Lumefantrine and Piperaquine: Mechanisms of Resistance

(EDCTP Funded PhD project)

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INTRODUCTION

Artemisinin Combination Therapy (ACT): *Current WHO recommendation*

1. Coartem (Artemether-Lumefantrine): first-line treatment in Kenya

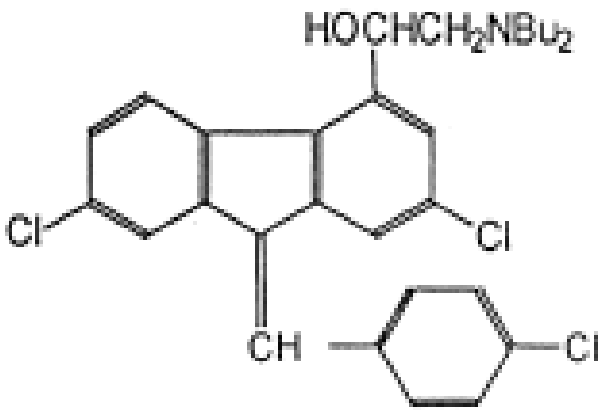
2. Artekin (Dihydroartemisinin-piperaquine)- undergoing clinical trials in Kenya

1. Coartem (Artemether-Lumefantrine):first-line treatment in Kenya

Safe and highly efficacious in the treatment of uncomplicated malaria

Falade et al,2005,Koram et al, 2005,Guthman et al, 2006

Lumefantrine(Benflumetol)



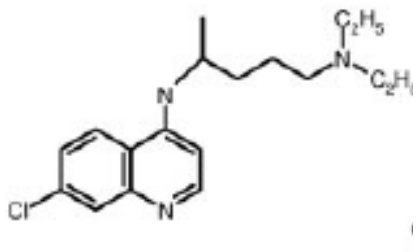
- Evidence that resistance to Lumefantrine (LM) could be selected rapidly..

Important to understand the mechanism of resistance to LM

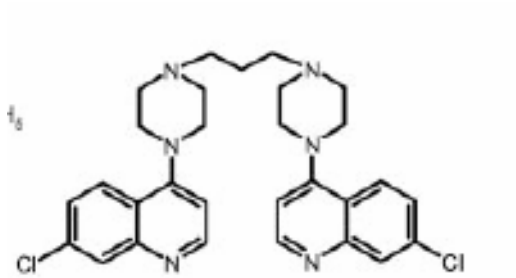
2. Artekin (Dihydroartemisinin-piperaquine) - Used in China, Vietnam

- ✓ Efficacious in treatment of multidrug resistant *P.falciparum* malaria
- ✓ Cheap and easy to administer
- ✓ **Potential alternative to Coartem (phase III clinical trials in Kenya)**

Piperaquine (PQ)



chloroquine



piperaquine

➢Used extensively as monotherapy in China, resistance emerged rapidly..

Important to understand the mechanism of resistance to PQ

THE PROJECT

Understanding the mechanisms of Piperaquine (PQ) and Lumefantrine (LM) resistance in *P. falciparum*

Main Goal

Identification of Molecular markers to monitor PQ / LM resistance

The Team

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Dr .Celine Caret, Sanger Institute, UK

HYPOTHESIS

- Mismatched pharmacokinetics between artemisinins and PQ / LM

→ Strong selective pressure for resistance

- PQ is Bisnoquinoline/LM is an amino-alcohol

→ Impact of CQ molecular markers on PQ / LM resistance?

Use of CQ resistance as framework to study LM / PQ resistance

OBJECTIVES OF THE PROJECT

- Assessment of PQ and LM activity in Kilifi over the years of this project
  - *Important to establish the baseline activity of Lumefantrine and Piperaquine as Coartem/Artekin go into widespread use*
- Assessment of selective pressure for resistance
  - *Is selective pressure for resistance to Coartem / Artekin exerted by LM/PQ*
- Search for molecular markers which can be used to predict Artekin / Coartem efficacy
- Assess the effect of artemisinin derivatives on auditory function in children

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CLINICAL TRIAL (IN PROGRESS)

Efficacy of Artekin (PQ/DHA) versus Co-artem (LM/ART) in Pingilikani , Kilifi -2006

