



Towards Understanding the Mechanisms of Lumefantrine Resistance in *Plasmodium falciparum*

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Fifth EDCTP Forum, 12-14 October 2009, Arusha

Artemether-Lumefantrine(Coartem)

- ✓ Recommended by WHO in many parts of Africa
- ✓ Emergence of Lumefantrine tolerance *in vivo* reported

In Kenya:

Coartem-First line for uncomplicated malaria 2006/2007



Context

- No baseline data on LM/DHA activity
- No data on frequency of *Pfmdr1/Pfcrt* mutations
- Mechanisms of resistance -molecular markers of resistance?

Objectives

1. Baseline data on activity of Chloroquine (CQ), Lumefantrine (LM) , dihydroartemisinin(DHA), piperaquine (PQ)
2. Understand mechanisms of LM resistance

Main Goal

Identify molecular markers to monitor LM (thus Coartem) resistance

Hypothesis

1. Mismatched pharmacokinetics between artemether and LM

➤ *Long acting LM will exert main selective pressure for resistance..*

2. Quinoline like structure-Similar to CQ in structure

➤ *Impact of quinoline molecular markers on selection of resistance*

CQ framework/reference to study LM resistance

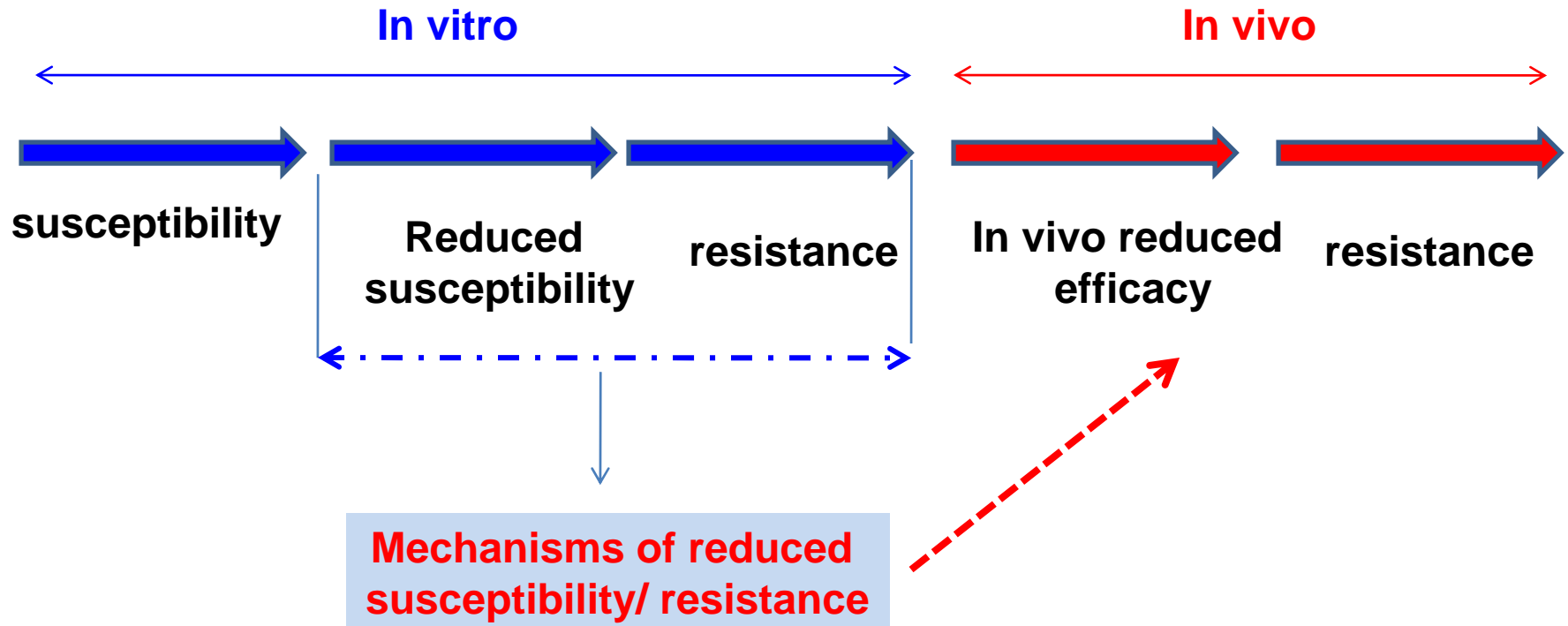
studies of Lumefantrine resistance in *P.falciparum*

❑ well-established resistant strains not available.

wait until establishment of in vivo resistance?

***NOT the best approach!!

Experience: Resistance arises in a stepwise fashion, *in vitro* models useful!



In Vitro Work-Methodology

✓ **Clinical trial framework**

adaption of clinical isolates to long term culture (>100 isolates)

Artekin (DHA /PQ) vs Coartem Clinical Trial (Phase III), 2006/2007, Kilifi, Kenya

