Global situation, update and next steps of artemisinin resistance

XVth Annual RAVREDA Meeting

Bogota, Colombia, May 3, 2016

Global **Malaria** Programme

World Health Organization

Outline

- Update on artemisinin resistance
- Situation in South America
- Next steps

Definitions

- Artemisinin (partial) resistance = delayed clearance does not meet the current conventional WHO 1973 definition of antimalarial drug resistance:
  - 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;
- Multidrug resistance (MDR) requires resistance to more than two operational antimalarial compounds of different chemical classes;
- ACT failure: treatment failure rate following treatment with an ACT due to the partner drug failure, regardless of the presence artemisinin resistance;
- ACT resistance: resistance to both compounds i.e. partial artemisinin resistance and partner drug resistance.

Artemisinin resistance definition

- *Suspected* endemic artemisinin resistance is defined as:
  - ≥ 5% of patients carrying *K13* resistance-validated 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 parasite clearance half-life of ≥ 5 hours after treatment with ACT or artesunate monotherapy.
- *Confirmed* endemic artemisinin resistance is defined as ≥ 5% of patients fulfil both of the following criteria:
  - ≥ 5% of patients carrying K13 resistance-validated 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 parasite clearance half-life of ≥ 5 hours.

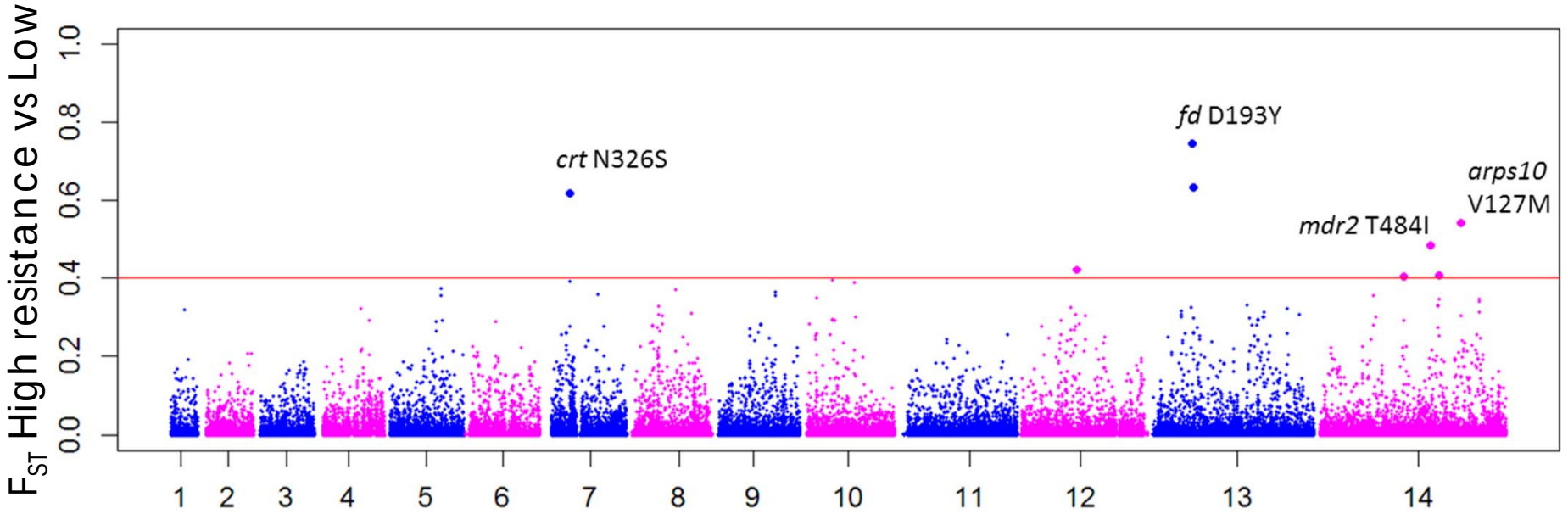
Associated and validated K13 resistance mutations 2015

