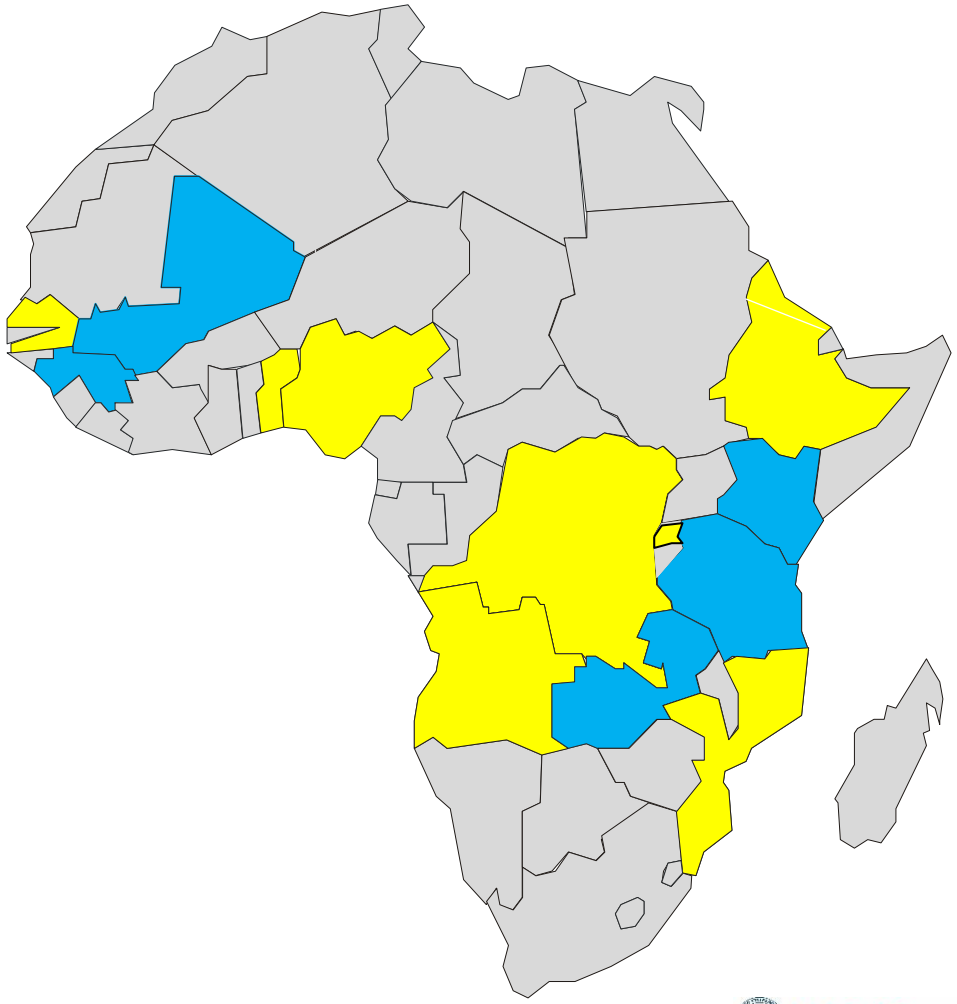
	Day										
	0	1	2	3	7	14	21	28	35	42	Any other
Procedure											
Clinical assessment	X	X	X	X	X	X	X	X	(X)	(X)	(X)
Temperature	X	X	X	X	X	X	X	X	(X)	(X)	(X)
Blood slide for parasite count	X		X	X	X	X	X	X	(X)	(X)	(X)
Urine sample	(X)										
Blood for:											
genotyping	X				X	X	X	X	(X)	(X)	X
haemoglobin or haematocrit	(X)					(X)		(X)		(X)	(X)
molecular markers	(X)				(X)	(X)	(X)	(X)	(X)	(X)	(X)
in vitro test	(X)										
antimalarial blood concentration	(X)				(X)	(X)	(X)	(X)	(X)	(X)	(X)
Treatment											
Medicine to be tested	X	(X)	(X)								
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)			(X)

Parentheses denote conditional or optional activities. For example, treatment would be given on days 1 and 2 only for 3-day dosing. On day 1, the patient should be examined for parasitaemia if he or she has any danger signs. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms. On extra days, blood slides may be taken routinely or at the request of the clinical staff.