223 children

6-59 months age

≥ 5 kg

Artekin (PQ/DHA) – n = 149

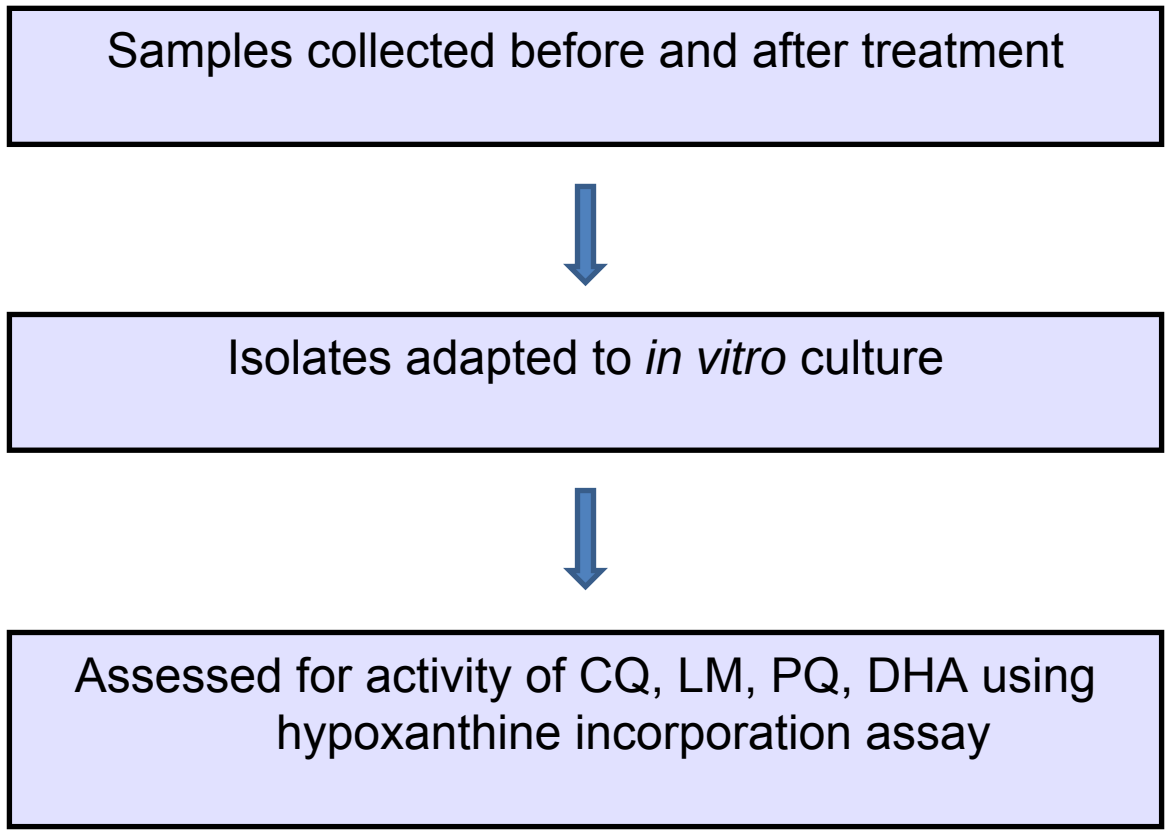
- **25** parasitological failures (17%) at **day 42** of treatment