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

Possible “permissive” or compensatory background mutations

GWAS confirms key role for K13



Mutation	BD	WSEA	ESEA		
			HR	IR	LR
kelch13	0%	33%	79%	27%	2%
arps10 V127M	0%	61%	92%	42%	12%
fd D193Y	2%	81%	95%	35%	3%
mdr2 T484I	6%	78%	88%	46%	23%
crt N326S	31%	100%	94%	38%	10%

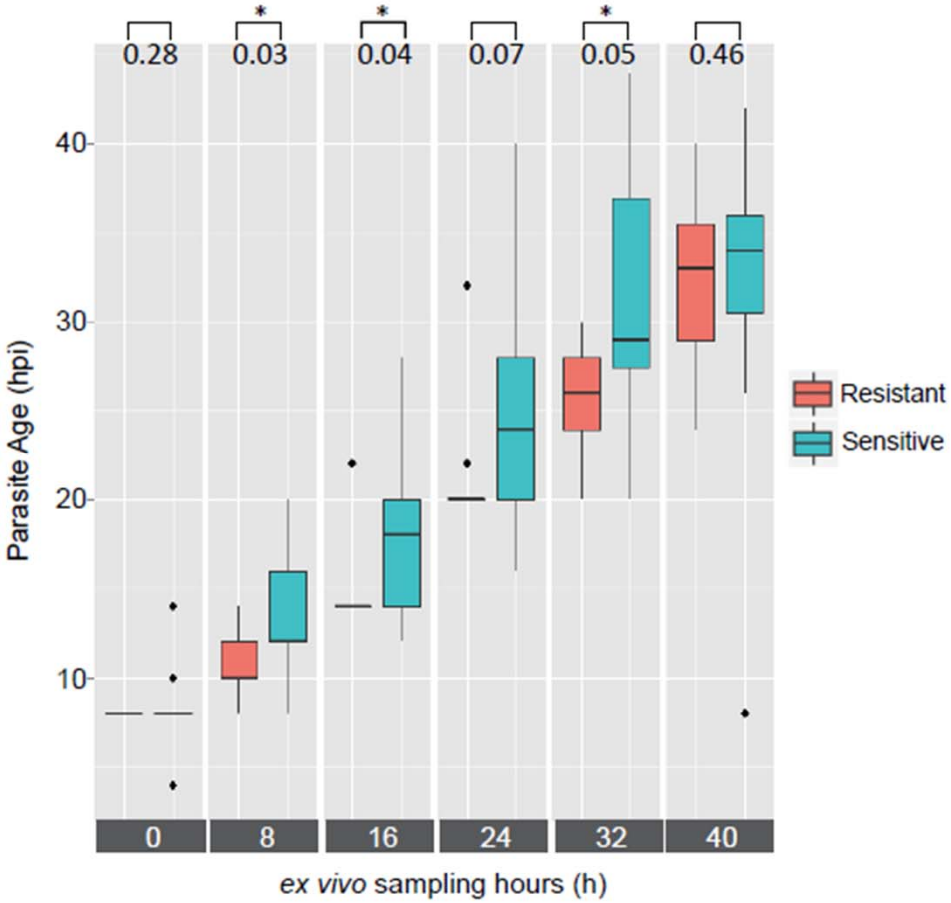
Note: differentiation is on both sides of SEA! E.g.

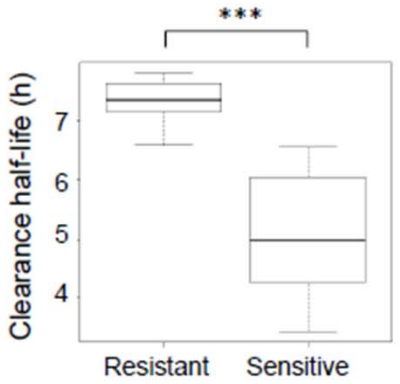
- W. Thailand vs. Bangladesh
- W. Cambodia vs. Laos

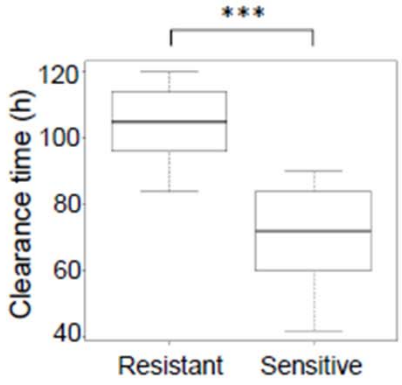
Miotto O et al. (2015) *Nature Genetics*

Parasite factors contributing to in vivo artemisinin partial resistance

Rings have an extended ring stage.