Decision-making process based on TES results



Countries with recent or upcoming PMI-funded TESs



2016

Ongoing or planned for 2017

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PARMA Network

Objectives

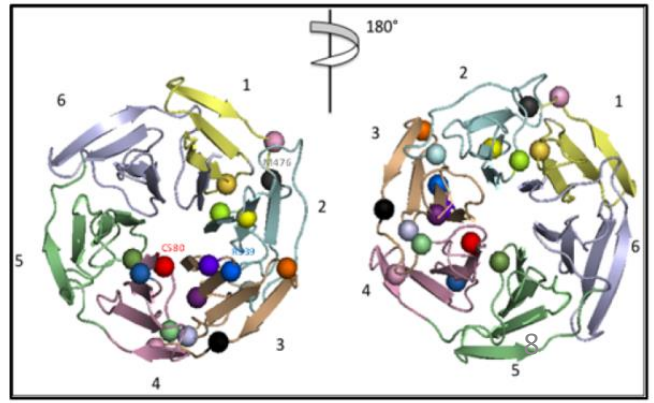
1) To assist PMI countries in testing malaria samples from TESs for genetic markers associated with antimalarial resistance

2) To support training and capacity building of African collaborators who possess the sufficient infrastructure:

- Laboratory (e.g., real time PCR, thermocyclers, gel electrophoresis)
- Bioinformatics (e.g., computer with sufficient memory and processing power)

PARMA Network

- PMI follows WHO technical guidance:
  - To incorporate resistance testing into existing monitoring
- k13 and other resistance testing at the CDC
- Technology transfer
- Results shared with NMCPs, who are encouraged to share the results with WHO and other interested partners in a timely manner



Using phenotypic and genotypic data together

Country example: Angola

Plucinski *et al. Malar J (2017) 16:62*

Table 4 Efficacy of first-line anti-malarials in three therapeutic efficacy monitoring sites in Angola, 2015