**Isolates taken before and after treatment*

✓ **In vitro assays-hypoxanthine incorporation method**

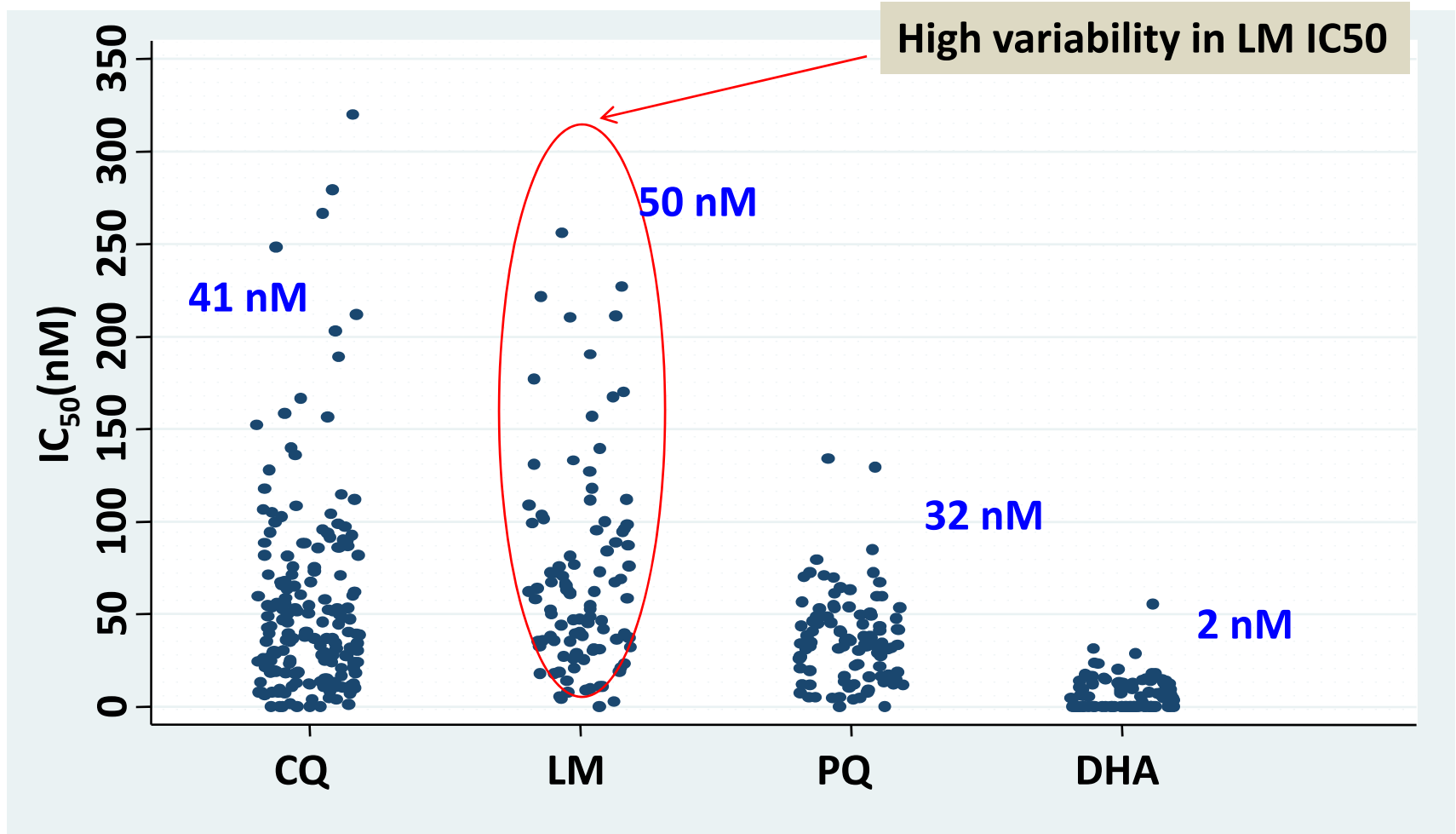
Assessment of activity of lumefantrine(LM), piperaquine (PQ), dihydroartemisinin (DHA), (chloroquine (CQ)-control

✓ **Genotyping**

- *Pfcrt* at codon-76 (*mutant= CQ resistance)
- *Pfmdr1* at codon-86(*mutant associated with CQ resistance)
- *Pfmdr1* copy number variation

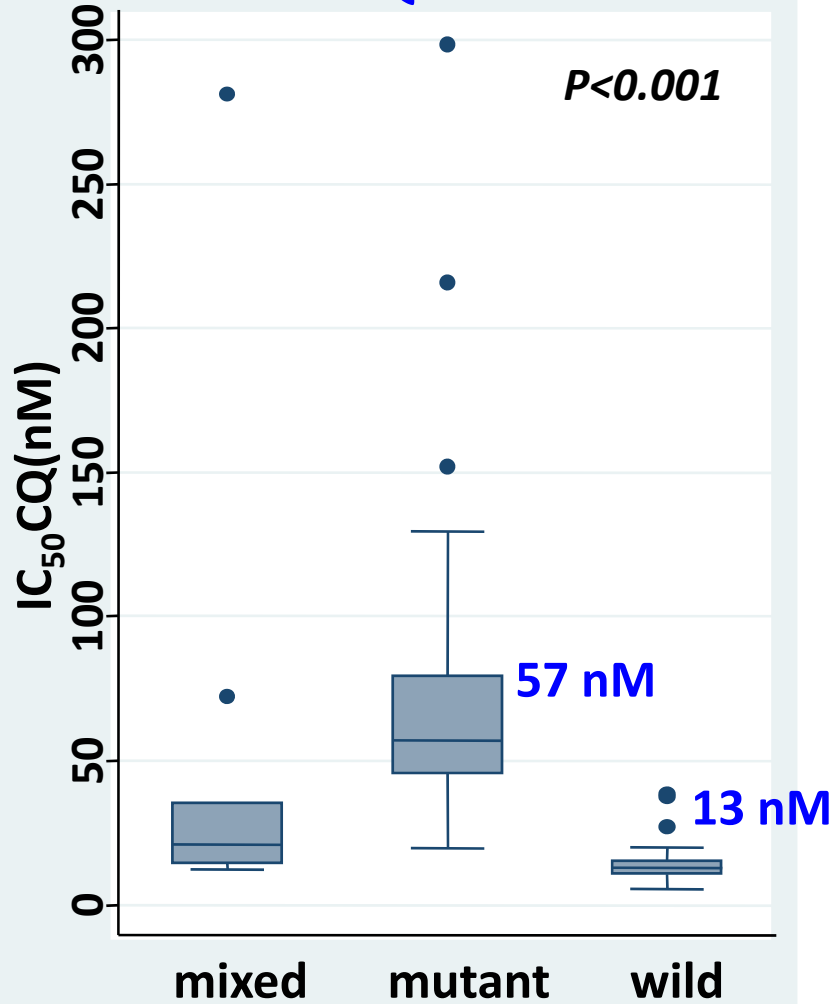
In vitro activity of CQ, LM, PQ DHA in Kilifi(n=115)