Mok S et al. (2015), *Sciences*

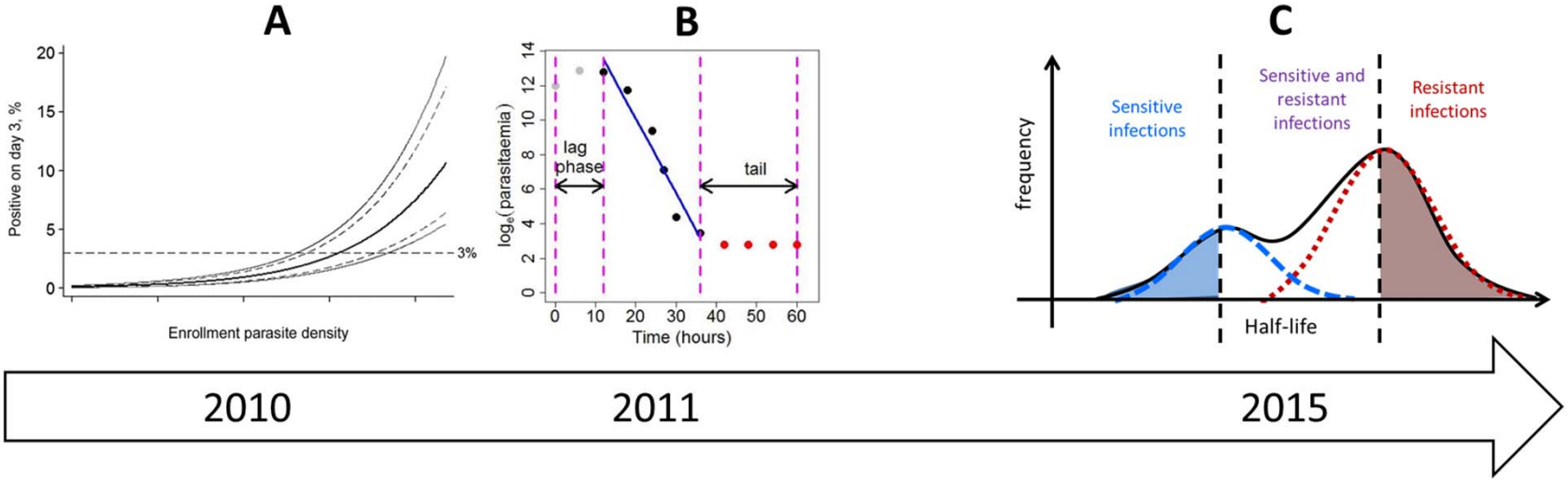
Day 3 vs slope

Day 3

- The proportion of patients who are parasitaemic after 3 days of treatment is highly dependent
  - on the initial parasitemia;
  - immunity of the patients;
  - the skills of the microscopists;
  - the methodology used for slide reading;
  - D3 ≠ 72 hours (day 3 overestimates positivity rate).