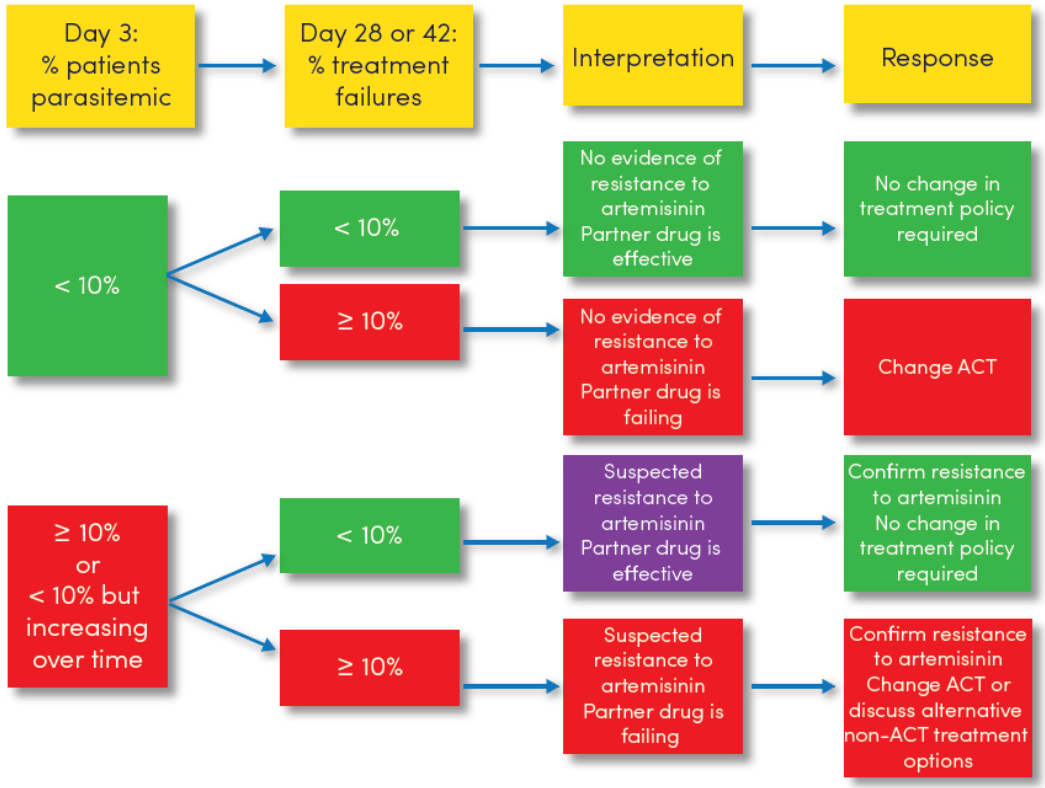
	Efficacy (95% confidence intervals)					
	Benguela		Zaire		Lunda Sul	
	ASAQ[a]	AL[a]	AL[a]	DP[b]	DP[b]	ASAQ[a]
Uncorrected						
Per-protocol Day 28	90.4 (81–96)	89.4 (80–95)	75.3 (65–83)	95.6 (88–99)	100 (94–100)	100 (92–100)
Per-protocol Day 42	–	–	–	81.9 (72–89)	100 (94–100)	–
Kaplan–Meier estimate Day 28	90.3 (84–97)	89.6 (83–96)	76.0 (68–85)	95.5 (91–100)	100	100
Kaplan–Meier estimate Day 42	–	–	–	82.5 (75–91)	100	–
PCR-corrected						
Per-protocol Day 28	99.9 (95–100)	96.1 (89–99)	86.5 (77–92)	98.8 (94–99)	100 (96–100)	100 (94–100)
Per-protocol Day 42	–	–	–	98.5 (92–99)	100 (96–100)	–
Kaplan–Meier estimate Day 28	99.9 (95–100)	96.3 (91–100)	88.1 (81–95)	98.8 (96–100)	100	100
Kaplan–Meier estimate Day 42	–	–	–	98.8 (96–100)	100	–

Per-protocol efficacy defined as proportion adequate clinical and parasitological response (ACPR), Kaplan–Meier estimate calculated from estimate of survival function

ASAQ artesunate–amodiaquine, *AL* artemether–lumefantrine, *DP* dihydroartemisinin–piperaquine

After excluding re-infections, out of 81 subjects in the Zaire artemether-lumefantrine arm:

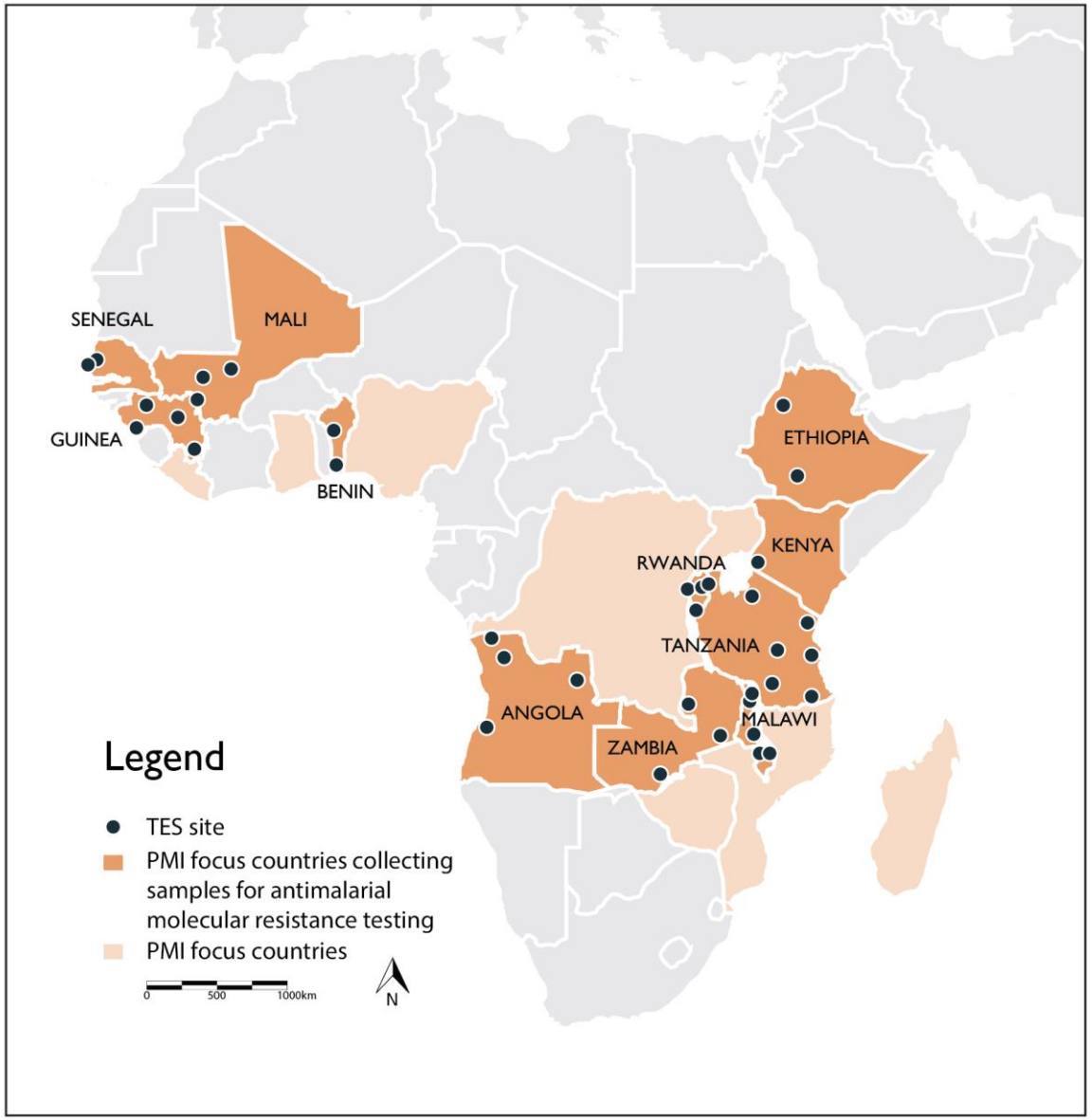
- 3 early failures
- 8 late failures



- *k13* (artemisinin): all wildtype
- *mdr1* (lumefantrine): majority with NYD and NFD haplotypes, associated with resistance

Current PARMA sites

- Currently in discussions
  - DRC
  - Mozambique
  - Madagascar
- Outside partners testing K13
  - Liberia (WHO)
  - Uganda
- Testing within the country
  - Nigeria
  - Ghana
- To be determined
  - Zimbabwe



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Over 2200 samples evaluated to date

Country	Year	Study type	Markers assessed
Angola	2015	TES	*k13 (n=389), mdr1(n=528)*
Guinea	2015	TES	*k13 (n=371), mdr1 (n=401)*
Malawi	2014	TES	*k13 (n=128), pfdhfr (n=128), dfdhps(n=128)*
Mali	2016	TES	*k13 (n=310), mdr1 (n=302)*
Senegal	2011-2014	observational surveillance	*k13 (n=249), mdr1 (n=249), pfcrt (n=249)*
Tanzania	2016	TES	*k13 (n=387), mdr1 (n=365)*
Zambia	2016	TES	*k13 (n=216), mdr1 (n=216)*

As of May 2017 and counting each sample only once regardless of number of mutations analyzed

Preliminary results

Many countries are still analyzing their data for publication and presentations at the upcoming ASTMH 2017 Meeting

(5 abstracts submitted)

Artemisinin resistance and *k13*

- Defined as delayed parasite clearance
- A molecular marker, *k13*, has been identified
- Suspected endemic resistance
  - Phenotypic or genotypic evidence
- Confirmed endemic resistance
  - Phenotypic and genotypic evidence
- High treatment failure rates in the Mekong associated with concomitant resistance to partner drug

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

World Health Organization

Which *k13* mutations matter?