Coartem (LM/ART) – n = 74

- **20** parasitological failures (27%) at **day 42** of treatment

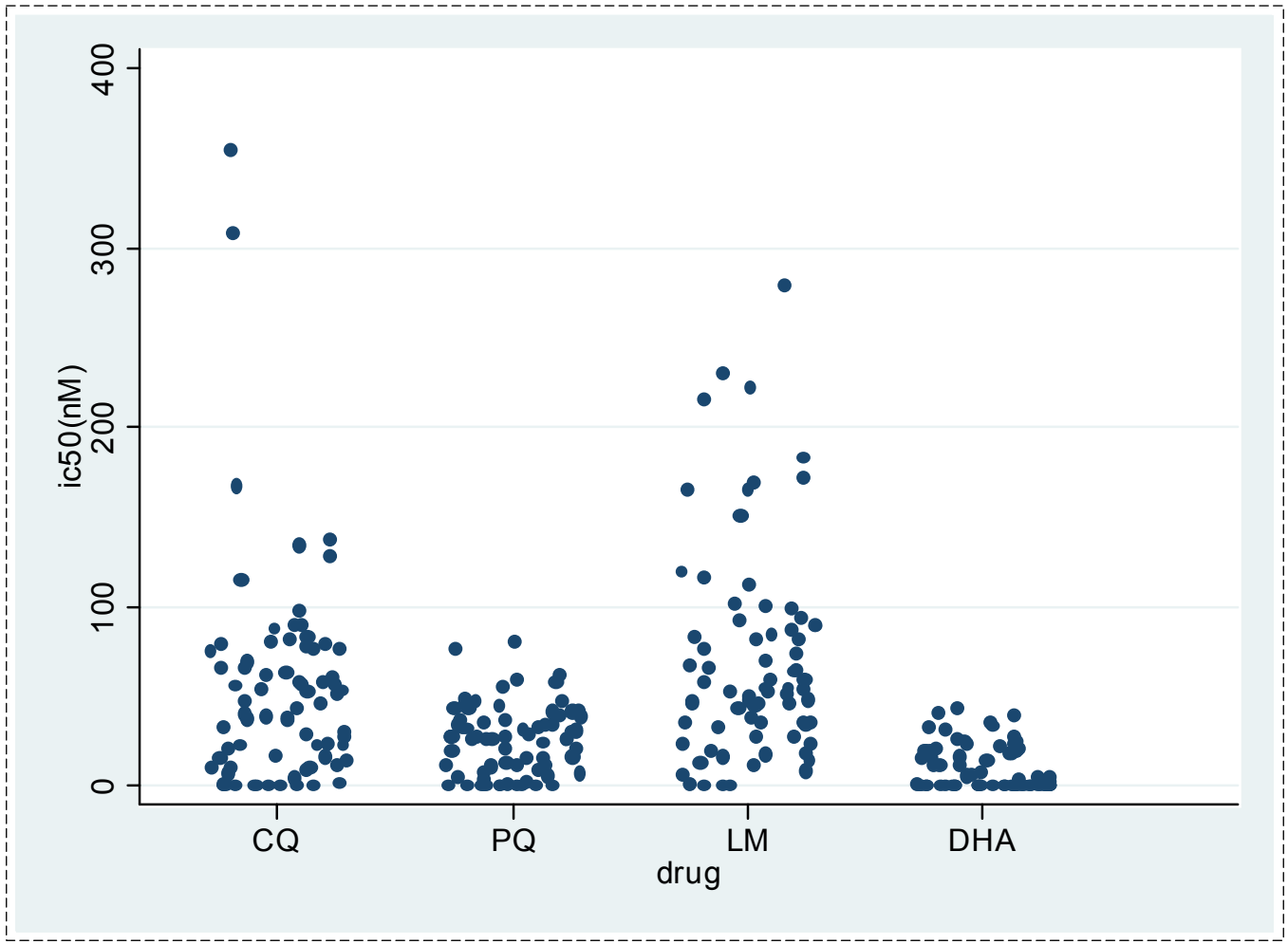
*Rates not PCR corrected

METHODS


Samples collected before and after treatment
Isolates adapted to *in vitro* culture
Assessed for activity of CQ, LM, PQ, DHA using hypoxanthine incorporation assay

Distribution of IC50(nM) for CQ, PQ, LM,and DHA

(n=73)



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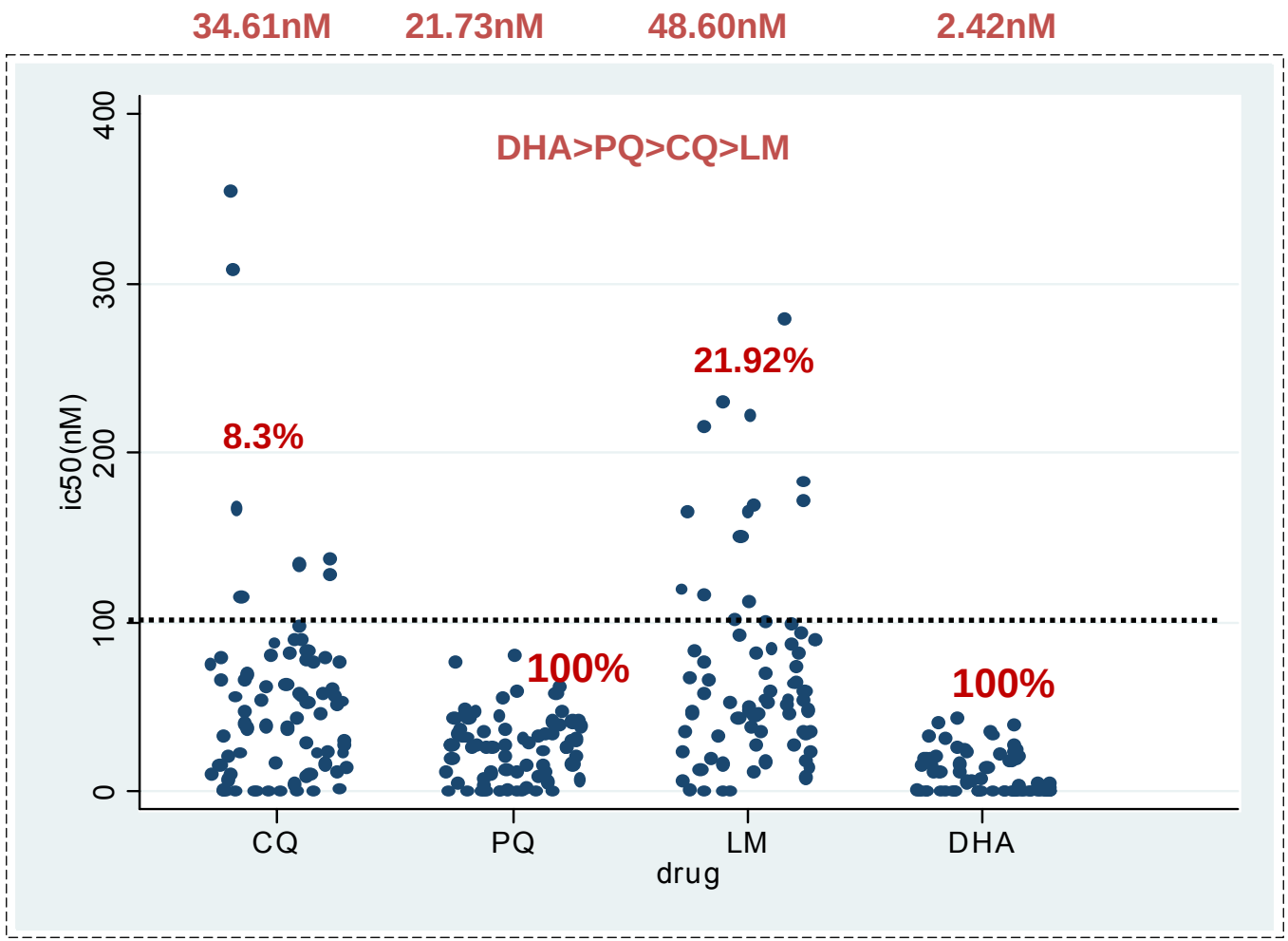