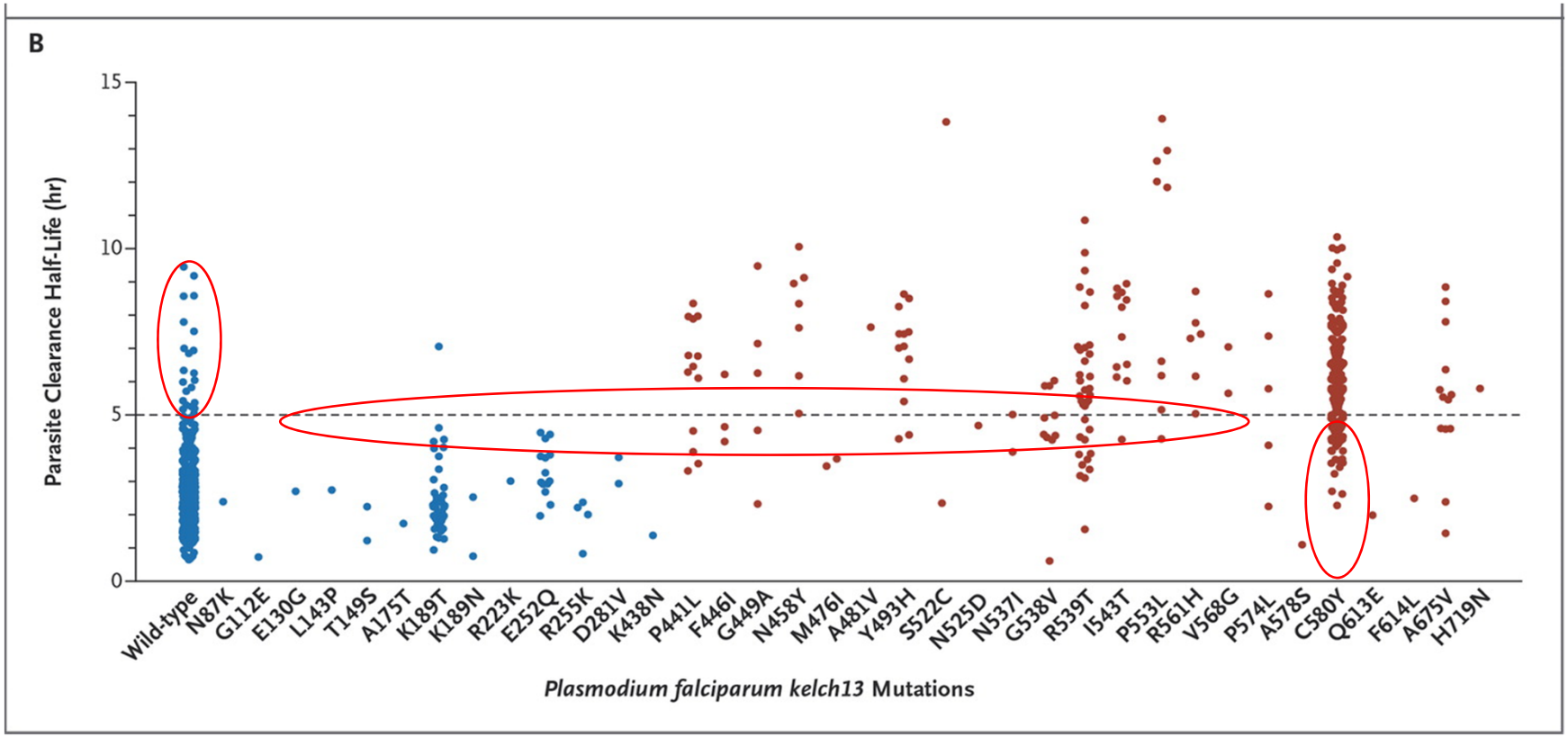
Slope

- Slope half-life is not influenced by initial parasitemia but still by the skills of the microscopists and the methodology used for slide reading;
- Highly dependent on immunity (variation up to 1 h);
- Limitations of the tool in real life:
  - low parasitemia;
  - rapid clearance;
- Does not take into consideration the lag phase or the the tail;
- Ideal samplings are too complicated for routine surveillance and 12-hourly sampling overestimates slope half-life.



	2010	2011	2015
RESULT ->	Stepniewska *et al.* (2010) analysis of recrudescence probability leads to the WHO definition of potential artemisinin resistance if >10% of patients are still parasitaemic by microscopy on day-3.	Flegg *et al.* (2011) propose the peripheral blood parasite half-life derived from the log-linear parasite clearance curve which is robust to initial parasitaemia and lag-phase.	White *et al.* (2014) propose a population-level definition of resistance which is robust to uncertainty of resistance status of individual infections.
CAVEAT->	This definition may not be suitable for populations with high or low parasitaemia on admission.	This definition leads to distributions of half-lives which appear to overlap.	Generalisation to a high transmission setting as in regions of Sub-Saharan Africa will need additional evaluation.