Recent isolates: 2006-2007

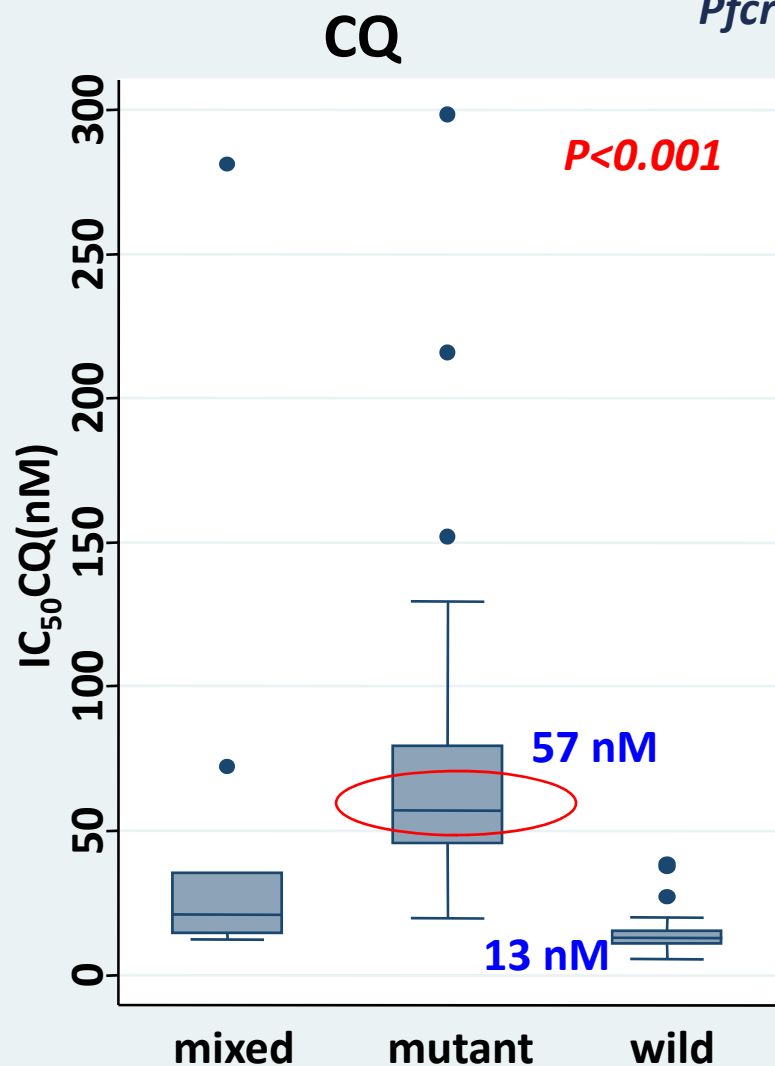


Pfcrt76

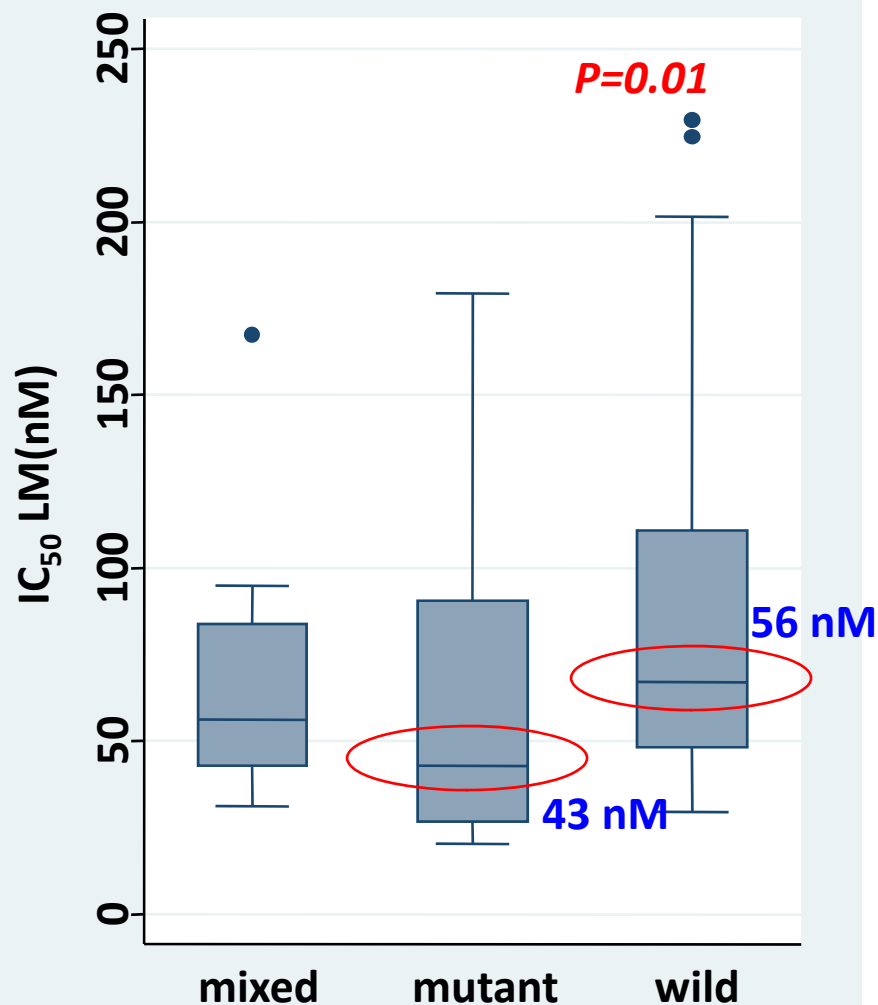