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Assessment of LM and PQ Selective pressure

Drug	Mean Ic50(nM) (Day 0) N=45	Mean Ic50(nM) *(Recurrent) N=28	*p value* (k wallis)
Lumefantrine (LM)	54.33	90.32	*p=0.0051*
Chloroquine	58.52	38.99	*p=0.0074*
Piperaquine (PQ)	28.03	25.98	*p=0.376*

✓Mean IC50 of LM significantly higher for recurrent parasites-selective pressure for resistance

Similar results reported by Sisowath et al,2005, Dokomajilar et al,2006

✓Mean IC50 of CQ significantly lower in these recurrent parasites

Negative correlation between CQ and other amino alcohols reported by Barnes et al,1992,Cowman et al,1994

* Data on recurrent parasites has been analyzed irrespective of drug allocation

Chloroquine Activity in Kilifi

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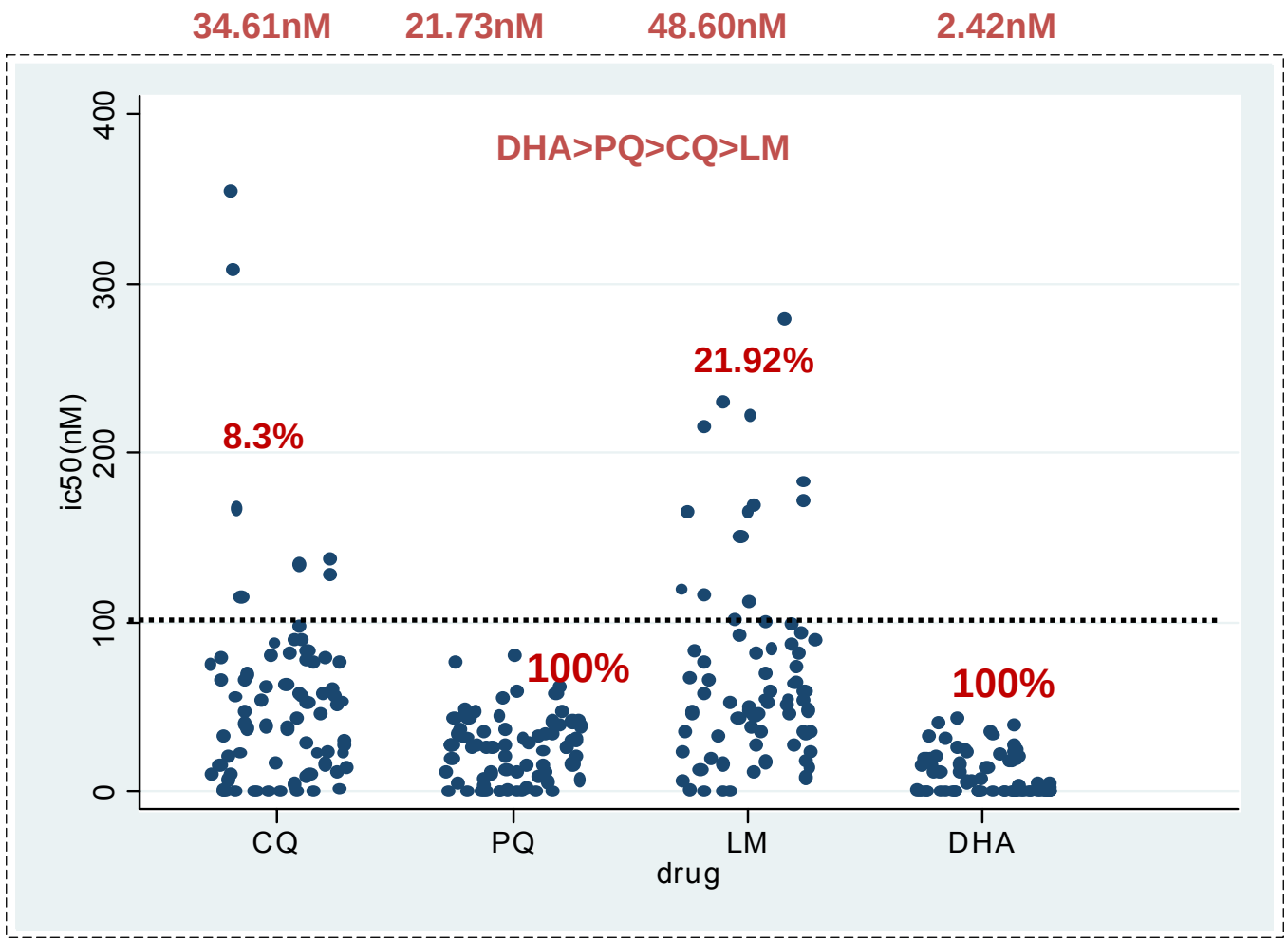
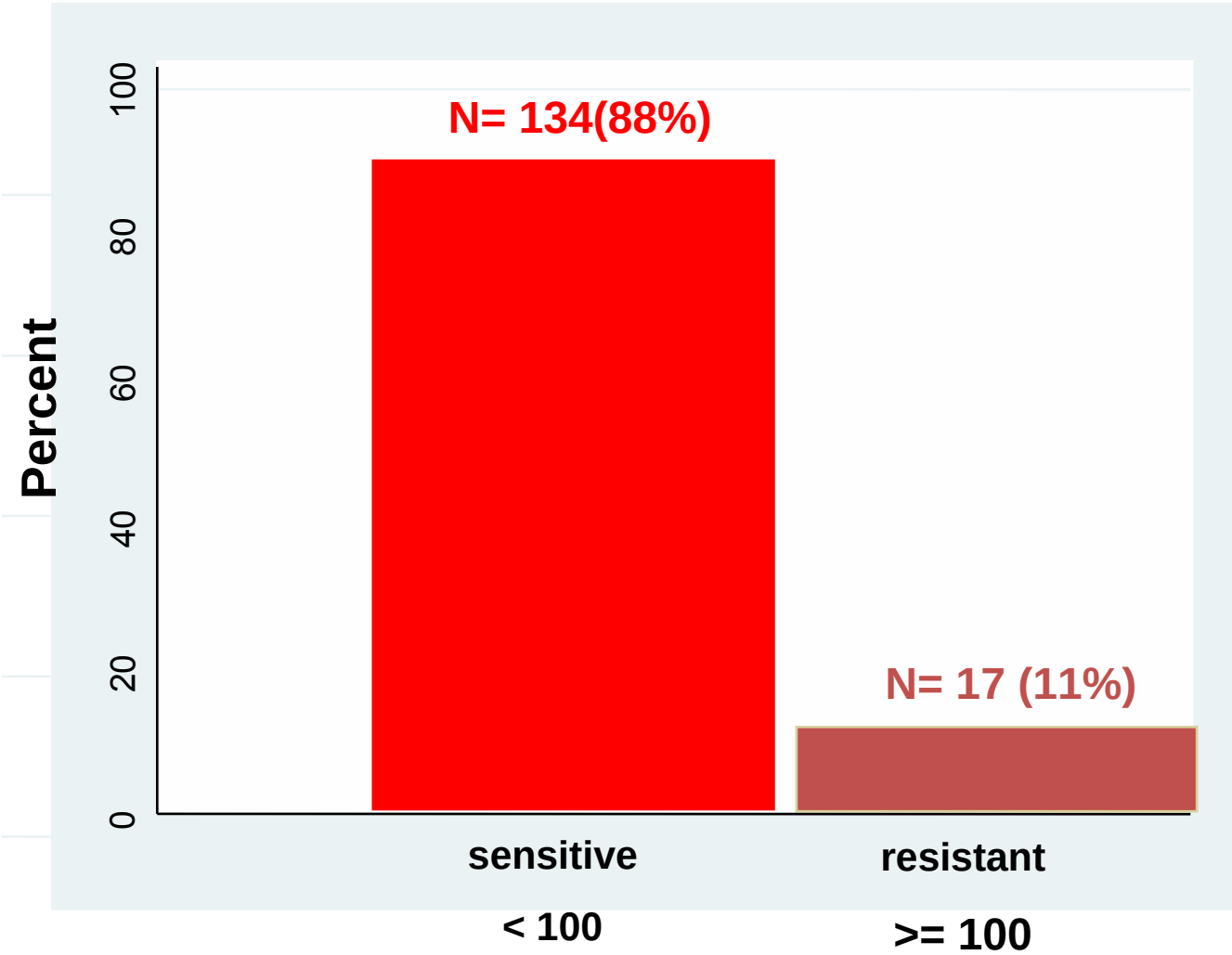
(n= 73)

