CURRENT SITUATION OF RESISTANCE TO ANTIMALARIAL DRUGS IN AMAZONIA

PAST AND CURRENT DRUGS

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- 24th March 2015

POUR LA RECHERCHE, POUR LA SANTÉ,
POUR DEMAIN

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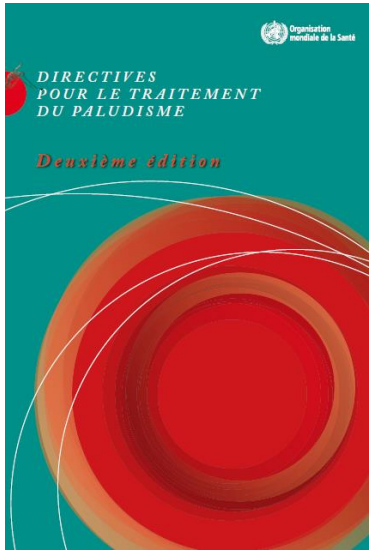
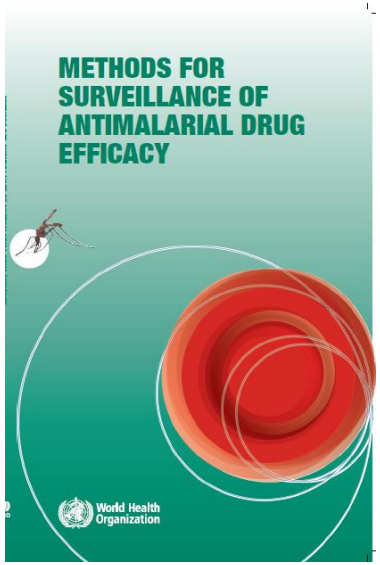
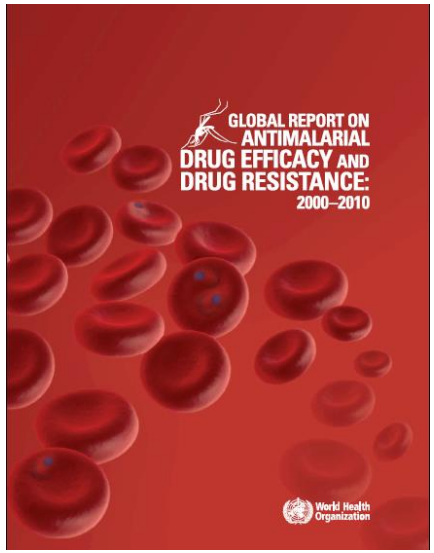
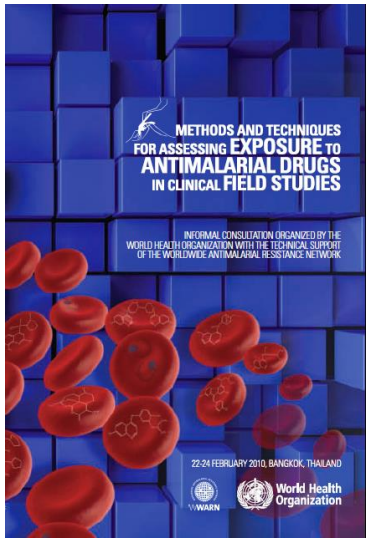
1. Why should we monitor parasite resistance?

- To quickly detect emergence
- To adapt therapeutic recommendations
- To adapt control strategies









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2. Tool box: Methods and outputs

Therapeutic efficacy studies (TES)

- Therapeutic responses on Day 28 or 42
- Parasite clearance time

In vitro / *ex vivo* drug testing

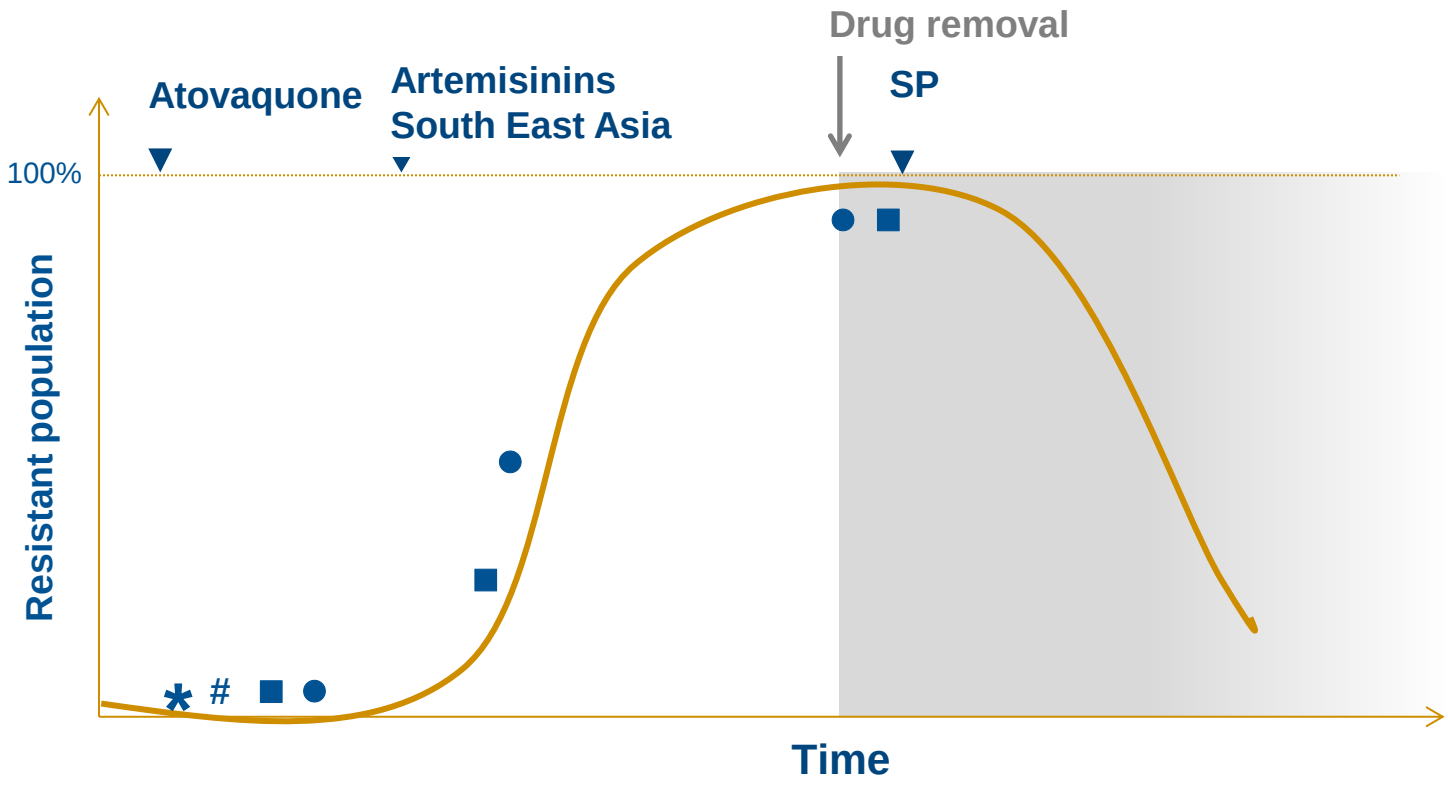
- Standard method: inhibitory concentration 50%
- Ring survival assay after 6 hours of exposure to DHA