Cambodia Lao PDR Vietnam	China Myanmar Thailand	Africa

- In SE Asia, distinct alleles originating from multiple independent events of emergence have been observed
- In Africa, non-synonymous mutations are rare and diverse

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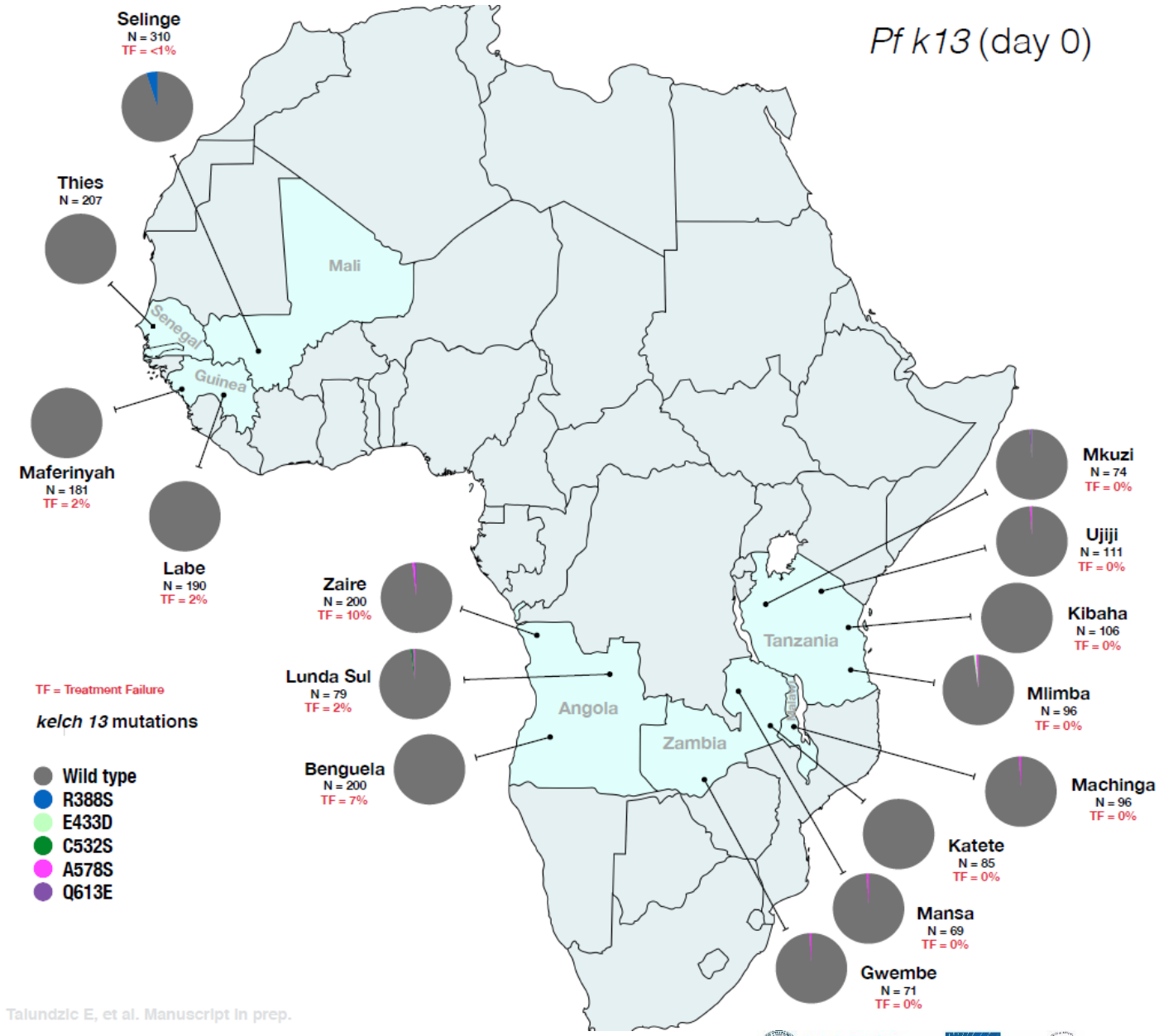
- No confirmed or candidate markers of artemisinin resistance (i.e., known SNPs in the *k13* gene) were detected
- Low level of other polymorphisms were observed (e.g., A578S)
  - Not associated with resistance
  - Frequently found in Africa



pfmdr1 gene

- *P. falciparum* multidrug resistance transporter 1
- Mutations (NYD, NFD) and copy number variation associated with susceptibility/resistance to many ACT partner drugs…
  - Lumefantrine
  - Amodiaquine
  - Mefloquine
- …as well as other antimalarials
  - Chloroquine

