

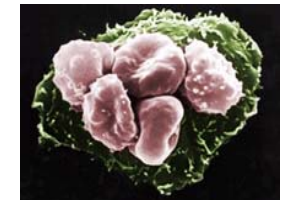
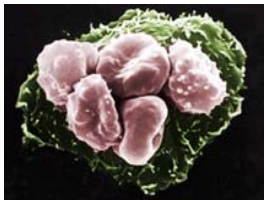


MALARIA:

Current Issues in Drug Treatment and Resistance and the Discovery of Resistance Determinants

David A. Fidock, Ph.D.

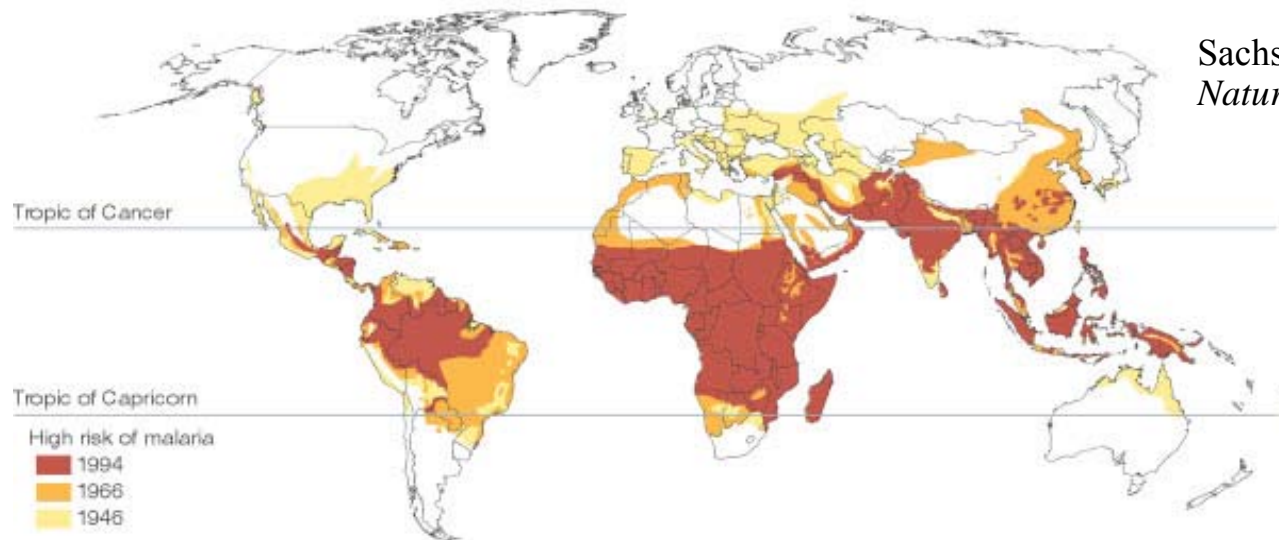
**Depts. of Microbiology and of Medicine
Division of Infectious Diseases
Columbia University Medical Center**



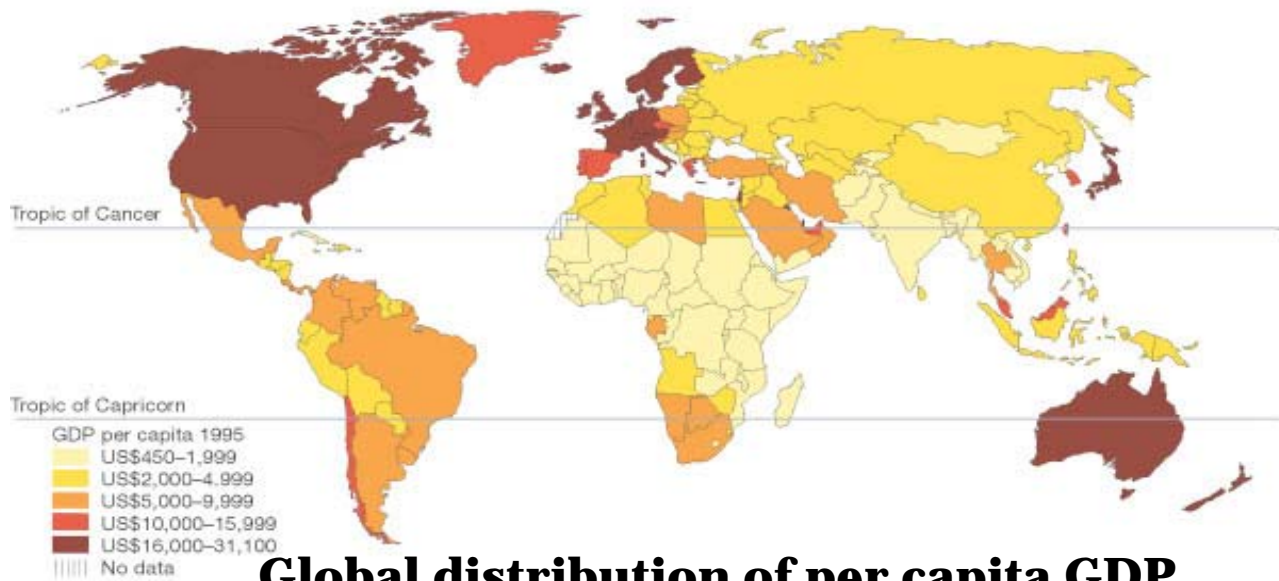
MALARIA

- 300 - 500 million cases annually
- 1.5 - 3 million deaths yearly
90% are African children
40% world's population at risk
- Caused by *Plasmodium falciparum*, *P. vivax*, *P. ovale* or *P. malariae*, transmitted by *Anopheles*
- *P. falciparum* - vast majority of malarial deaths (anemia, cerebral malaria)