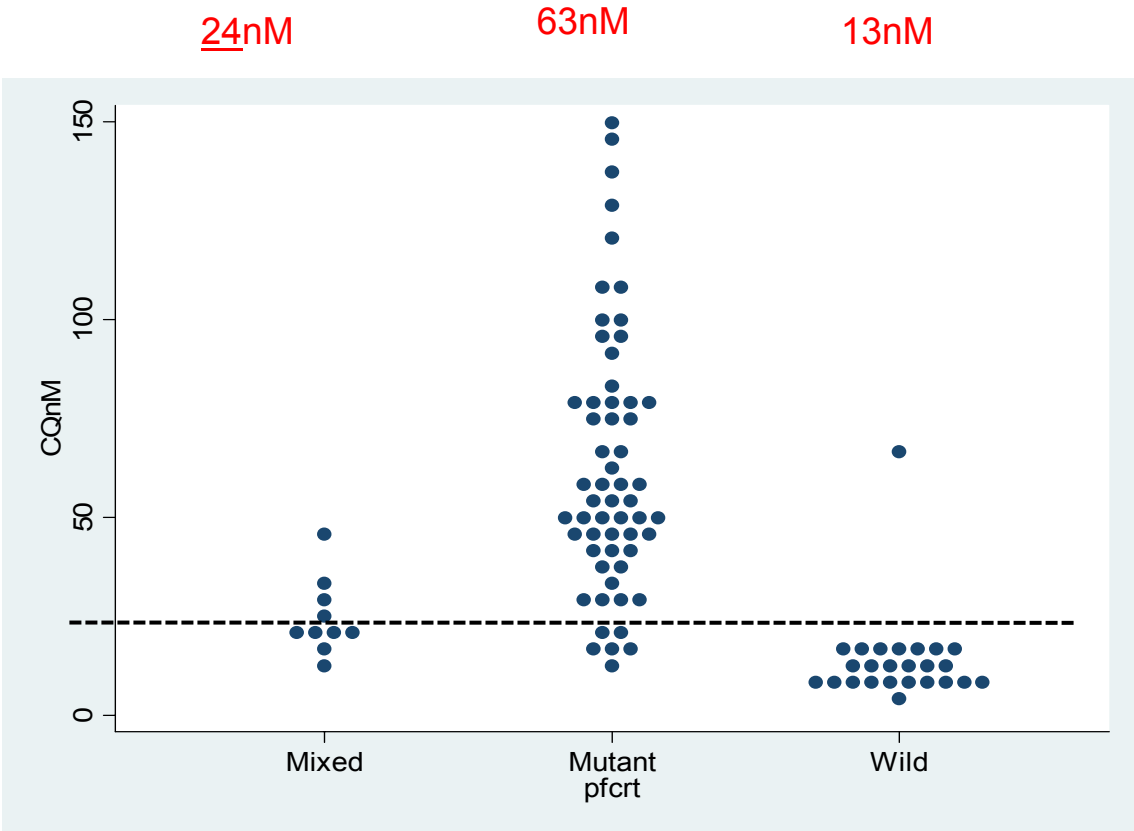


Chloroquine sensitivity in Kilifi isolates using 100nM cut off point

N= 151



Relationship between Chloroquine IC50 and pfcrt -76 genotype in Kilifi



Cut off point of 25nM appropriate for Kilifi…

Need for a cut-off point tailored for each site has been proposed ,Durand R, 2001

Chloroquine resistance before and after its withdrawal in Kenya

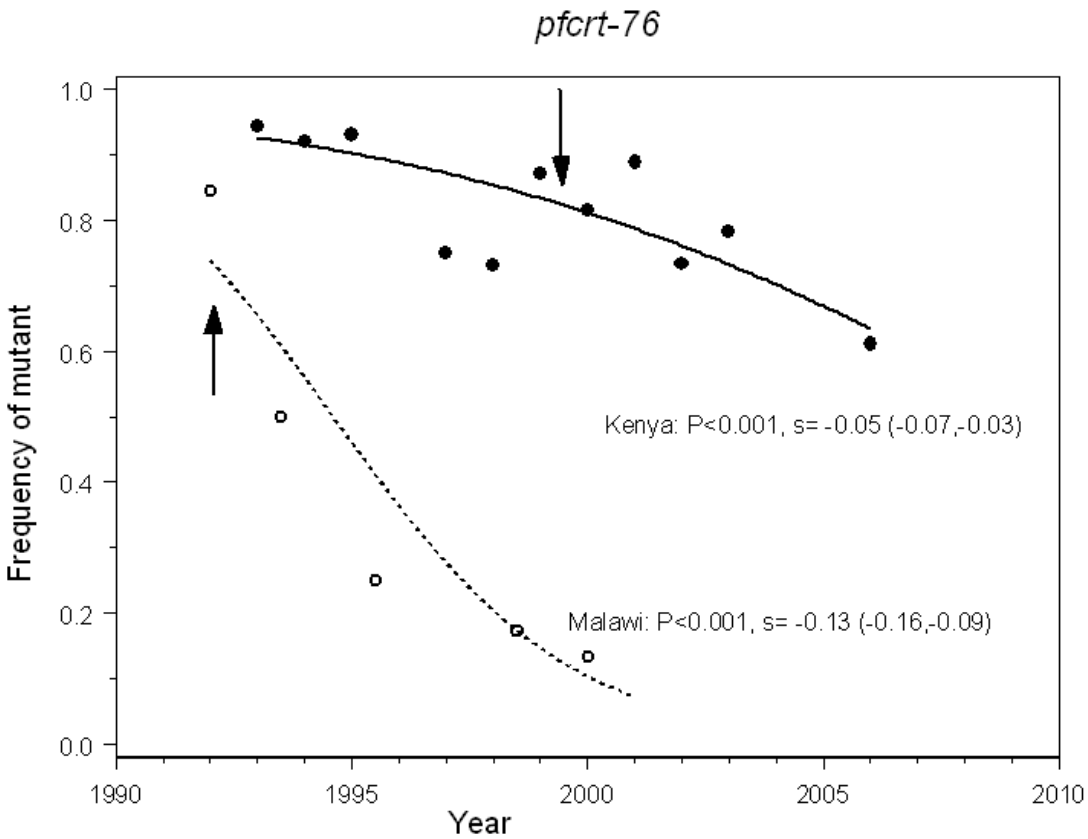
1998: change of policy from CQ to SP as treatment of choice in Kenya