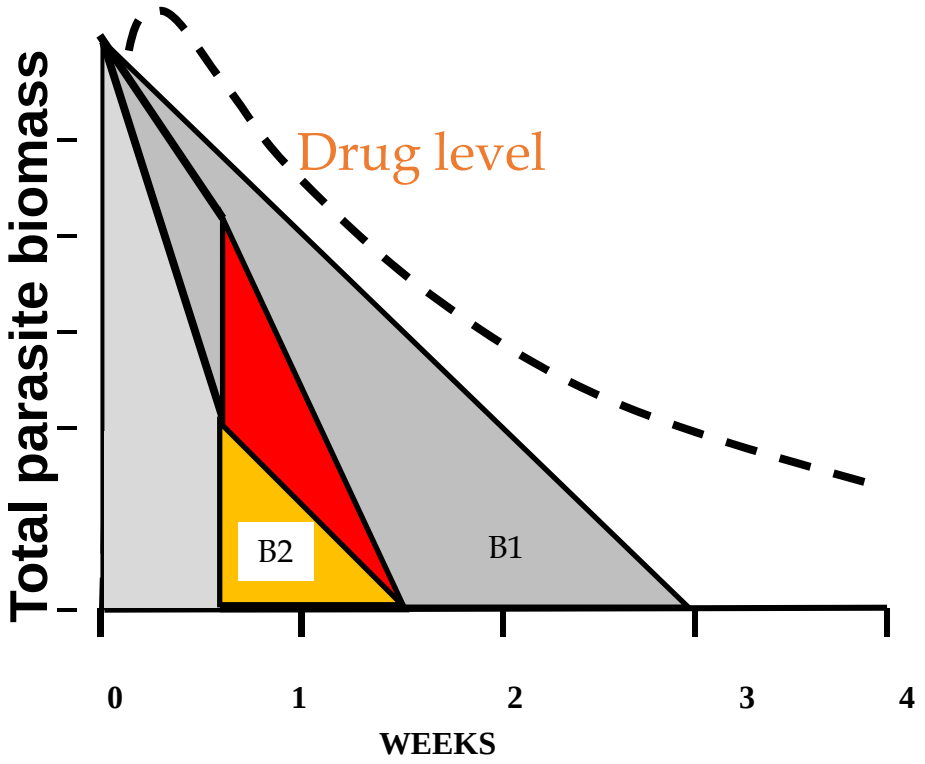
Correlation between K13 mutations and parasite clearance half-lives



Ashley EA et al. (2014) *N Engl J Med*

Consequences of artemisinin resistance

- Artemisinin resistance could become total
- Implication for the treatment of severe malaria
- Development of multidrug resistance (partner drug resistance)
  - Slow parasite clearance in patients treated with an ACT causes more parasites to be exposed to the partner medicine alone, increasing the risk of de novo resistance to the partner medicine;
  - But selection of resistance to partner drug is correlated with half-life of the partner drug



Actions against artemisinin resistance

- 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 and to 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.

Clinical outcome after ACT treatment according to sensitivity pattern of each component

Artemisinin*	Partner drug	Treatment outcome
Sensitive	Sensitive	Treatment success (ACPR)
Resistance (partial - delayed clearance)	Sensitive	Treatment success (ACPR) China, Laos, Myanmar
Sensitive Sensitive	Resistance (low grade)** Resistance (high grade)	Treatment success (ACPR) Treatment failure (ASSP, India)*
Resistance (partial - delayed clearance)	Resistance	Treatment failure (Cambodia, DP; Thailand, ASMQ)