Sachs & Malaney, 2002
Nature 415: 680



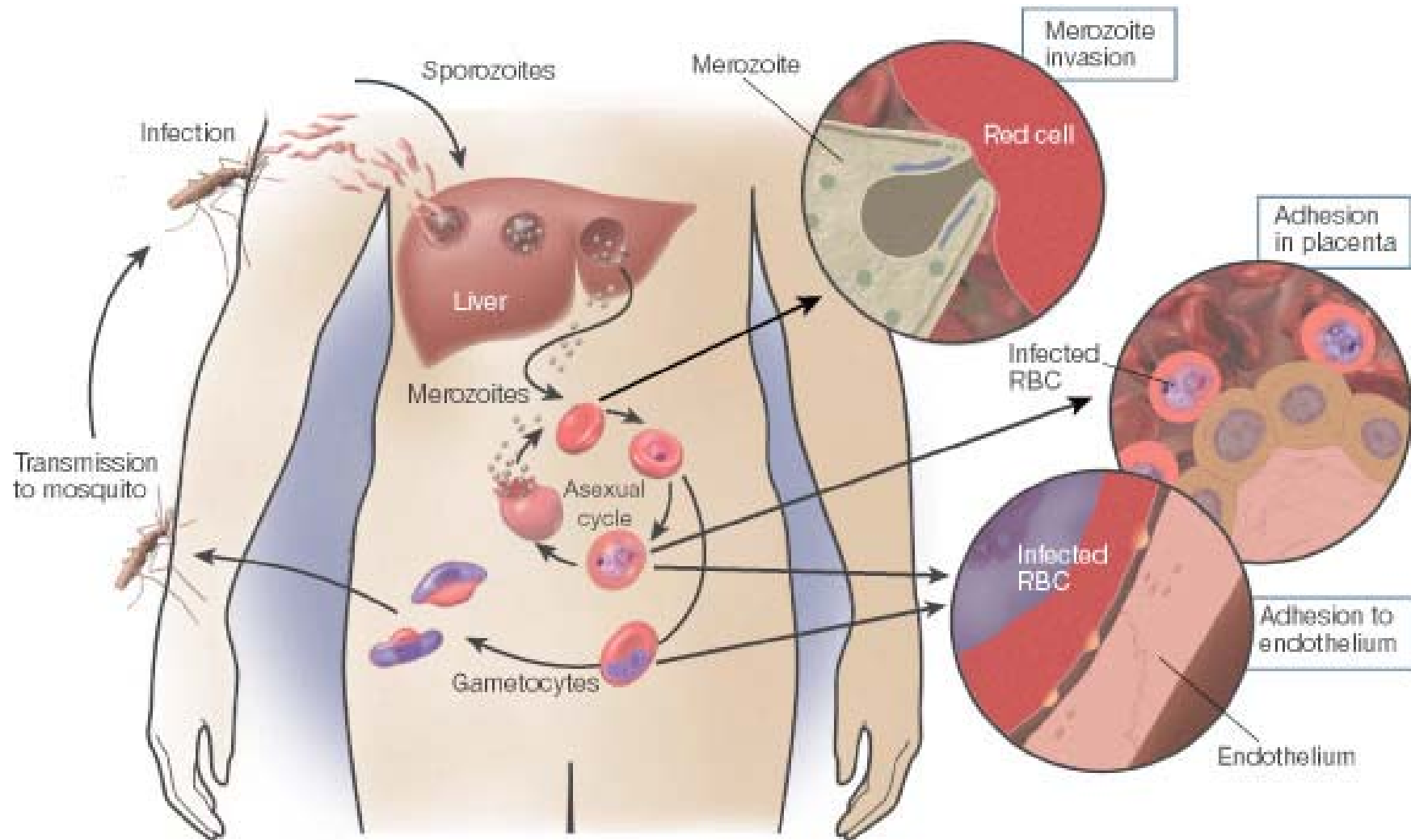
Global distribution of malaria



Global distribution of per capita GDP

**MALARIA - DISEASE OF THE POOR.
COMPOUNDED BY SPREAD OF MULTIDRUG RESISTANCE**

Parasite life cycle and pathogenesis of falciparum malaria



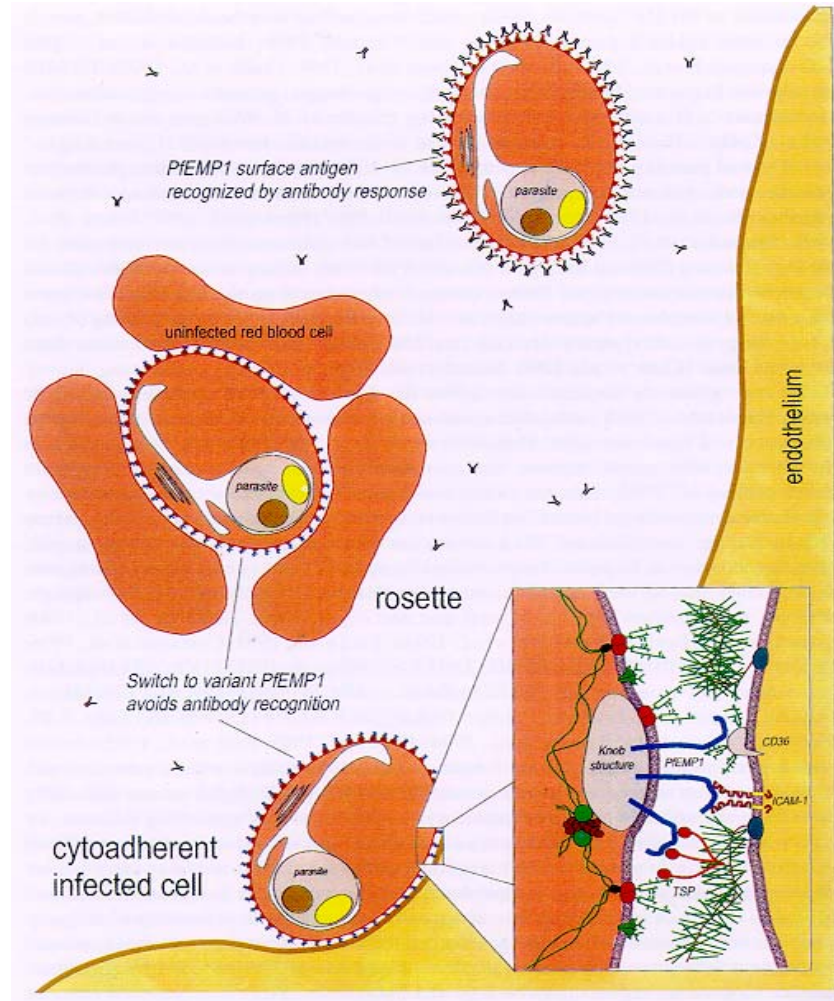
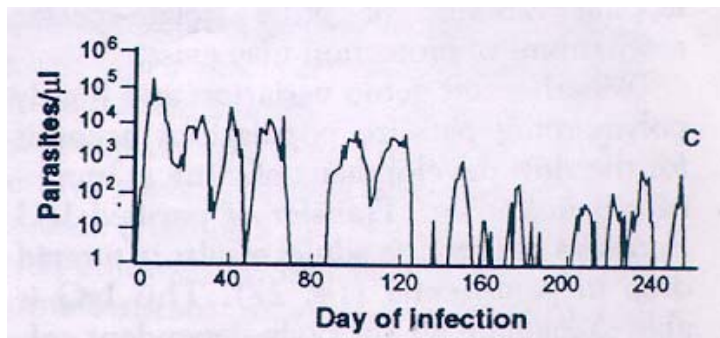
Molecular basis of *P. falciparum* pathogenesis: a primary role for PfEMP1

PfEMP1 exported to erythrocyte surface, encoded by *var* gene family

PfEMP1 mediates cytoadherence, rosetting, sequestration

Target of strain-specific immunity

Causes waves of parasitemia, coincident with antigen switching



Infected RBC cytoadherence and merozoite release

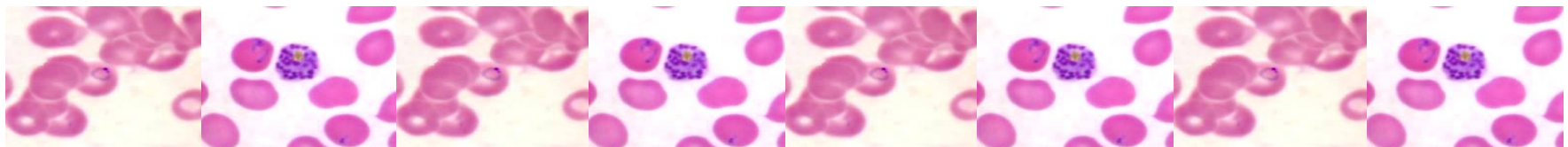
QuickTime™ and a
Sorenson Video decompressor
are needed to see this picture.

Clinical features and possible mechanisms of disease

Syndromes	Clinical features	Disease mechanisms