As part of previous and current work

✓Analysed frequency of chloroquine resistant genotypes in Kilifi over years (1993 to 2006)

✓Compared this with data observed in Malawi over the same period

Observed population frequencies of mutant pfcrt-76 over time in Kenya and Malawi



Reversal of chloroquine resistance observed in Kilifi but at a slower pace than that reported in Malawi

Mwai et al, 2007 Chloroquine resistance before and after its withdrawal in Kenya: a study in Kilifi, an area of high transmission-submitted for publication

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Search for molecular markers of LM and PQ resistance

Need to establish isolates that are resistant to LM/PQ

1. Assessing the in vitro activity of LM/PQ in Kenyan isolates

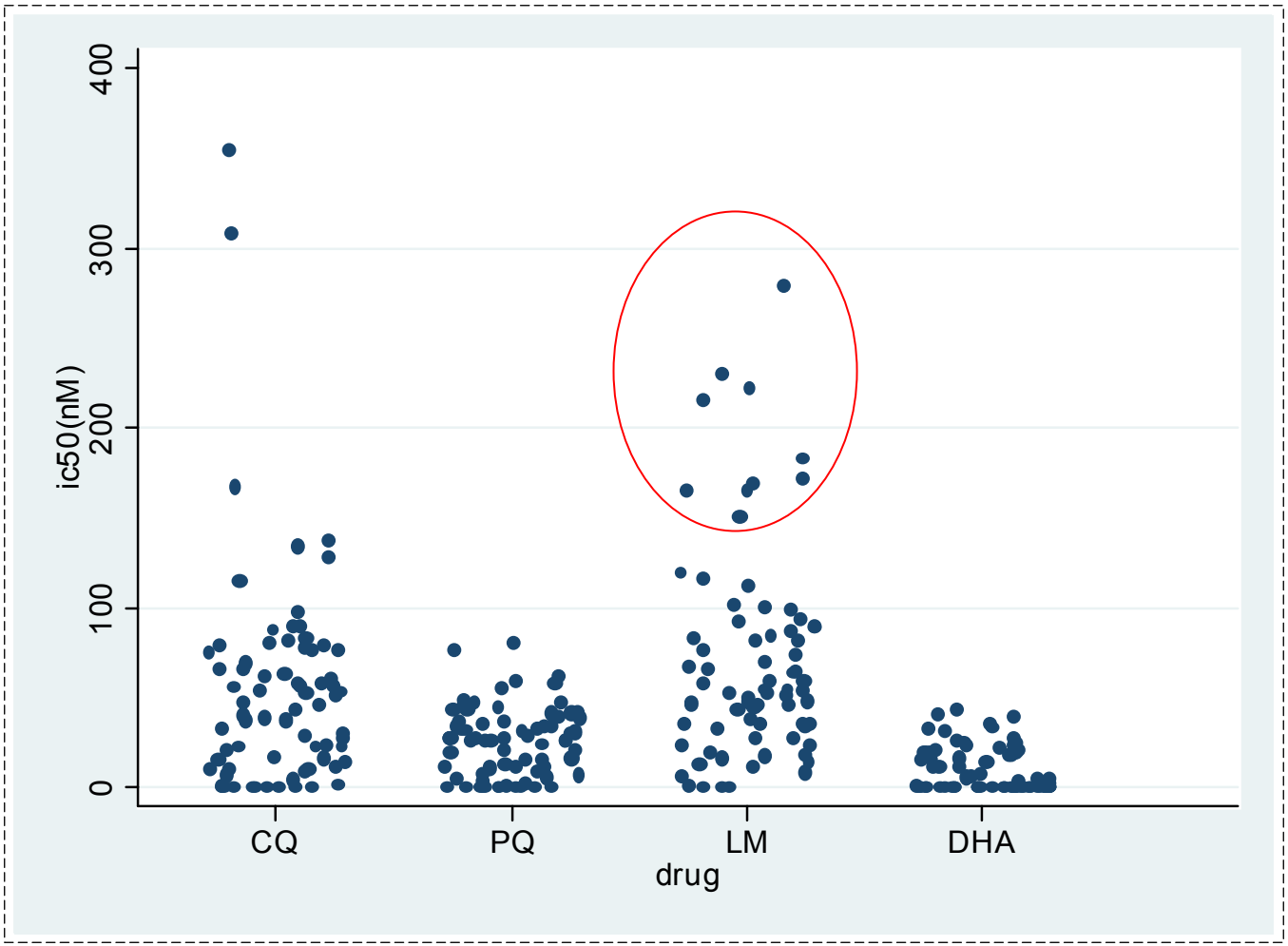
   - *identify isolates that have decreased susceptibility to LM / PQ*