CQ



Pfcrtr76



LM

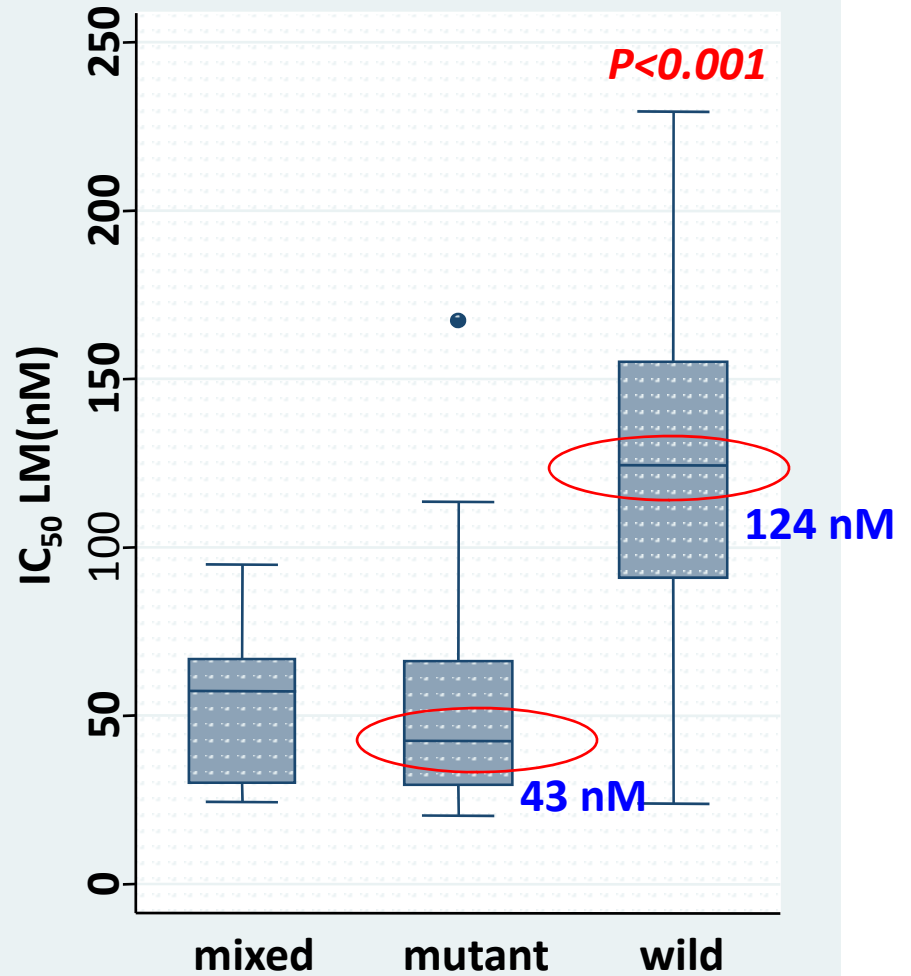
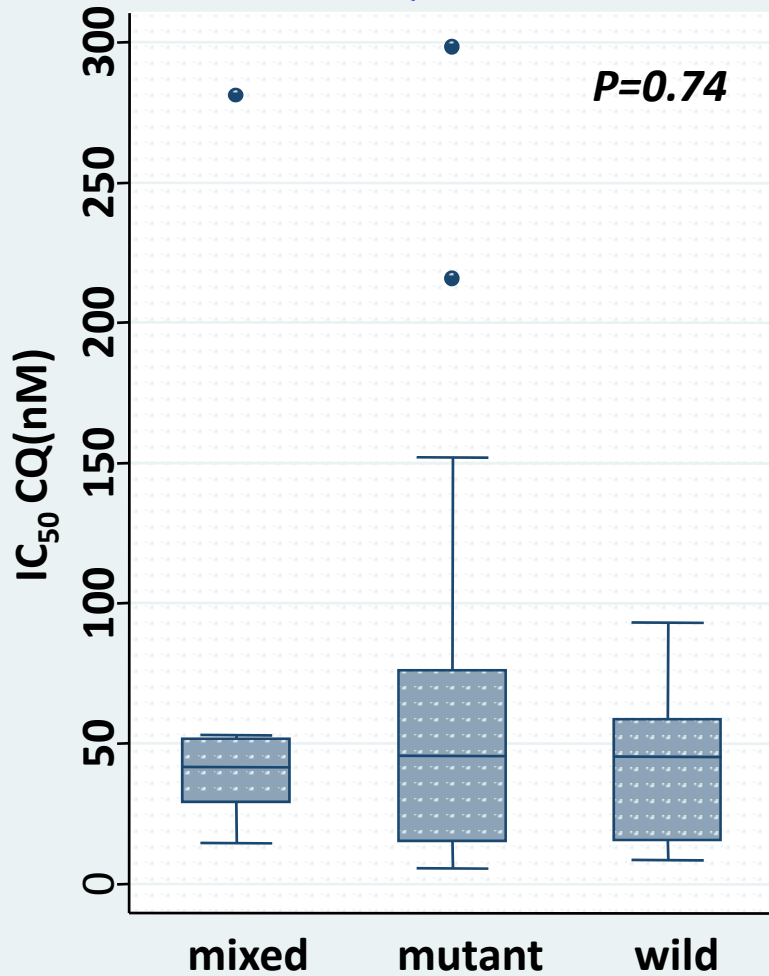