Molecular marker of resistance

- Validated genes
- Microsatellites and SNP polymorphisms around

3. Resistance and genotype

Adapted from **Wiesh** 2011, *Lancet* 11:236-247



Mutations emerge, are selected or not, and spread

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4. Resistance and molecular marker for resistance

Advantages

- Easy to analyze
- Easy for monitoring of resistance
- Useful to determine the resistance origin

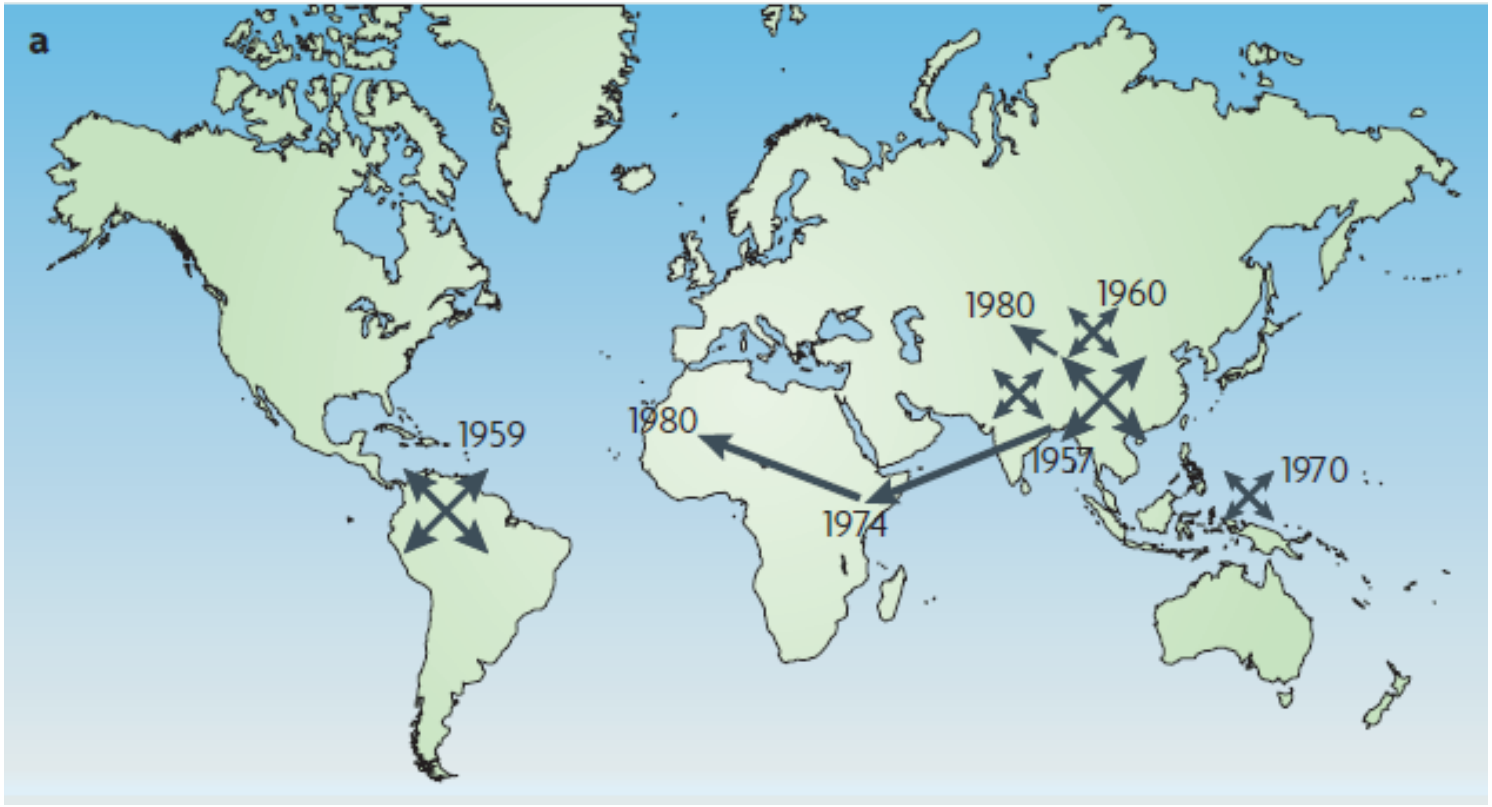
Limitations

- Should be carefully linked to resistance
  - ✓ Therapeutic efficacy studies
  - ✓ *In vitro* testing
- Are they informative for ever?