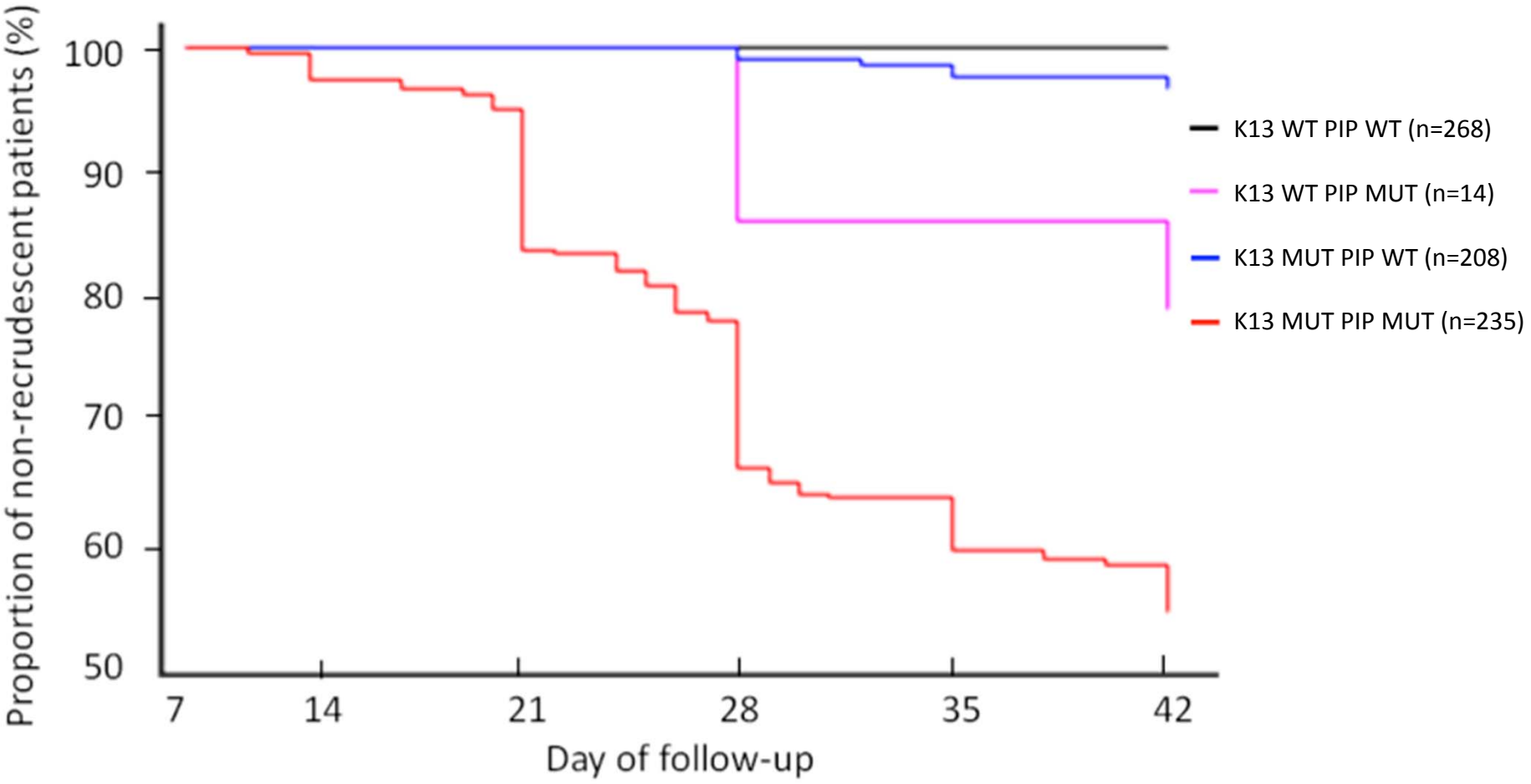
* **A 3-day treatment with artesunate used as monotherapy may cure up to 50% of patients;**

**** For amodiaquine and SP, treatment response was still adequate despite 20-30% of AQ or SP resistance in absence of artemsinin resisitance**

Relation between partner drug efficacy and K13 mutations

Year	Site	ACT	N	Efficacy 28/42 days (%)	K13 mutant (%)	*Pfmdr1* (n > 1) (%)
2011	Pailin, Cambodia	Artesunate-mefloquine	29	100	75.9 (C580Y)	6.9
2012-13	Dak Nong, Viet Nam	Dihydro-piperaquine	33	100	72.7 (C580Y; Y493H)	N/A
2014	Yingjiang county, Yunnan, China	Dihydro-piperaquine	23	100	91.3 (F446I)	N/A
2014-15	Champassak province, Lao PDR	Artemether-lumefantrine	29	93.2	83.3 (C580Y; R539T)	N/A