Wild type *Pfcrtr76* = reduced LM susceptibility

Pfmdr186

CQ

LM

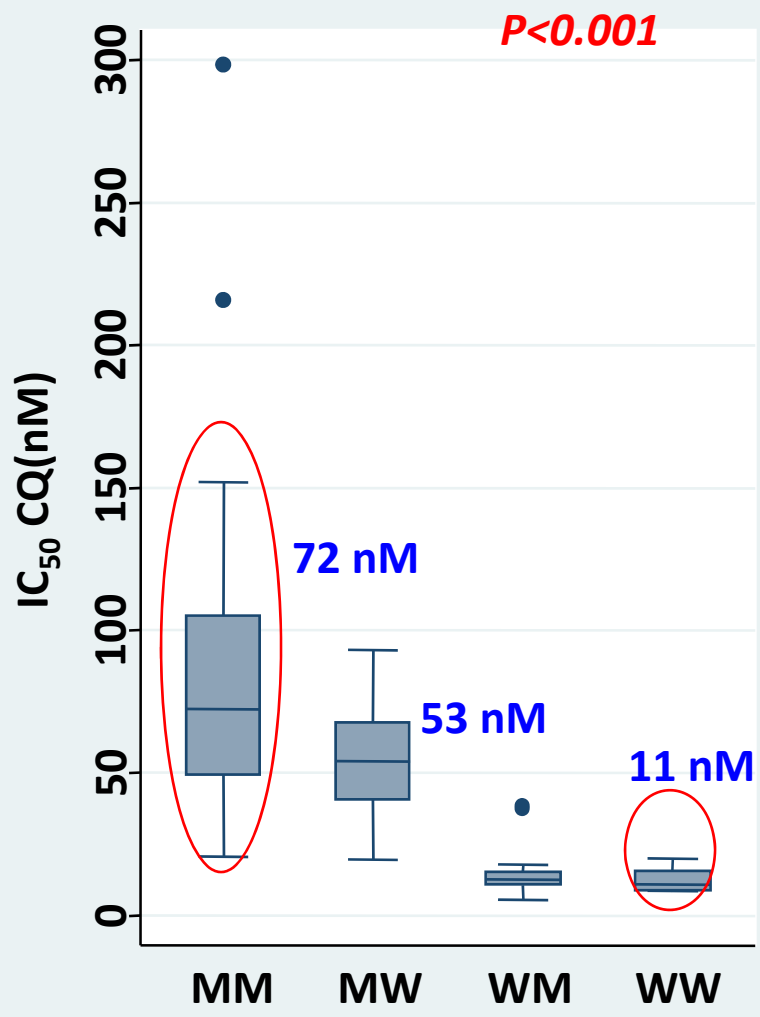


Wild type *Pfmdr186* = reduced LM susceptibility

Pfcrt **Mut**-Pfmdr1**Mut**= **MM**
Pfcrt **Wild**-Pfmdr1**Wild**=**WW**

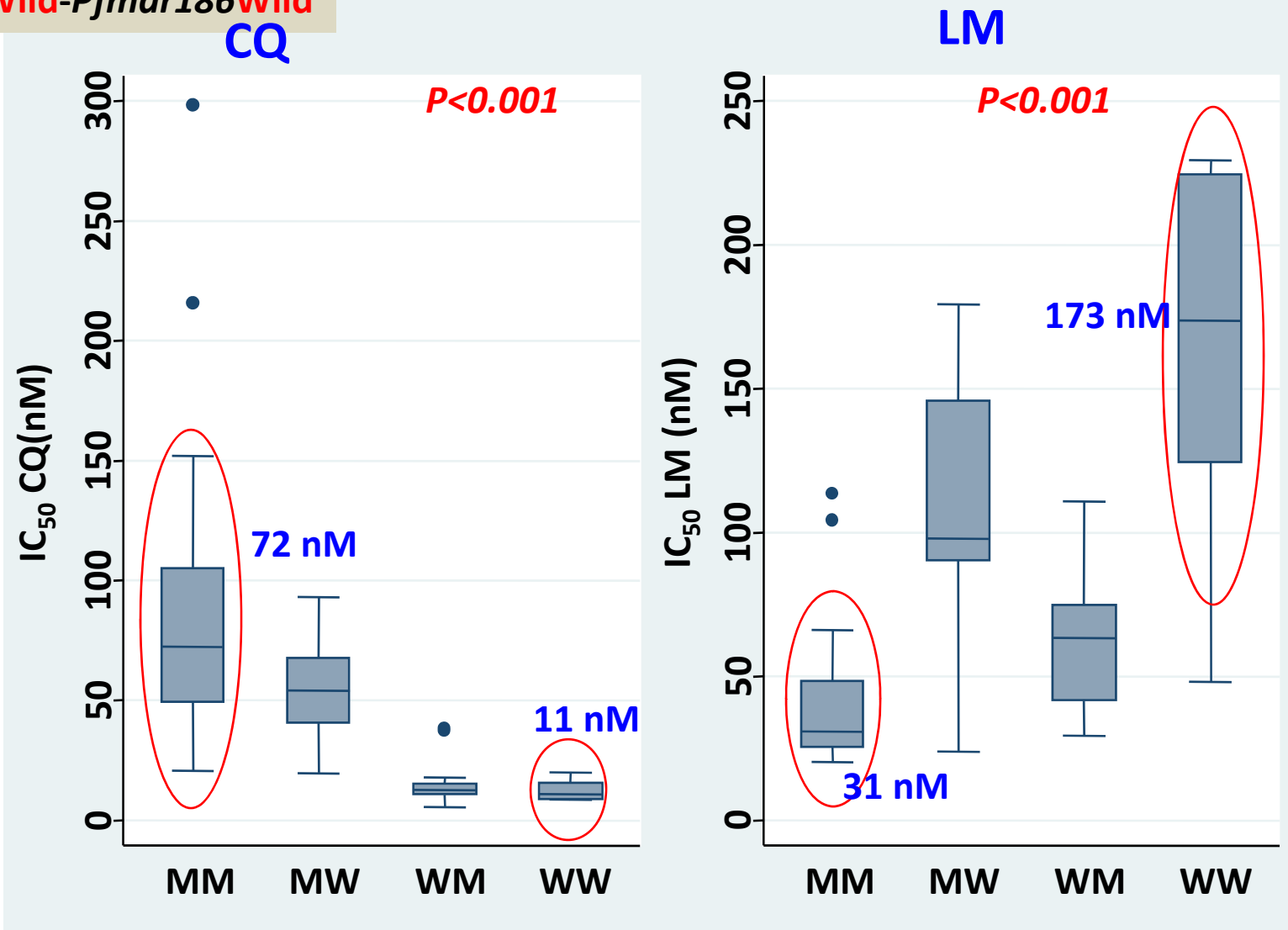
Pfcrt-76+Pfmdr1-86

CQ



MM= *Pf*crt76 Mut-*Pf*mdr186Mut
WW = *Pf*crt76 Wild-*Pf*mdr186Wild