5. Chloroquine resistance

Dondorp 2010, *Nature Rev Microbiol*

Emergence : SEA and South America



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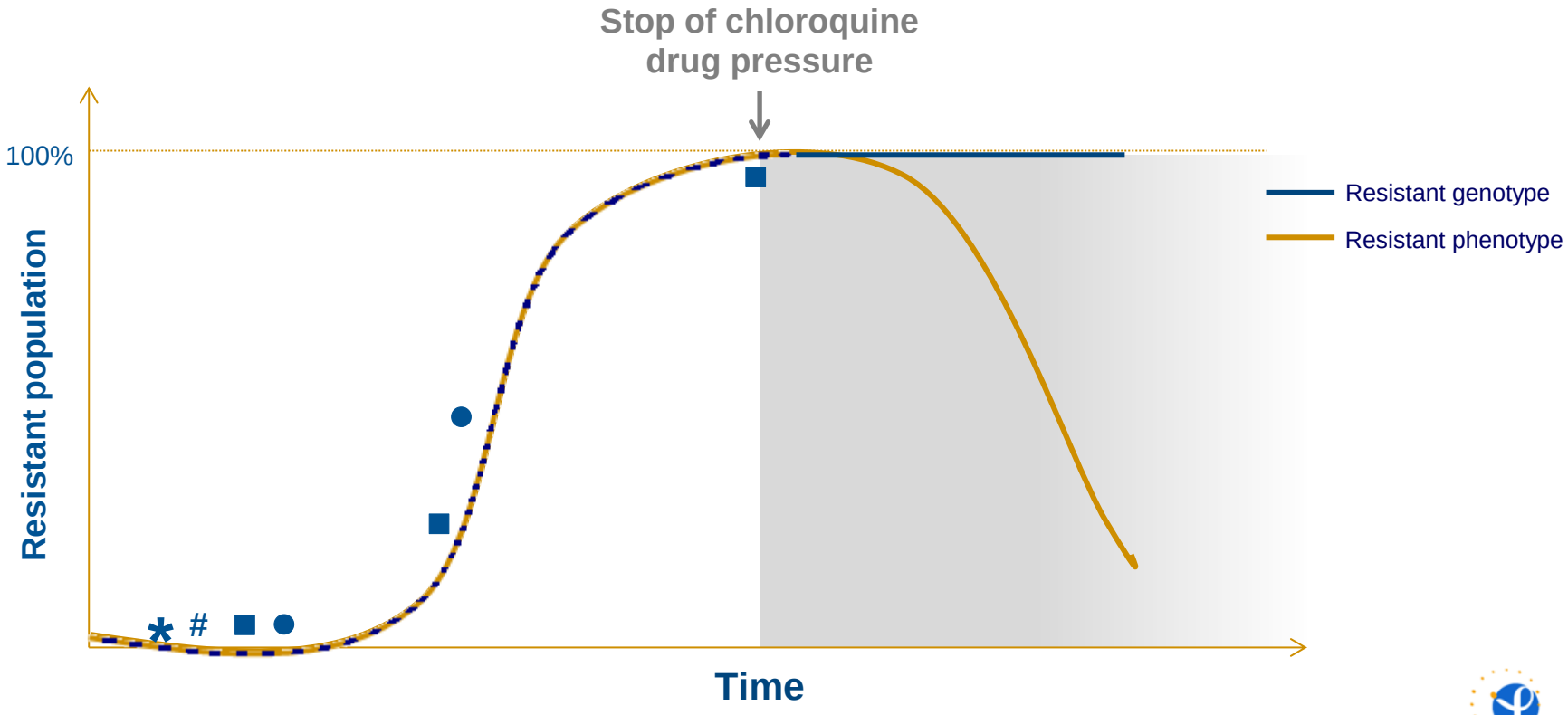
6. The historical perspective regarding chloroquine

Chlroquine resistance evolution in French Guiana

- Fixation of *pfcrt* alleles associated with resistance
- Discordance between genotype and phenotype, Why?



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7. Fixation of standard drug-resistance allele

Legrand 2012, *Antimicrob Agents Chemother*

- **Chloroquine was abandonned in 1995**

- **Resistant marker prevalence between 1997 and 2012**

Gene	Haplotype	Prevalence %	n	
Pfcrt	**SVMNT**	**97.5%**	**1028 / 1054**	
codons 72-76	CVMNK	1.6%	17 / 1054	**~90% of imported cases (Haiti, Africa)**
	CVIET	0.9%	9 / 1054	
Pfmdr1	**NFCDY**	**90.7%**	**701 / 773**	
codons 86,184,1034,1042,1246	NFSDY	8.7%	67 / 773	