Role of each markers in DHA-PIP efficacy in Cambodia (N = 725)



Courtesy D. Ménard, IP Cambodge: PIP resistance molecular marker

Situation of *Pf* drug efficacy/resistance in PAHO

- ACTs remain highly efficacious were tested:

  http://www.who.int/malaria/areas/drug_resistance/drug_efficacy_database/en/

- Chloroquine is highly efficacious in the MesoAmerica;
- K13
  - KARMA project
    - N=1256: Brazil, Colombia, French Guiana, Peru; only 1 mutant in Manaus 2013 (A481V);
  - MalariaGEN
    - N= 27: Colombia and Peru; all WT;
  - CDC and WHO data
    - Guyana and Suriname: 5% of C580Y in Guyana in 2010.
  - Haiti: N=82, 2010-2013; all WT.

Why conduct Therapeutic Efficacy Studies (TES)?

- Surveillance of therapeutic efficacy (also called in vivo test) over time is an essential component of malaria control and provides
  - important information for determining whether first- and second-line drugs are still effective: and
  - the evidence-base to ministries of health to update their national malaria treatment policies;
- The WHO currently recommends monitoring the efficacy of first-line and second-line ACTs every 2-3 years in all falciparum-endemic countries. The results of TES make it possible to determine 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*; and
  - proportion of treatment failure by 28-day or 42-day follow-up (depending on the partner drug half-life in the specific ACT); a treatment failure rate exceeding 10% should prompt a change in the national antimalarial treatment policy.

Past history

- RAVREDRA was created in 2001 and was successful in implementing monitoring drug efficacy which led to the adoption of ACTs as first line treatment in the Amazon;
- PAHO is using a slightly adapted protocol (threshold of inclusion criteria: parasitemia 250/ul instead of 1000/ul);
- Challenges:
    - ACT considered to be highly efficacious;
    - Reduce number of cases making TES difficult to implement;
    - Modification of the protocol not based on evidence (day-3 protocol; modifications introduced by research institutes);
    - Difficult to confirm emergence of artemisinin resistance.

Next steps

- Revitalize TES under RAVEDRA umbrella;
    - Countries to be targeted: Brazil, Colombia, Guyana, Peru, Venezuela (ACTs) and Haiti (chloroquine + *pfcrt*);
    - Systematic monitoring K13 in all TES studies;
    - Guatemala, Honduras, Nicaragua and Dominican Republic should set up a routine surveillance system to monitor trend of *pfcrt* polymorphism relying a regional reference laboratory and local capacity strengthening;
    - MM in other countries?
- Based on previous results in Guyana, conduct a survey in zones of suspected artemisinin resistance;
- Avoid modification of standard protocols;
- Include monitoring of efficacy against *P. vivax*.

WHO Collaborating Centre (WHOCC)

- Role and functions
  - WHO Collaborating Centers are set up by WHO in support of its programme at the country, intercountry, regional, interregional and global levels;
  - In line with the WHO policy and strategy of technical cooperation, a WHO collaborating centre also participates in the strengthening of country resources, in terms of information, services, research and training, in support of national health development.

- WHOCC in the Region
  - Centre for Prevention and Control of Malaria: CDC;
  - Center for Surveillance of Antimalarial Drug Resistance: Institut Pasteur.