*Pf*crt-76+*Pf*mdr1-86



***Pf*crt76-*Pf*mdr186 wild type= decrease in LM susceptibility**

***Pfmdr1* and copy number variation (CNV)**

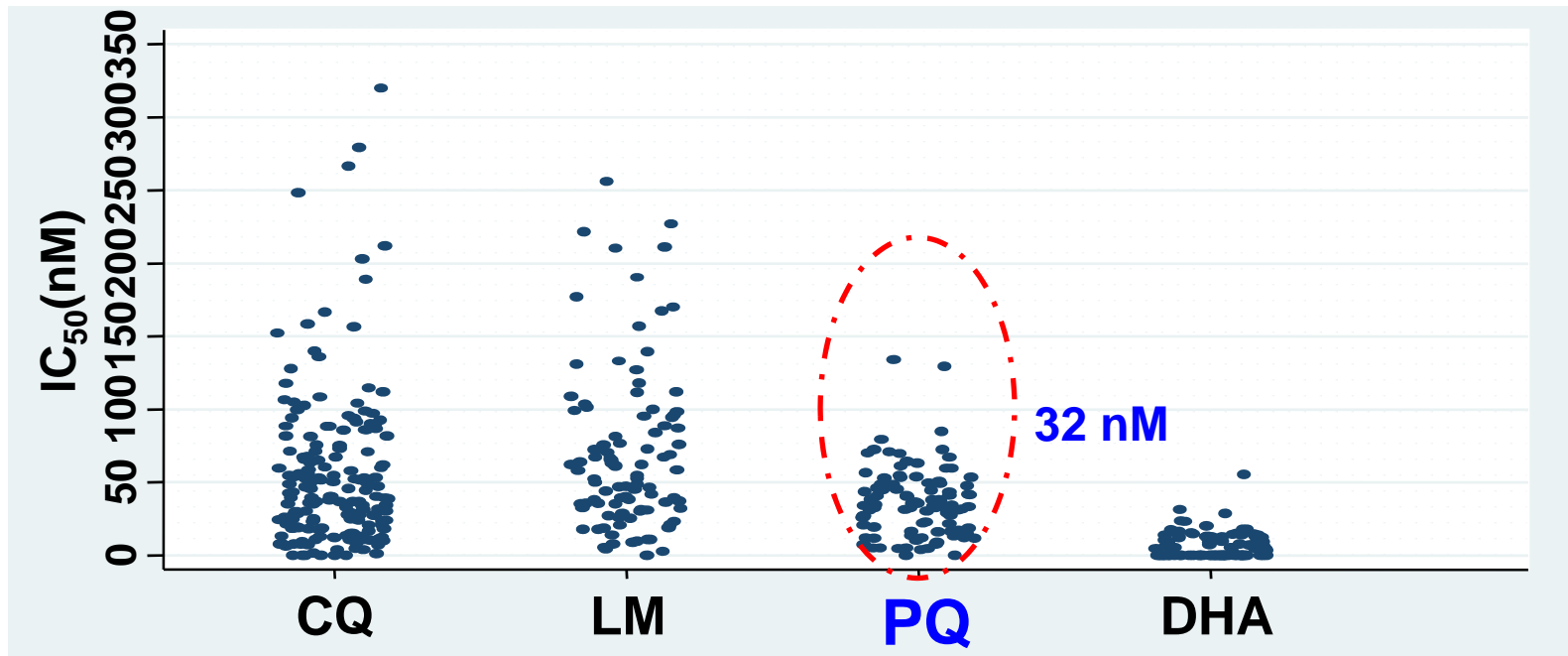
Increase CNV = decrease in LM activity (South East Asia)

We analyzed *Pfmdr1* CNV in Kenya

Out of 98 isolates, only one has more than 1 copy number..

low prevalence of CNV in line with previous reports

PQ:

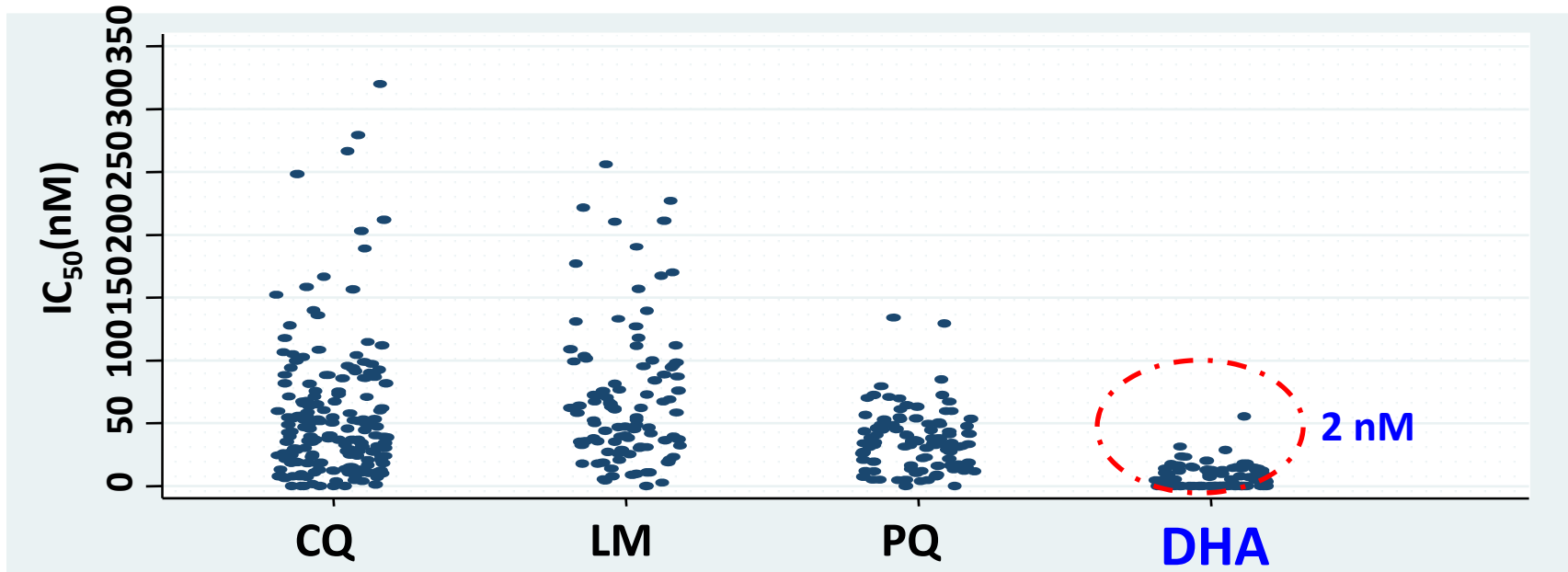


PQ= bis-chloroquine

Pfcr76 and *Pfmdr186*? No association with PQ activity
Pfcrt wild type= 36 nM and Pfcrt mutant= 38 nM

in line with previous reports..

DHA..



DHA: High activity in vitro..90% isolates $IC_{50} < 25nM$

Pfcrtr76 ? No association with DHA activity

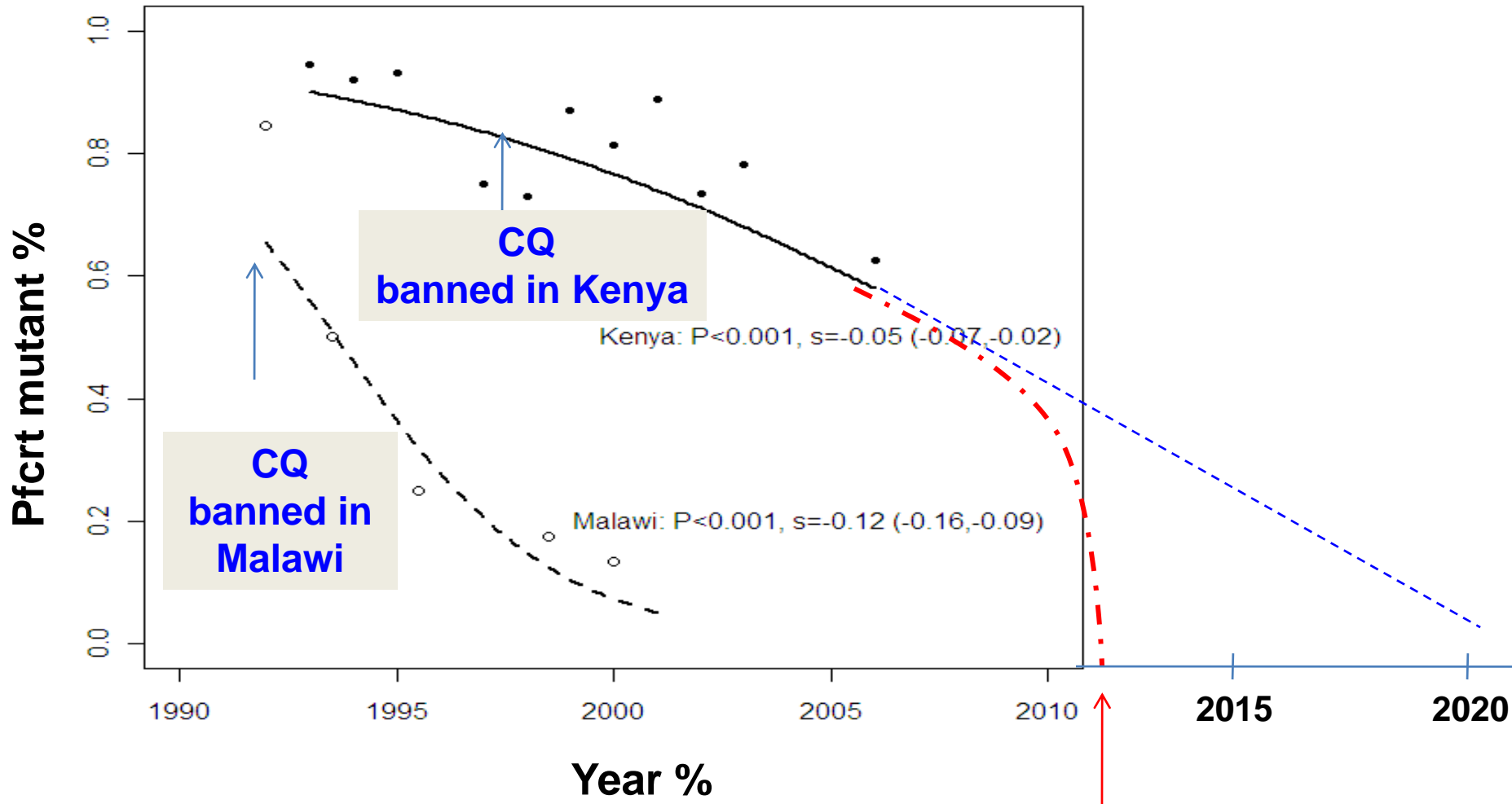
But *Pfmdr186*: wild type 3 nM versus 2 nM mutant (p=0.04).