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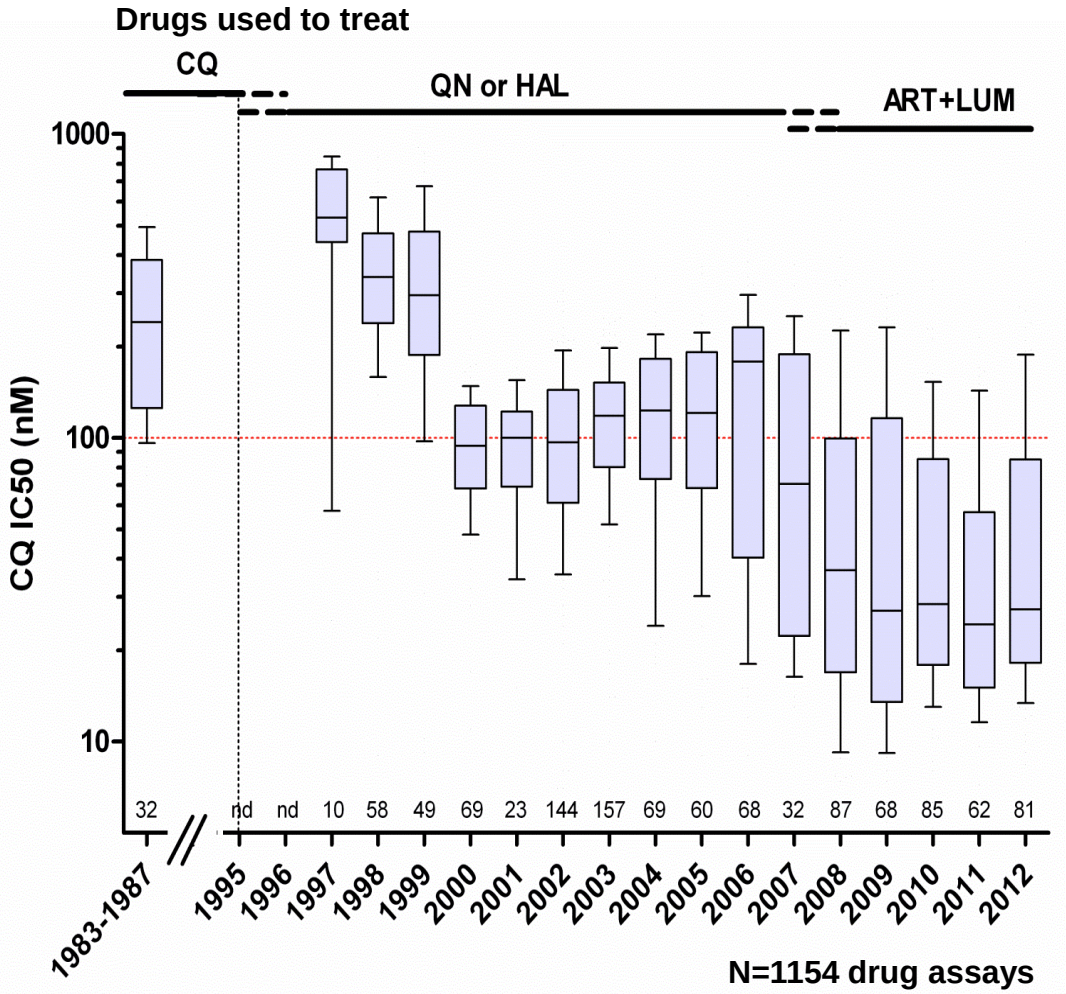
8. Evolution of chloroquine susceptibilities

Legrand E. et *al*. *Antimicrob Agents Chemother,* 2012
Dedet JP. et *al*. *Bull Soc Path Exot,* 1988



- **Prevalence of CQR isolates :**
  - **1990s: >90%**
  - **2012 : 25%**

→Standard molecular markers (*pfcrt* K76T) are uninformative

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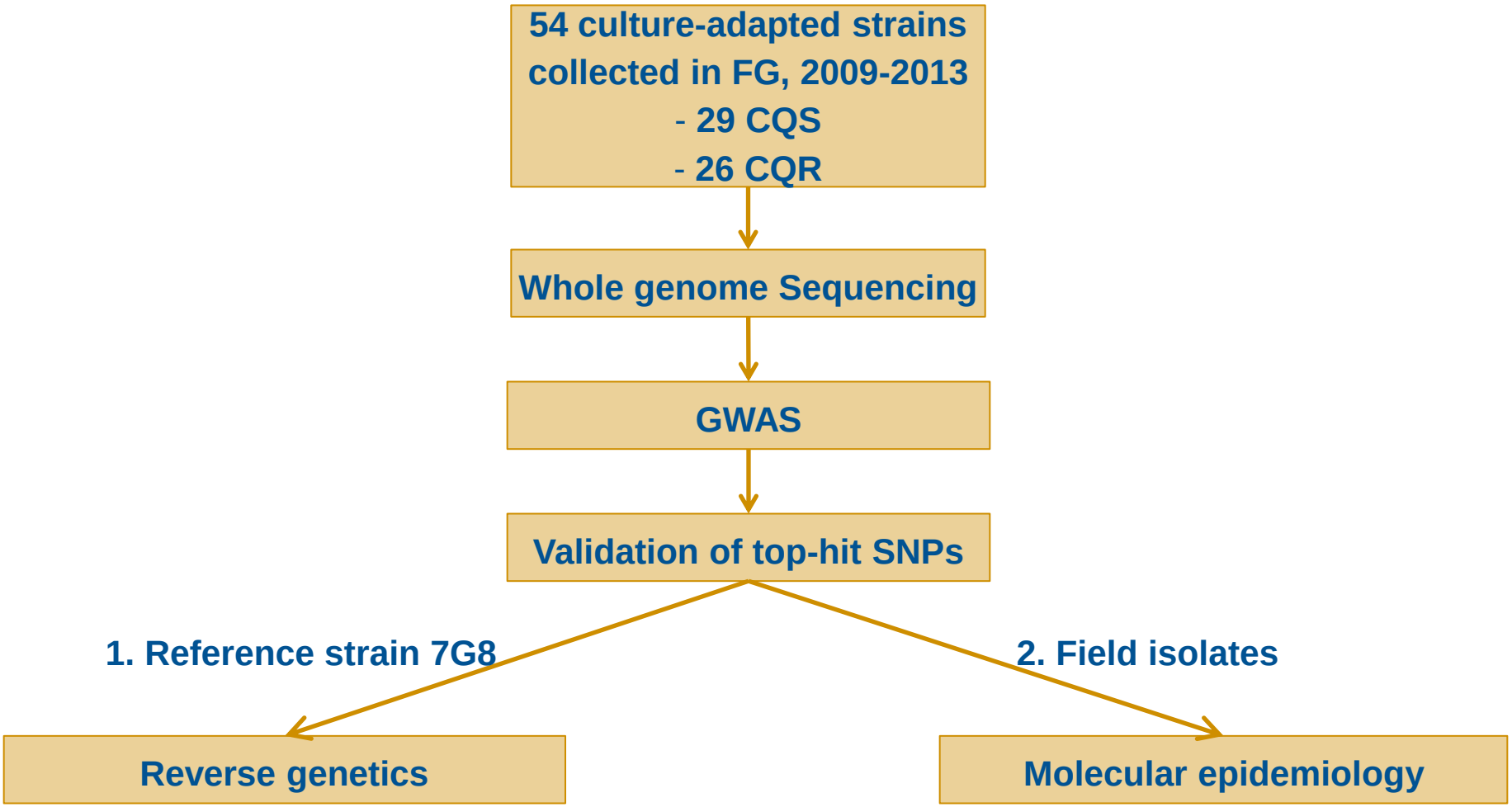
9. Process to identify the genetic markers