Severe anemia	Shock; impaired consciousness; respiratory distress	Reduced RBC production (reduced erythropoietin activity, proinflammatory cytokines); increased RBC destruction (parasite-mediated, erythrophagocytosis, antibody and complement-mediated lysis)
Cerebral malaria	Impaired consciousness; convulsions; long-term neurological deficits	Microvascular obstruction (parasites, platelets, rosettes); proinflammatory cytokines; parasite toxins
Metabolic acidosis	Respiratory distress, hypoxia, tachypnea; acidemia; reduced central venous pressure	Reduced tissue perfusion (hypovolemia, reduced cardiac output, anemia); parasite products; pro-inflammatory cytokines; pulmonary pathology (airway obstruction, reduced diffusion)
Other	Hypoglycemia; disseminated intravascular coagulation	Parasite products and/or toxins; proinflammatory cytokines; cytoadherence
Malaria in pregnancy	Placental infection; low birth weight and fetal loss; maternal anemia	Premature delivery and fetal growth restriction; placental mononuclear cell infiltrates and inflammation; proinflammatory cytokines

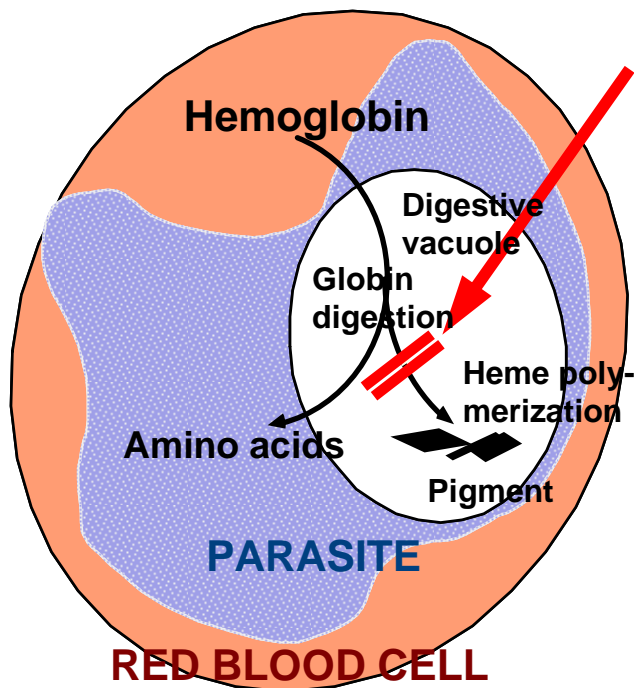
Why is malaria rampant?