Marginal modulation of DHA activity by *Pfmdr186* genotype
..in line with some previous reports *in vitro*..

Reversal of CQ resistance in Malawi and Kenya

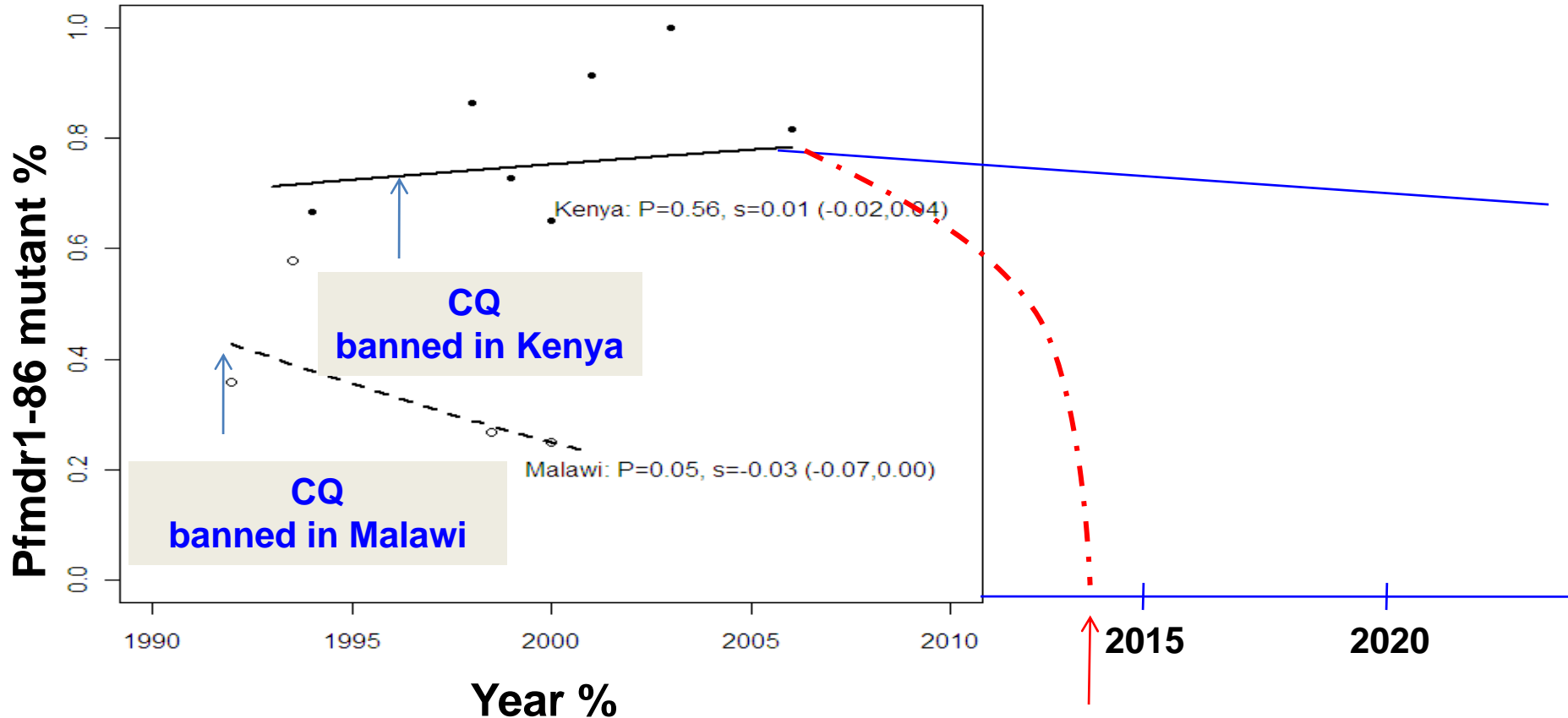
Reversal of CQ resistance in Kilifi/Malawi

pfprt-76



Kublin et al. 2003 JID 187, p1870
Mwai et al. 2009, Mal J 8:106

pfmdr1-86



**LM pressure=
a drastic drop in mutants**

Kublin et al. 2003 JID 187, p1870

Mwai et al. 2009, Mal J 8:106

Conclusion:

Coartem efficacious but.....

- * High spread of LM IC50 (2 to 250 nM)
- * 20% isolates: LM IC50 > 100 nM: they are wild *Pfmdr186/Pfcrt76*
- *inverse relationship of LM and CQ activity!!

✓ Confirmation

Use of LM= Increase in wild type *Pfcrt76/Pfmdr186*

****selection of CQ sensitive parasites**

Conclusion

❑ Isolates with decreased LM susceptibility emerging in vitro-selection of CQ sensitive parasites

➤ *Need for continuous monitoring of sensitivity/genetic markers*

❑ The role of other SNPs-genes-*Pfcr76/Pfmdr186* alone may not account for LM resistance

Related work ongoing:

-analysis of sequence data-role of other codons of *Pfcr76* and *Pfmdr1*

-Identification of novel **drug resistance gene candidates:**

-(Solexa sequencing/DNA microarrays)

Acknowledgements

Kemri-Wellcome Trust

Steven Muriithi

John Okombo

Abdi Diriye

Victor Masenno

Steffen Borrmann

Pete Bull

Jennifer Musyoki

Moses Mosobo

Whole of malaria culture lab

All the research participants

Sanger Insitute

Theresa Feltwell

Celine Carret

Chris Newbold

Dominic Kwaitotski

University Of Oxford

Prof Chris Newbold's Lab

Laura Alexander

University of Heidelberg

AG Borrmann Lab

Supervision

Dr Alexis Nzila

Prof Kevin Marsh

Funding

EDCTP

Kemri-Wellcome Trust Programme