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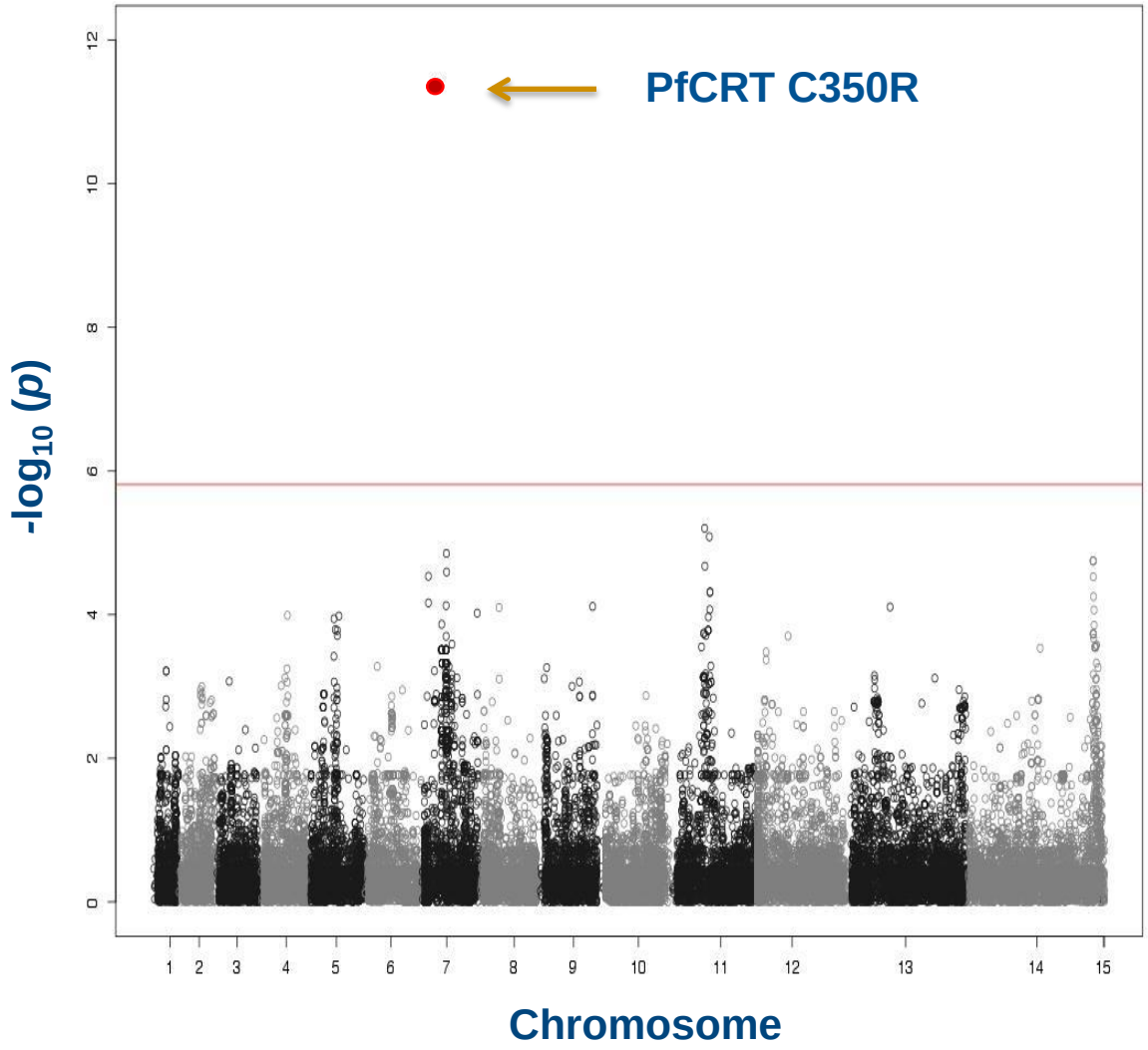
10. GWAS results