- Inter-tropical regions favorable for transmission (Africa: up to 300 infectious bites per year per individual)
- Immunity partial, takes years, labile, dependent on infection
- Vaccines: elusive (only irradiated sporozoites protect)
- **Chemotherapy** - principal axis for malaria control.
Compromised by global spreading of resistance to first-line antimalarials. Multidrug resistance rampant in SE Asia.



The demise of chloroquine

- Introduced 1940s, cornerstone of Malaria Eradication Program
- Gold standard for treatment of uncomplicated malaria
- Yearly consumption peaked at 1,300 tons (900 million doses)
- Safe, rapidly effective (3 day treatment), affordable
- Resistance arose within a decade. Affordable replacements rapidly failing

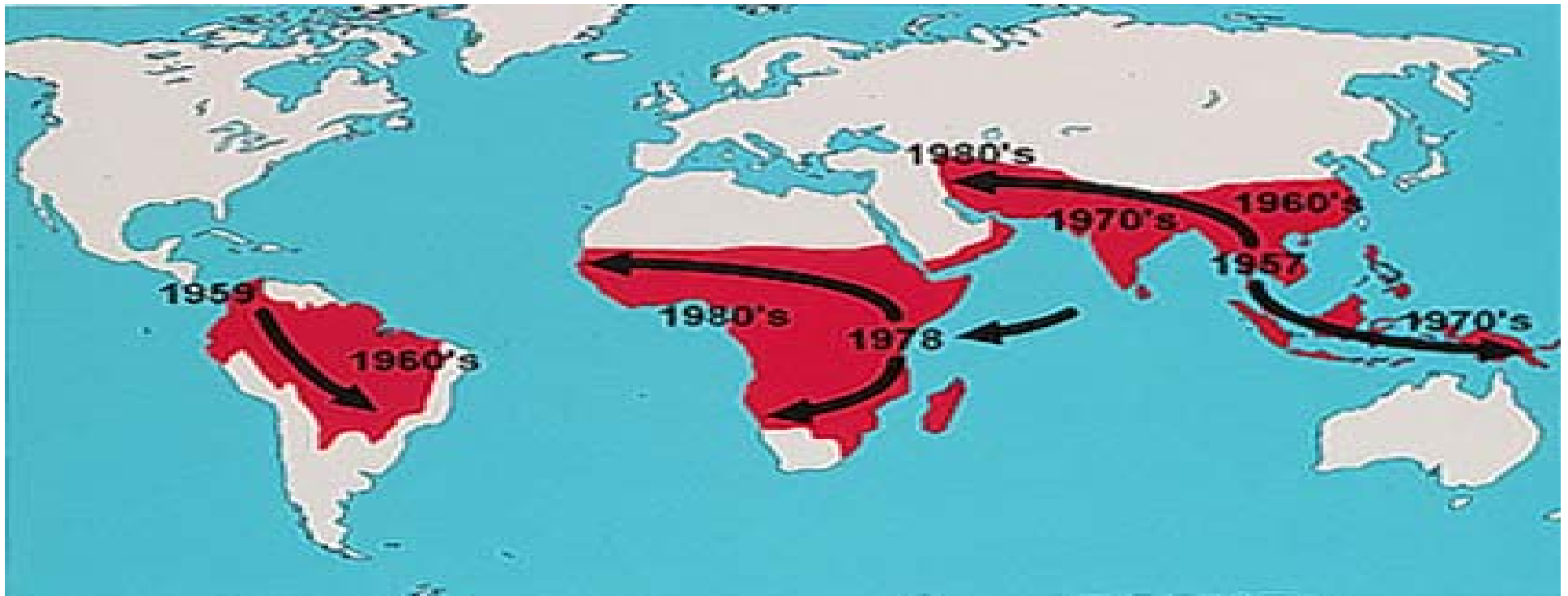


Weak base 4-aminoquinoline

Concentrates in digestive vacuole

Binds toxic heme moieties, causes membrane lysis, cell death

The search for the genetic basis of chloroquine resistance (CQR)



CQR - spread under massive drug pressure
Major cause of increased malaria mortality
and morbidity in sub-Saharan Africa