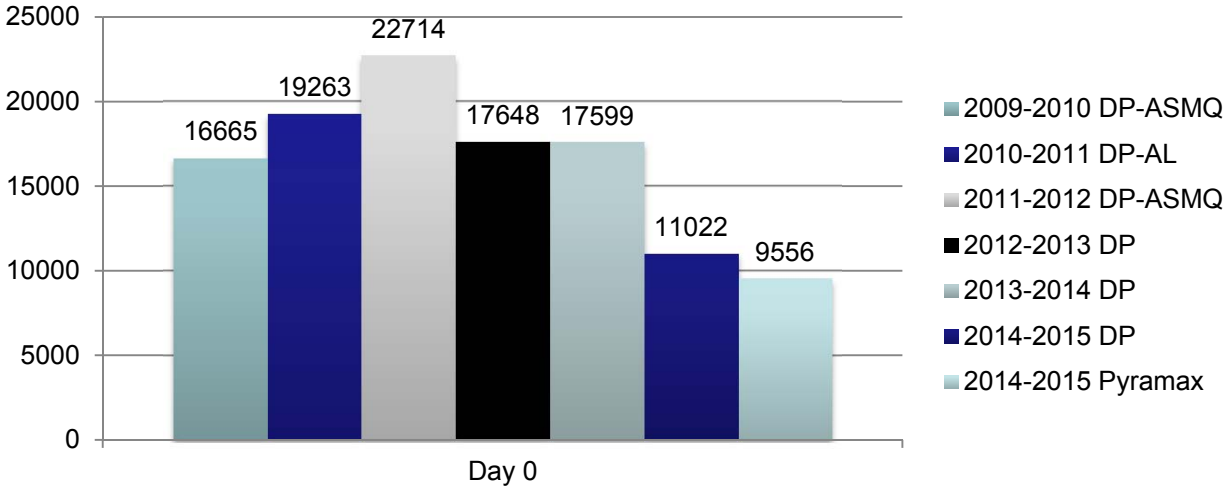
- Expectations
  - Support WHO/PAHO activities;
  - Implement WHO norms and standards;
  - Data sharing.

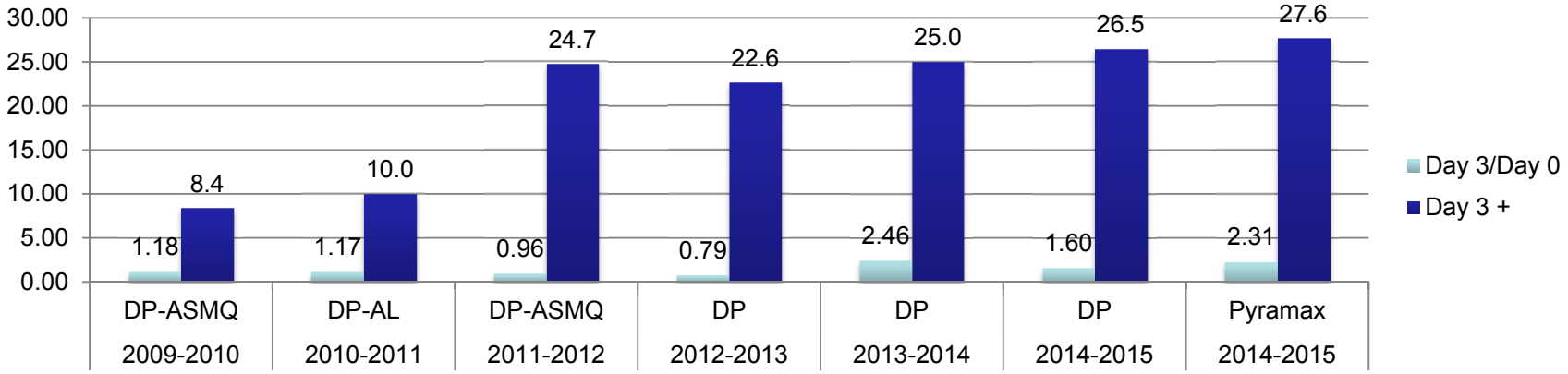
Thank you for your attention

Definitions of ‘candidate’ and ‘validated’ K13 mutations

- The criteria for determining whether a K13 propeller mutation is ‘validated’ or ‘candidate’ remain as per the suggestion of the ERG on K13 2014:
  - a statistically significant association ($p < 0.05$) between a K13 mutation and either a parasite clearance half-life ≥ 5 hours or 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 of 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 requirements 1 and 2 are met, ‘associated’ when either 1 or 2 are met.

Trend of parasitemia after ACT treatment in Cambodia 2009-2015





There is no evidence for recent emergence of higher levels of artemisinin resistance (WWARN, 2015).