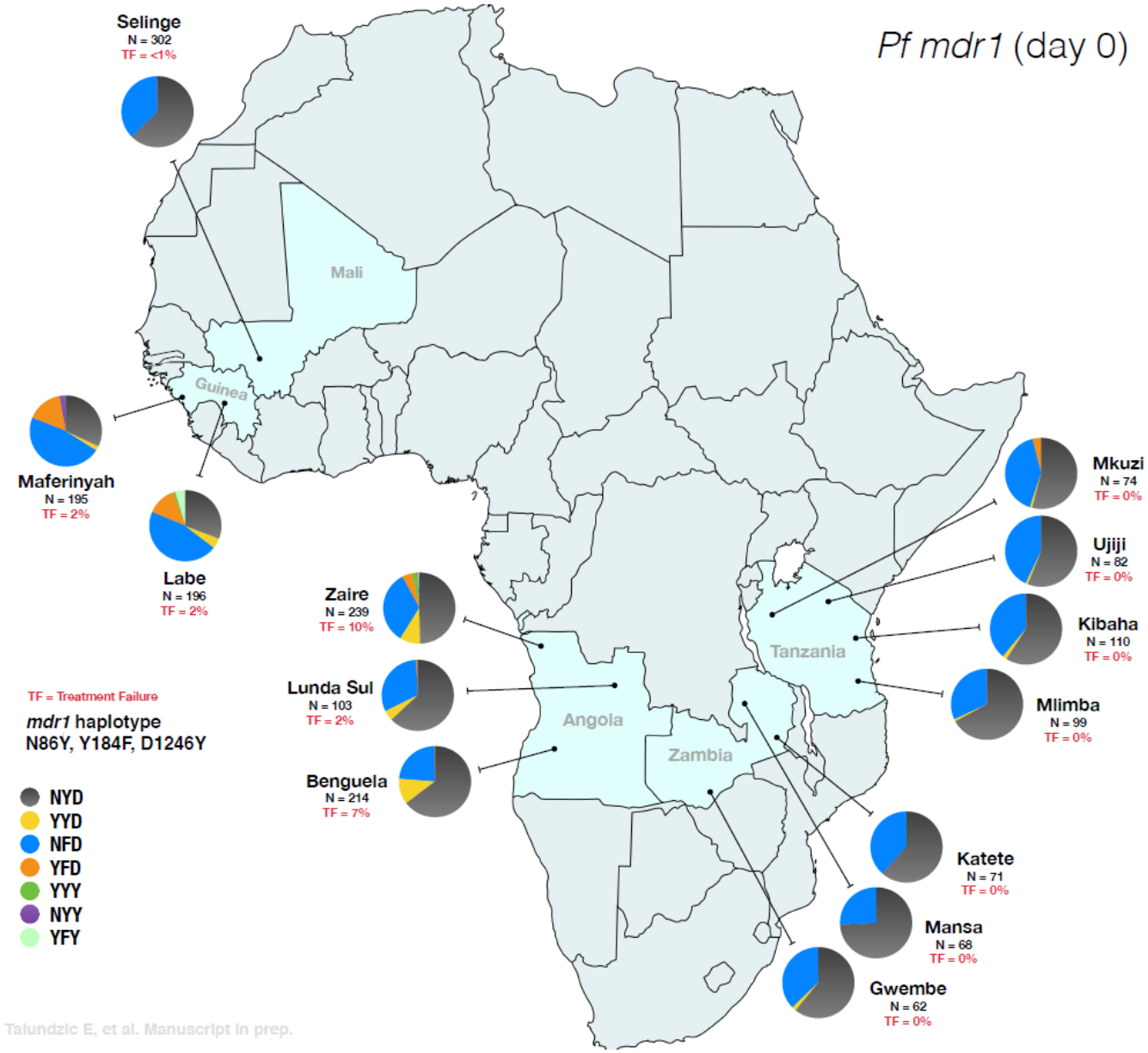
Treatment of uncomplicated P. falciparum malaria

Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended artemisinin-based combination therapies (ACT):

- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artesunate + sulfadoxine–pyrimethamine (SP)

Strong recommendation, high-quality evidence

- Mutations in the *pfmdr-1* gene associated with resistance to lumefantrine (partner drug)
- Important haplotypes associated with resistance
  - NYD
  - NFD



Other molecular marker associations

- *P. falciparum* chloroquine resistance transporter *(pfcrt)*
  - amodiaquine, chloroquine
- dihydrofolate reductase (*pfdhfr)*
  - pyrimethamine
- dihydropteroate synthase (*pfdhps)*
  - sulphadoxine
- *plasmepsin 2* and *3*
  - piperaquine

A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype–genotype association study

www.thelancet.com/infection **Published online November 3, 2016**

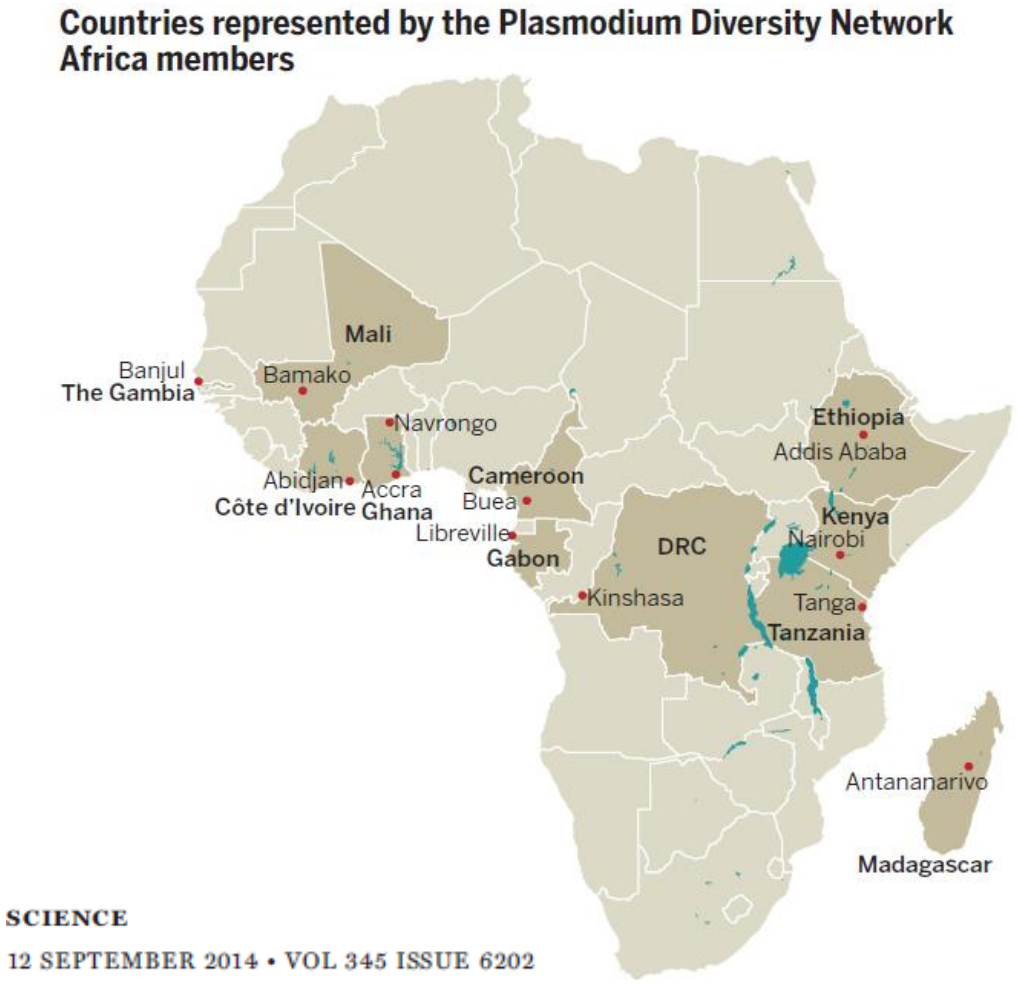
What if a concerning result is found?