Mapping the CQR determinant in a *P. falciparum* genetic cross

CQ-sensitive clone



CQ-resistant clone



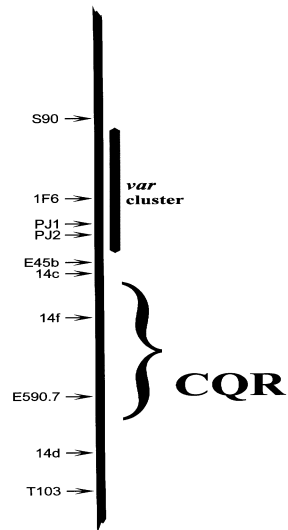
Mosquitoes

Chimpanzee

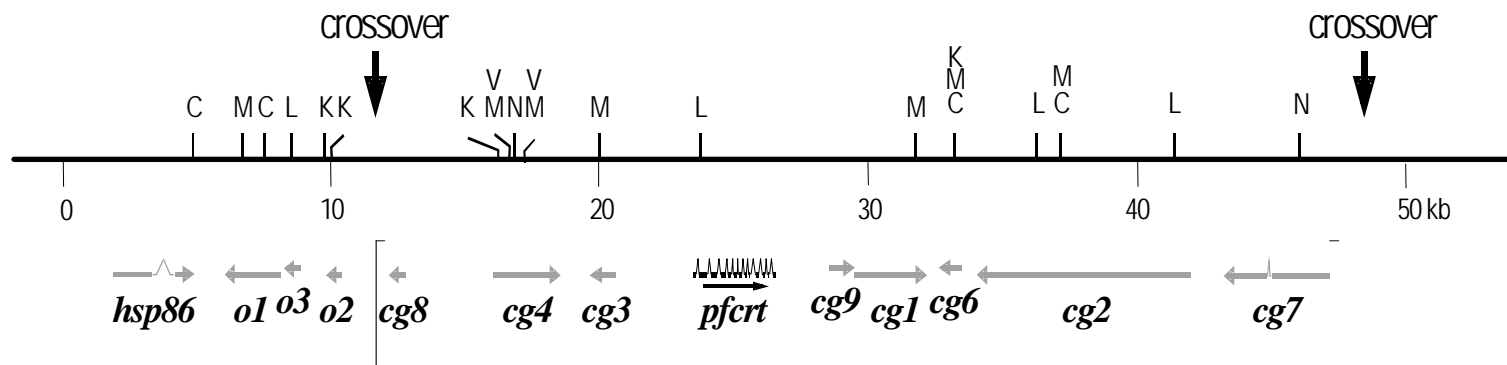
Clone independent progeny and determine drug responses

Map genetic locus

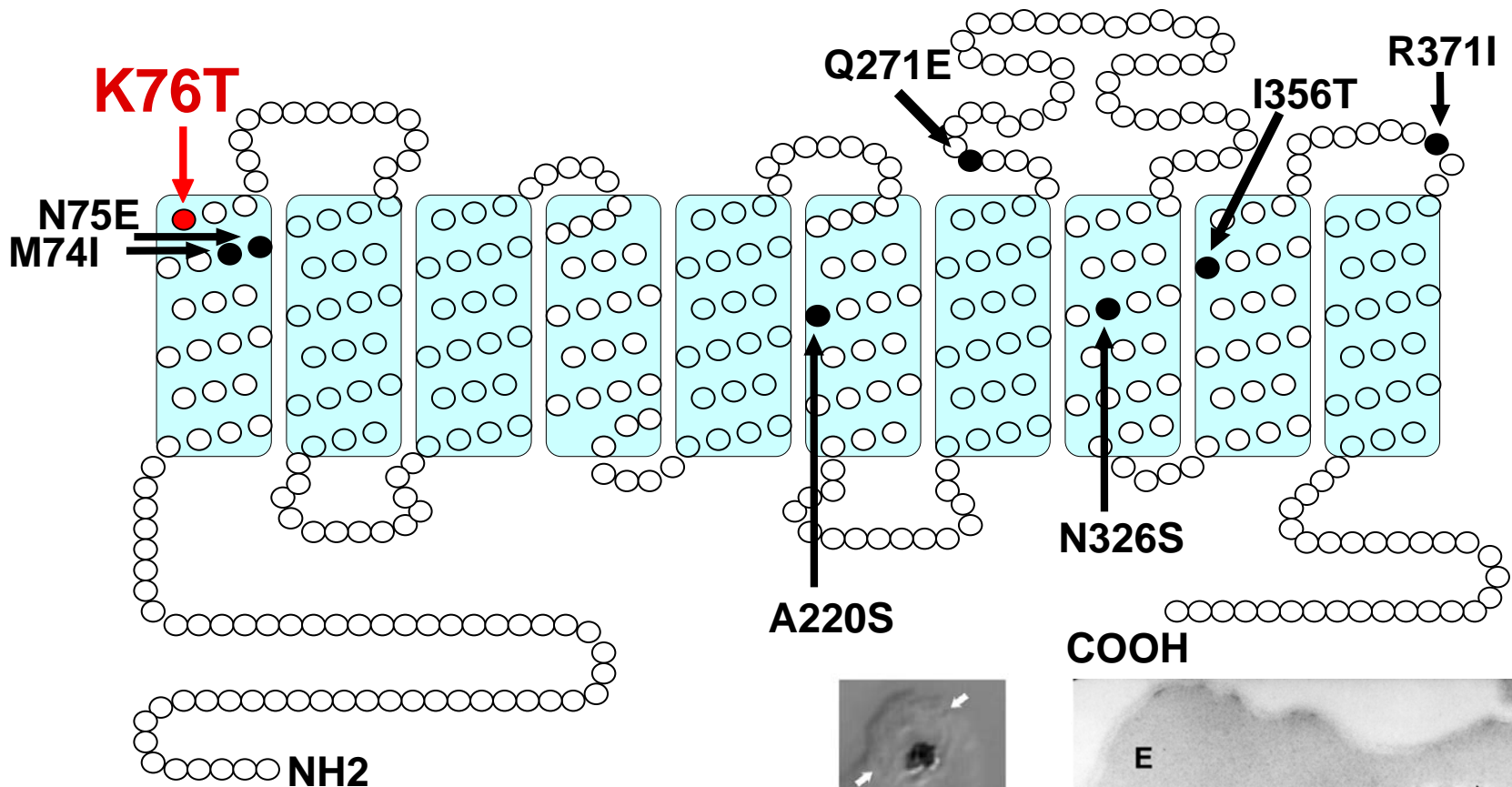
Identify gene(s)