2. Selection of resistance to LM/PQ in vitro by continuous culture in PQ/LM

   - *V1/S multidrug resistant reference isolate used*

3. Selection of resistance *in vivo* using an animal model-In collaboration with KEMRI Nairobi

Search for LM Markers in Isolates with reduced sensitivity to Lumefantrine



Important to characterize isolates with reduced sensitivity to Lumefantrine…

Work in progress

1. *Pfmdr1* copy number analysis

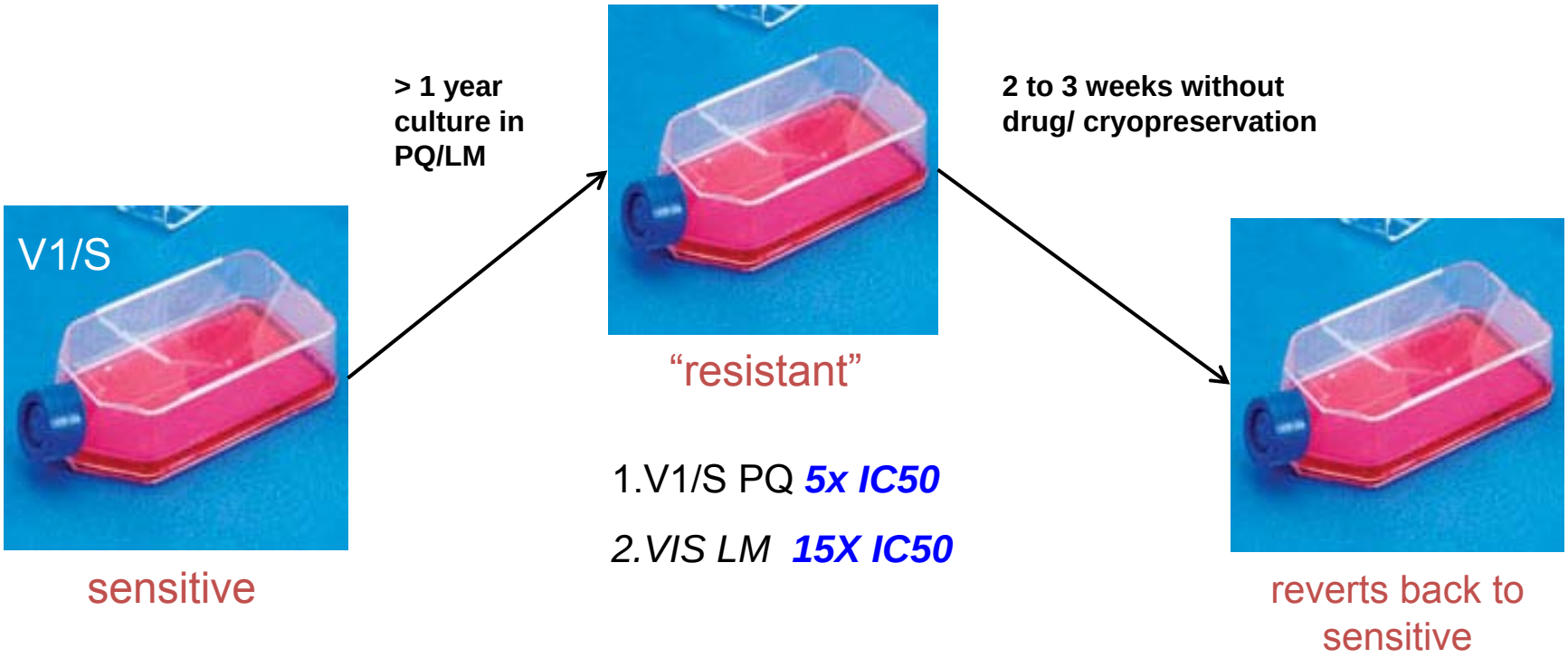
2. Sequencing of genes associated with quinoline resistance

- *Pfmdr1* (codons 86, 184, 1034, 1042, 1246) and *Pfcrt* Domain 1, 4 and 5 , *pfnhe* in collaboration with the Hygiene Institute, University of Heidelberg

3. Continued adaptation of field isolates *in vitro*, drug assays and assessment of selective pressure

Selection of LM/PQ Resistance *In Vitro*

Use of V1/S (multidrug resistant reference isolate)



Transient resistance: gene amplification/protein over expression

Work in progress

1. Sampling of V1/S LM and V1/S PQ for whole genome analysis / expression profiling using DNA microarrays-In collaboration with Sanger Institute

2. Continued selection of resistance *in vitro / in vivo*

3. Characterizing isolates with reduced sensitivity to LM

Summary

- Chloroquine and Piperaquine active against Kenyan Field isolates
  - *some isolates have reduced sensitivity to Lumefantrine - Need to characterize these isolates.*
- *Reversal of Chloroquine resistance in Kilifi but slower pace than in Malawi*
- Isolates with transcient resistance to Piperaquine / Lumefantrine obtained
  - *sampling for microarray analysis ongoing*

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Abdi Diriye
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