- Notify NMCP, WHO, and other relevant parties
- Plan follow-up confirmatory studies in consultation with WHO
  - Consider intensifying the number of TES sites
  - Continue to utilize molecular testing in future studies
  - Emphasize timely turnaround of testing results
- Engage with NMCP and WHO in reviewing treatment policy and options for first-line ACT (e.g., Angola)

Moving forward

- Disaggregate the results
  - Only Day 0 → point prevalence
  - Only Day of Late Treatment Failure → focus on failures
- Encourage countries to incorporate genetic results in their TES publications
- Better define significance of certain haplotypes and single nucleotide polymorphisms
- Next round of TES samples and technology transfer
  - Ethiopia, Rwanda, Benin, Mozambique, and DRC
- Increase collaboration and communication
- Emphasize sustainability

Increase collaboration and communication



World Health Organization

WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK

WWARN

Emphasizing sustainability

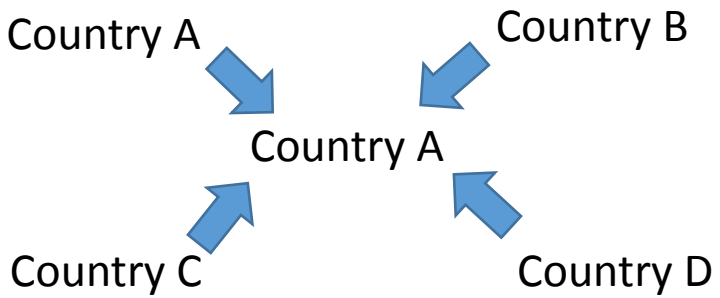
Step 1: Training at CDC (Atlanta)

Guinea
Kenya
Malawi
Mali
Senegal
Tanzania
Zambia
More to come

Step 2: Reinforce concepts in home country

Senegal
Tanzania
More to come

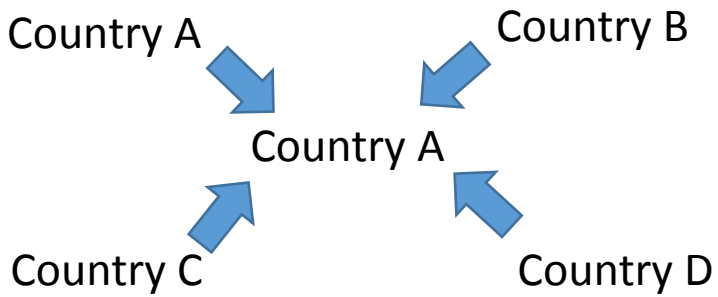
Step 3: Establish regional hubs



To be determined

For discussion

Regional Hubs

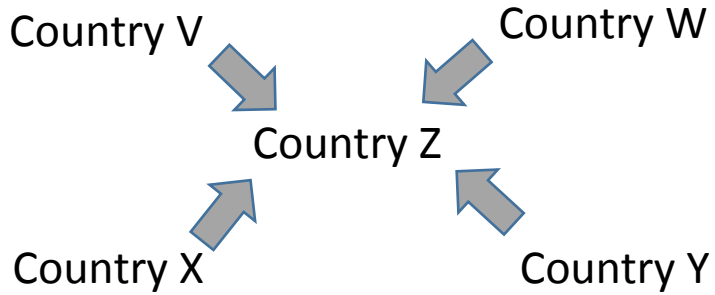


Qualifications

- Africa-based
- Adequate infrastructure and personnel
- Demonstrated ability to independently operate
- Desire to work collaboratively

Services offered at the hub

- Testing
  - All samples
  - Confirmatory (e.g., 10%)
- Training
- Technical support



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