Genetic mapping of the resistance determinant



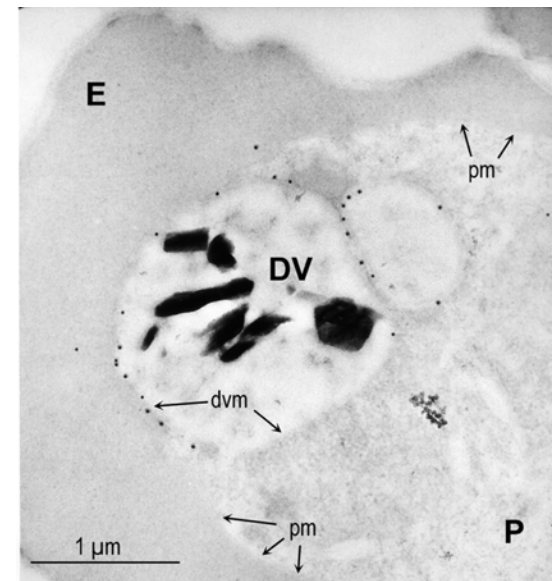
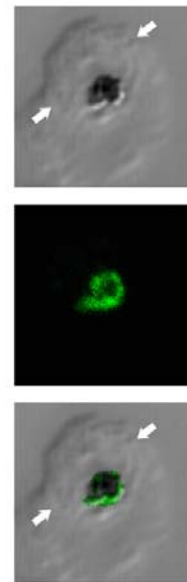
cg2: first candidate, no change in CQ response following allelic exchange...



PfCRT

8 mutations

10 transmembrane domains
On membrane of digestive vacuole (site of CQ action)

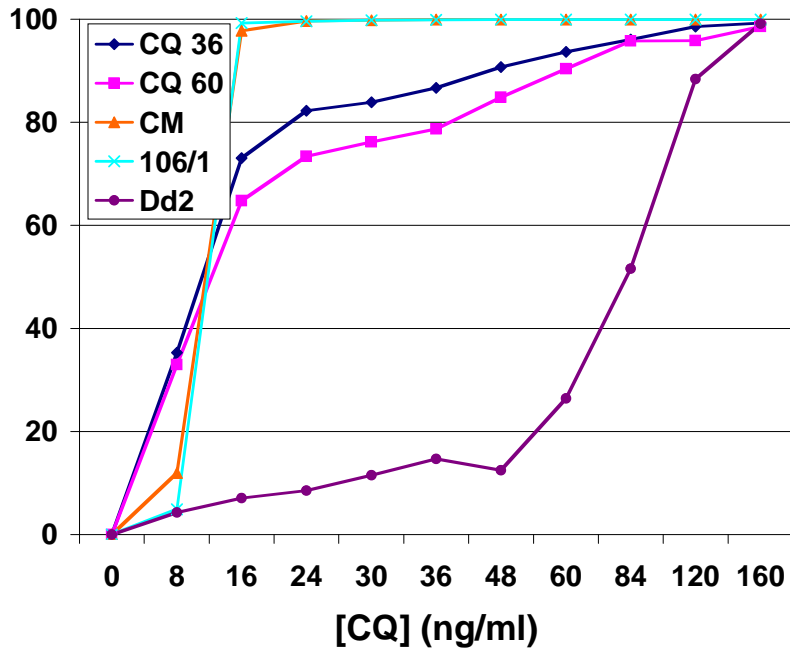


pfcrt mutations associated with CQR

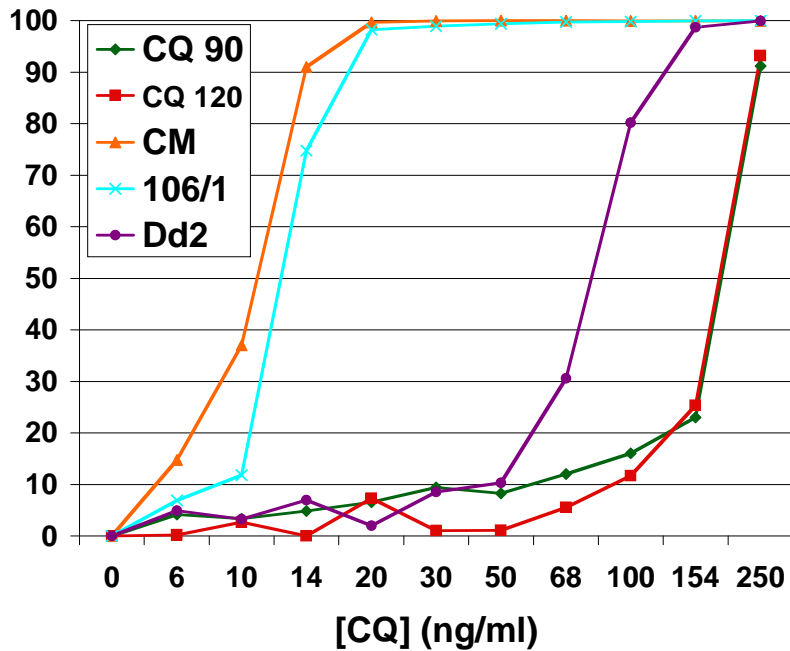
PfCRT position & encoded amino acid

Parasite type & origin	72	74	75	76	97	220	271	326	356	371
<u>Chloroquine sensitive</u>										
“wild type”	C	M	N	K	H	A	Q	N	I	R
106/1 (revertant?)	C	I	E	K	H	S	E	S	I	I
<u>Chloroquine resistant</u>										
SE Asia & Africa E1a	C	I	E	T	H	S	E	S	T	I
SE Asia & Africa E1b	C	I	E	T	H	S	E	S	I	I
Papua New Guinea	S	M	N	T	H	S	Q	D	L	R
South America W1a	S	M	N	T	H	S	Q	D	L	R
South America W1b	C	M	N	T	H	S	Q	D	L	R
South America W2	C	M	E	T	Q	S	Q	N	I	T

Are these mutations responsible for CQR?