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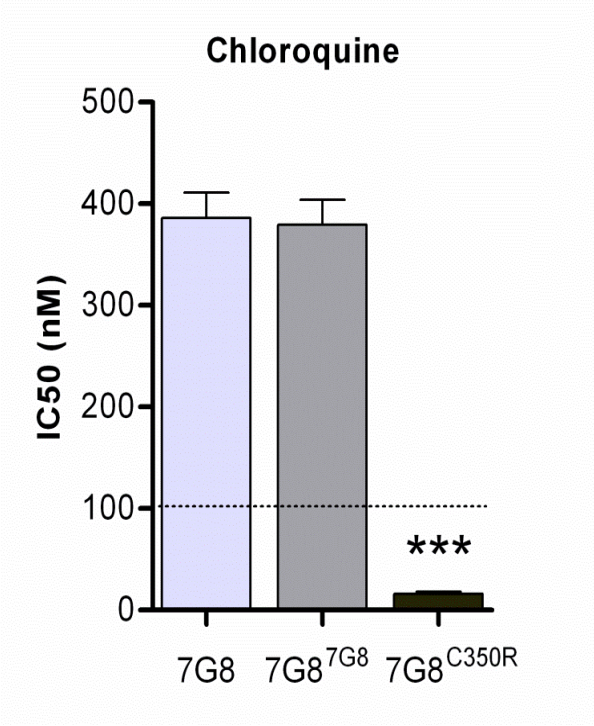
11. C350R validation

1. Reverse genetics

In the CQR Brazilian strain 7G8

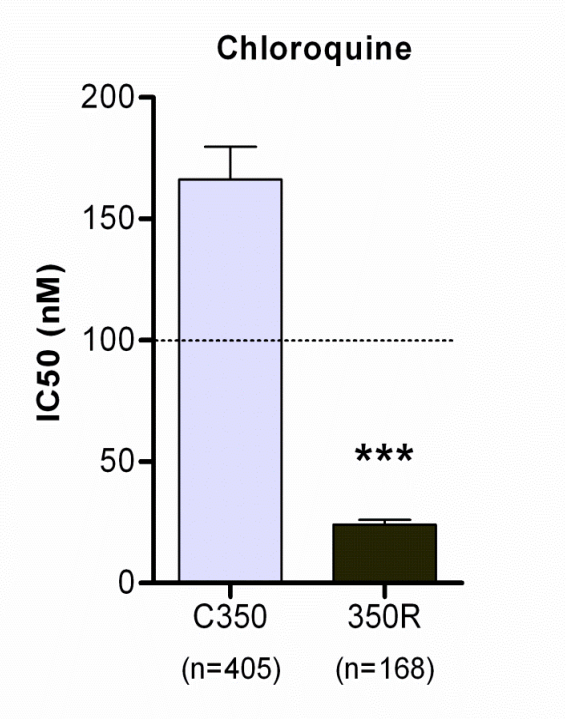
(8 drug assay replicates)



2. Molecular Epidemiology

In field isolates from FG

(1997-2012, n=573)



→ **K76T alone is no more a molecular marker for resistance**

→ **Standard molecular markers might not be informative after drug removal**

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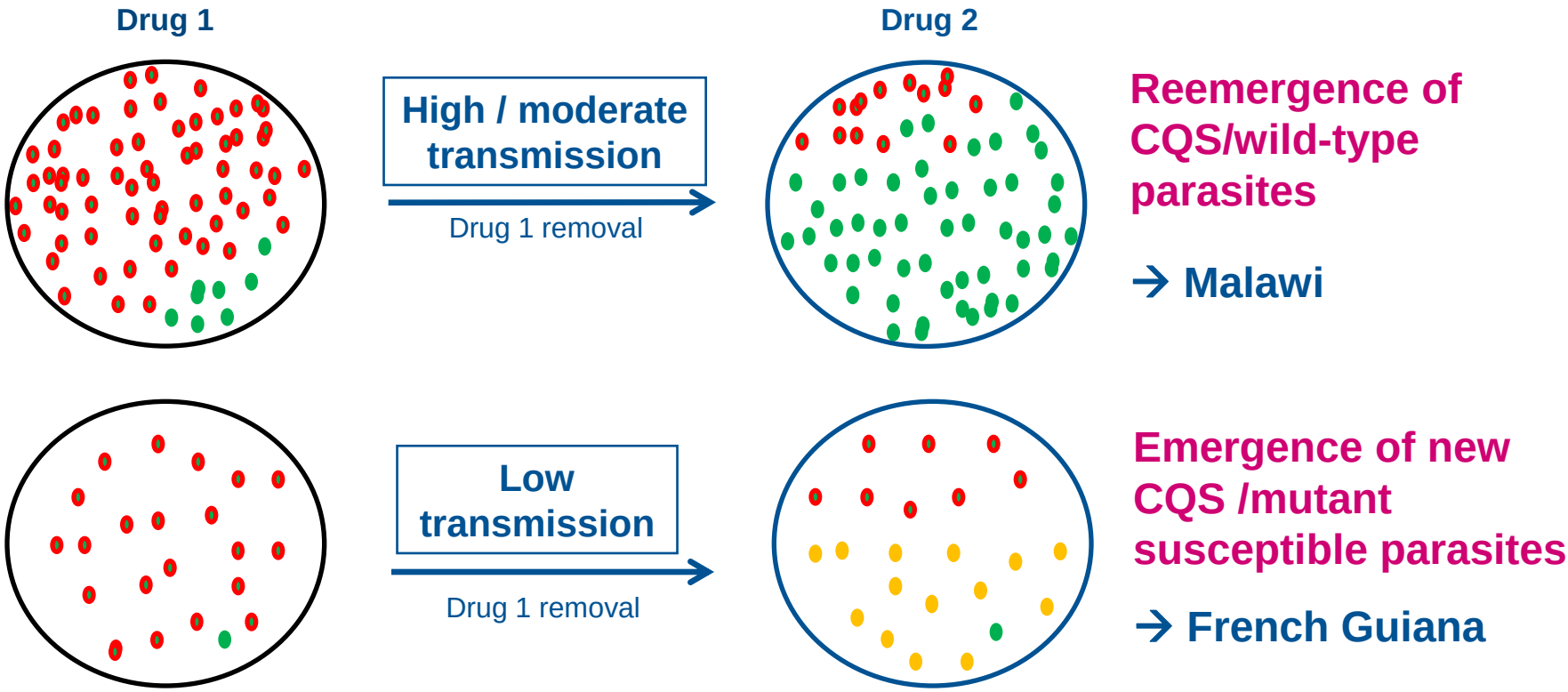
12. Genetic evolution after policy change



In low transmission settings :

→ **Phenotypic tests and/or efficacy studies should regularly confirm the usefulness of the molecular markers**

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13. Artemisinine resistance, where are we now?