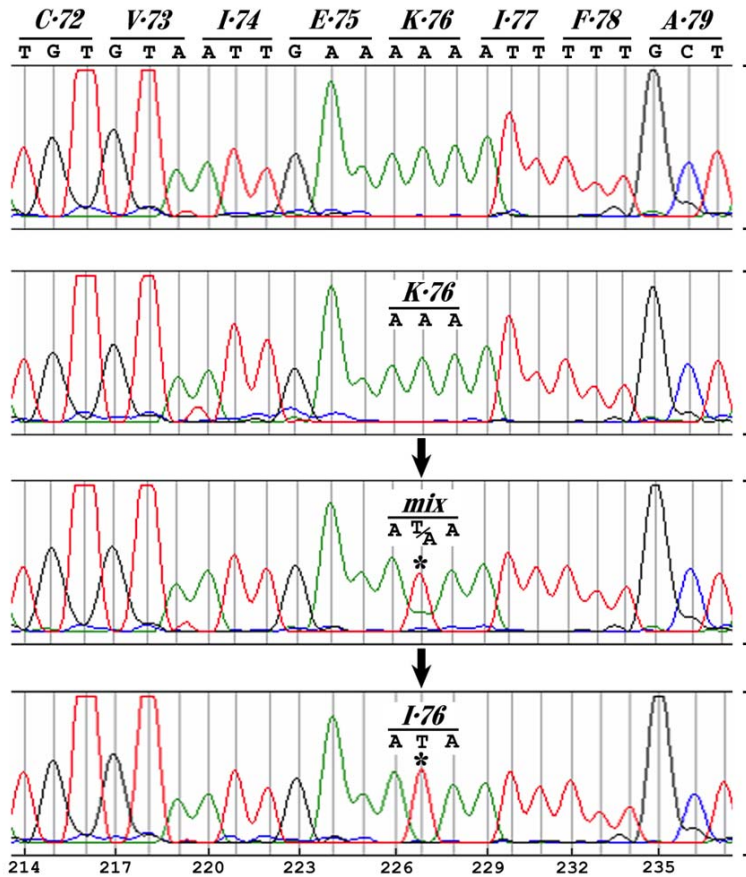


Day 73: slight shift in CQ-pressured lines co-expressing mutant *pfCRT*.



Day 108: highly resistant line appeared. Southern, PCR: mutant *pfCRT* plasmid absent.