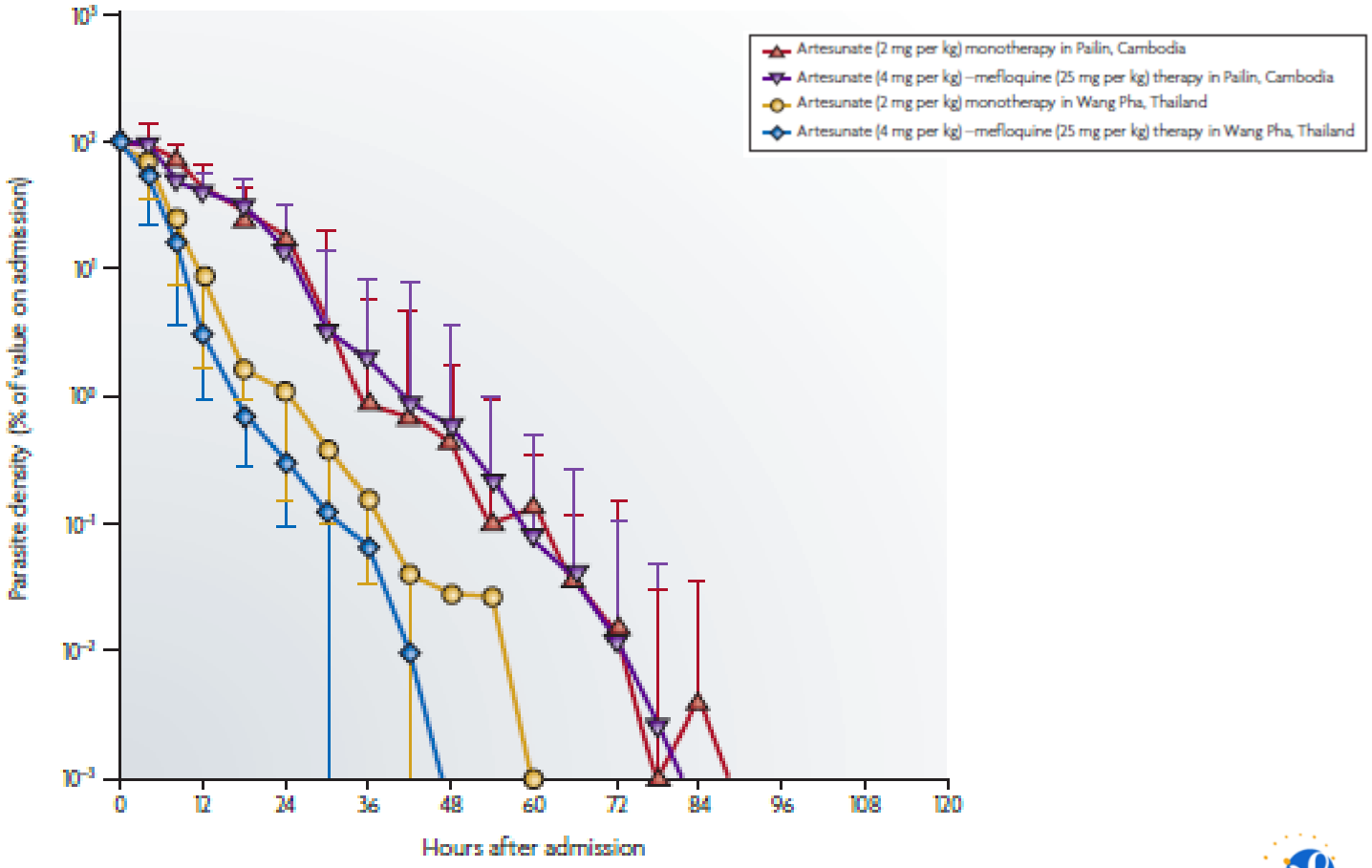
Emergence: SEA

Adapted from **Dondorp** 2010, *Nature Rev Microbiol*

14. *In vivo* phenotypic profile

White 2008, *Science*

A delay parasite clearance time



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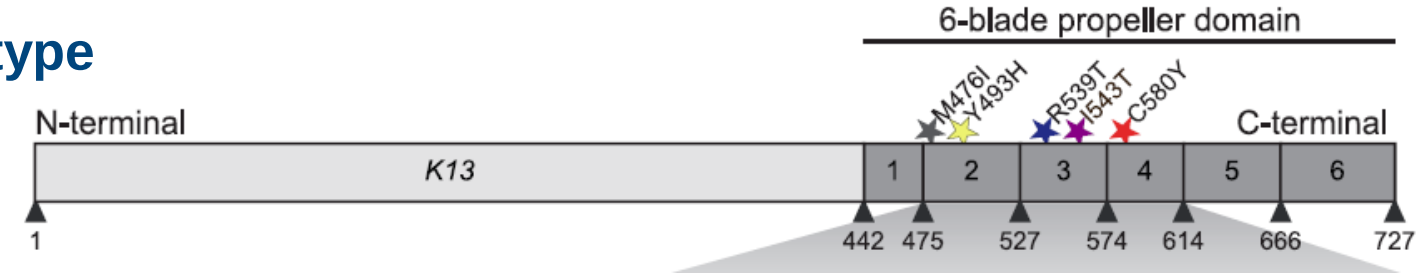
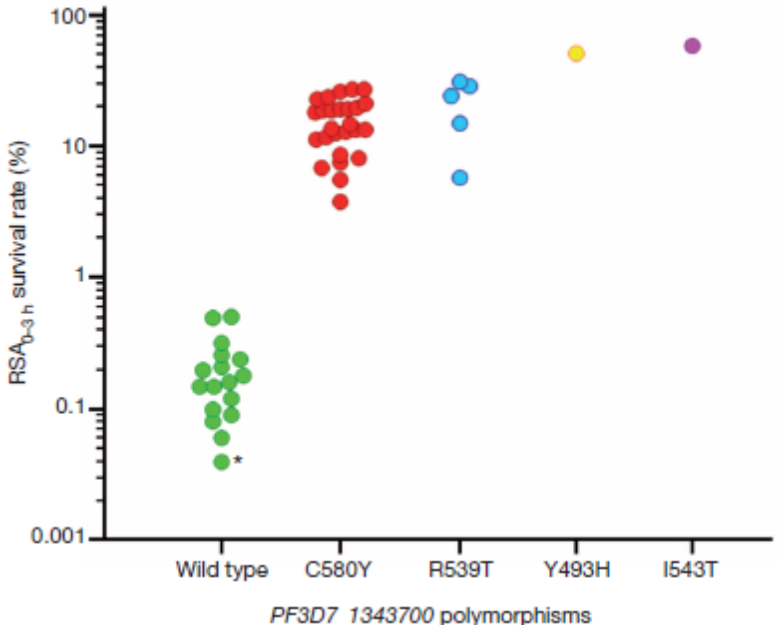
15. Genotype: mutations in active site of gene K13

Ariey et *al*., 2014, *Nature*
Straimer et *al.*, 2015, *Science*

Genotype



In vitro phenotype



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16. Is artemisinine resistance emerged on the Shield?

- **Suriname: 7.9% D3 positive – 63% PCT >5h**
- **Guyana: 0% D3 positive – PCT ND**
- **French Guiana: 5.7% D3 positive – PCT ND**
- **Brazil: 0 to 58% D3 positive**

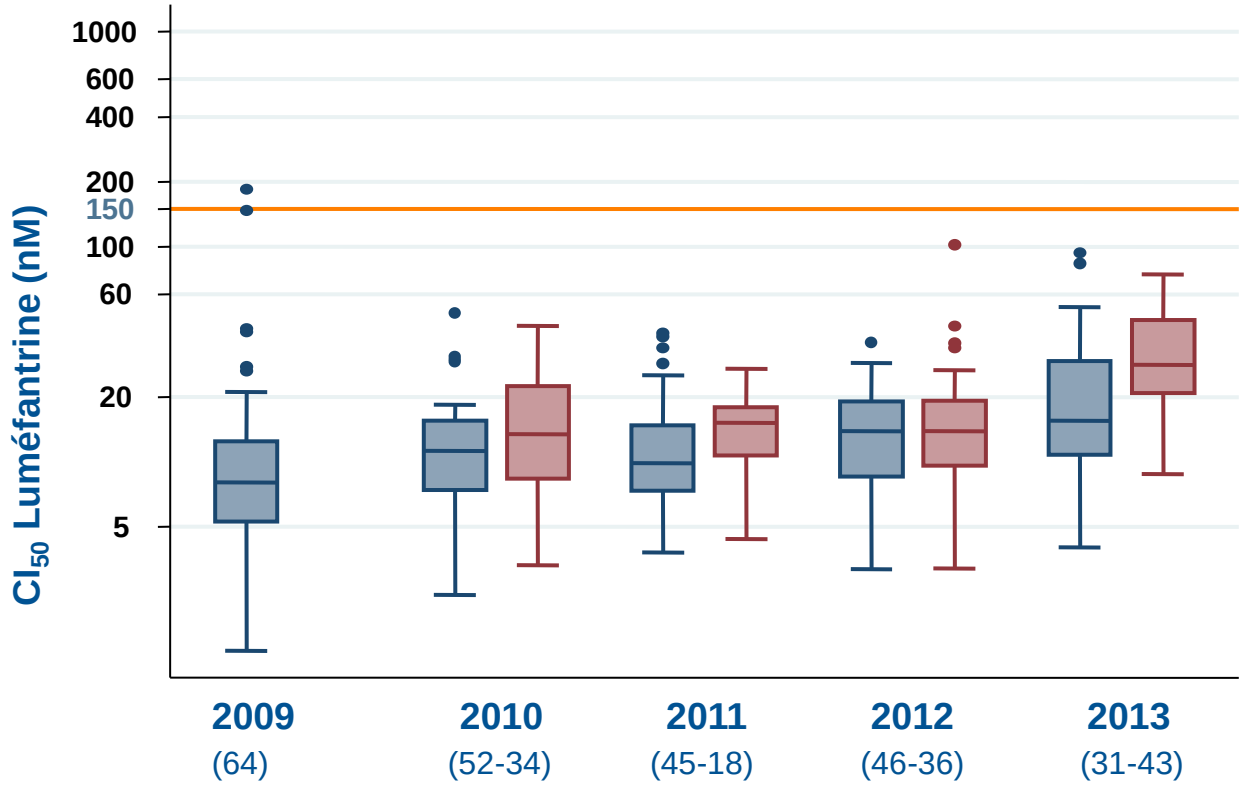
17. Is artemisinine resistance emerged on the Shield?

- **French Guiana**
  - 312 samples genotyped
  - No mutations in the active site
- **Guyana**
  - 73 samples from the last therapeutic efficacy studies (2014)
  - No mutations in the active site
- **Brazil, Manaus**
  - 133 samples
  - None of the five mutations

→ **Probably, not yet at a large extend**

18. Resistance to partner drugs

→ Parasites susceptibles to partner drugs in French Guiana



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Thank you for your attention

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