Selection of a CQ-resistant line mutated at *pfprt* position 76

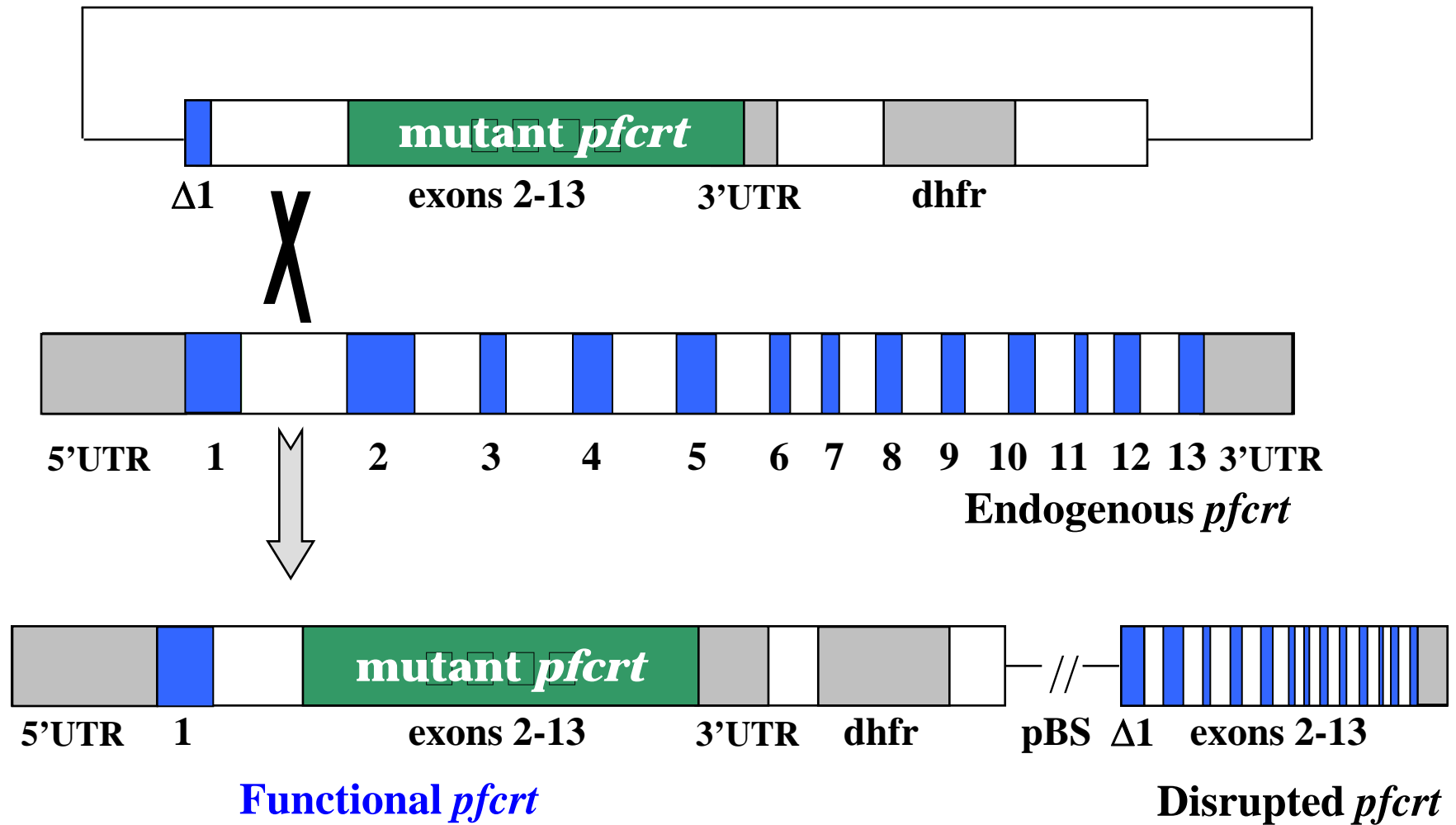


K76I mutation occurred, confirms linkage analysis assignment of critical role of codon 76.

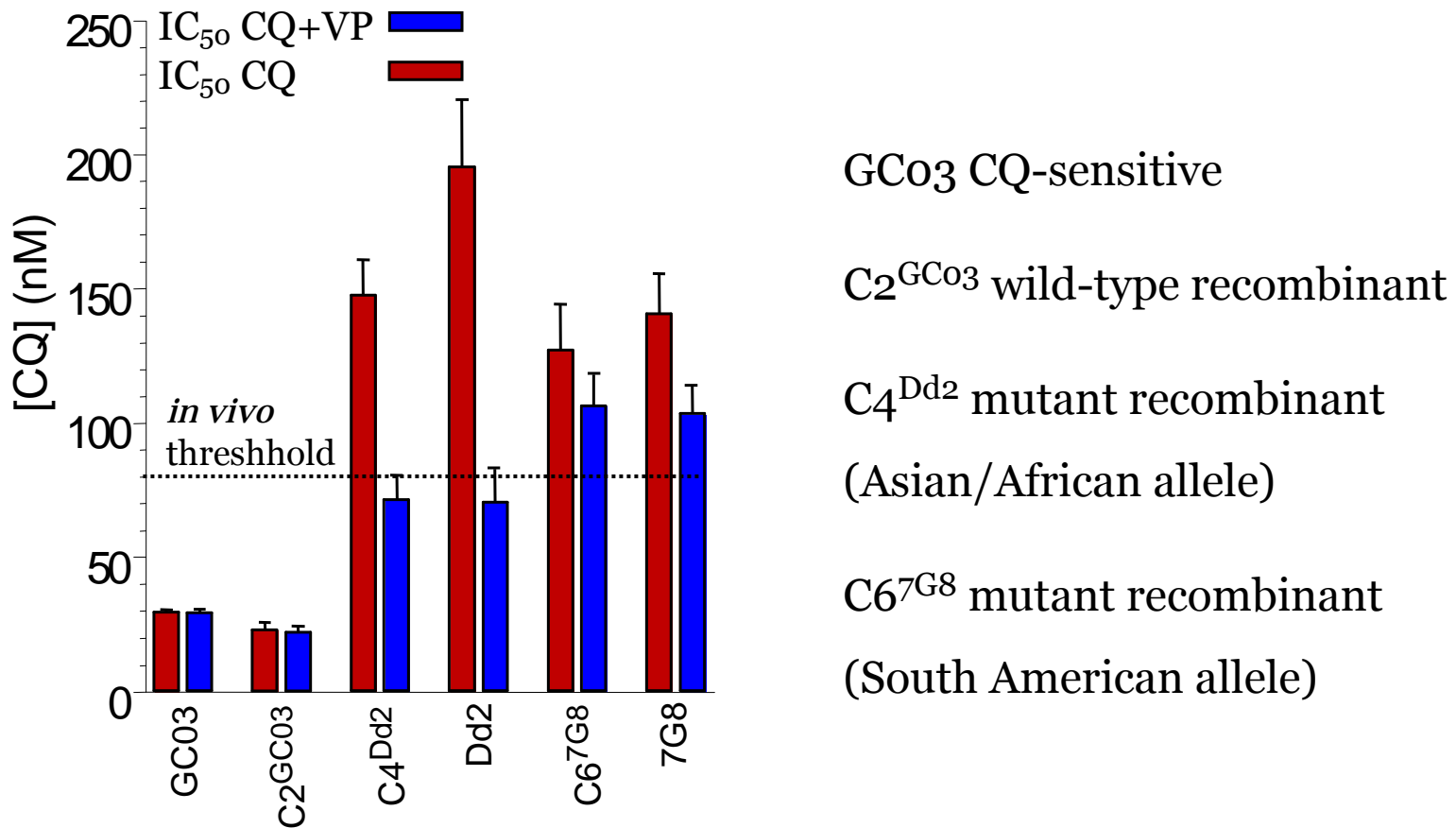
106/1 may have been *in vitro* revertant; CQ pressure may have selected for multiple changes.

Are *pfprt* mutations sufficient to confer CQR?

Allelic exchange strategy to replace wild-type with mutant *pfcr*

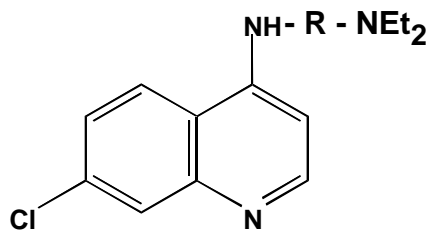
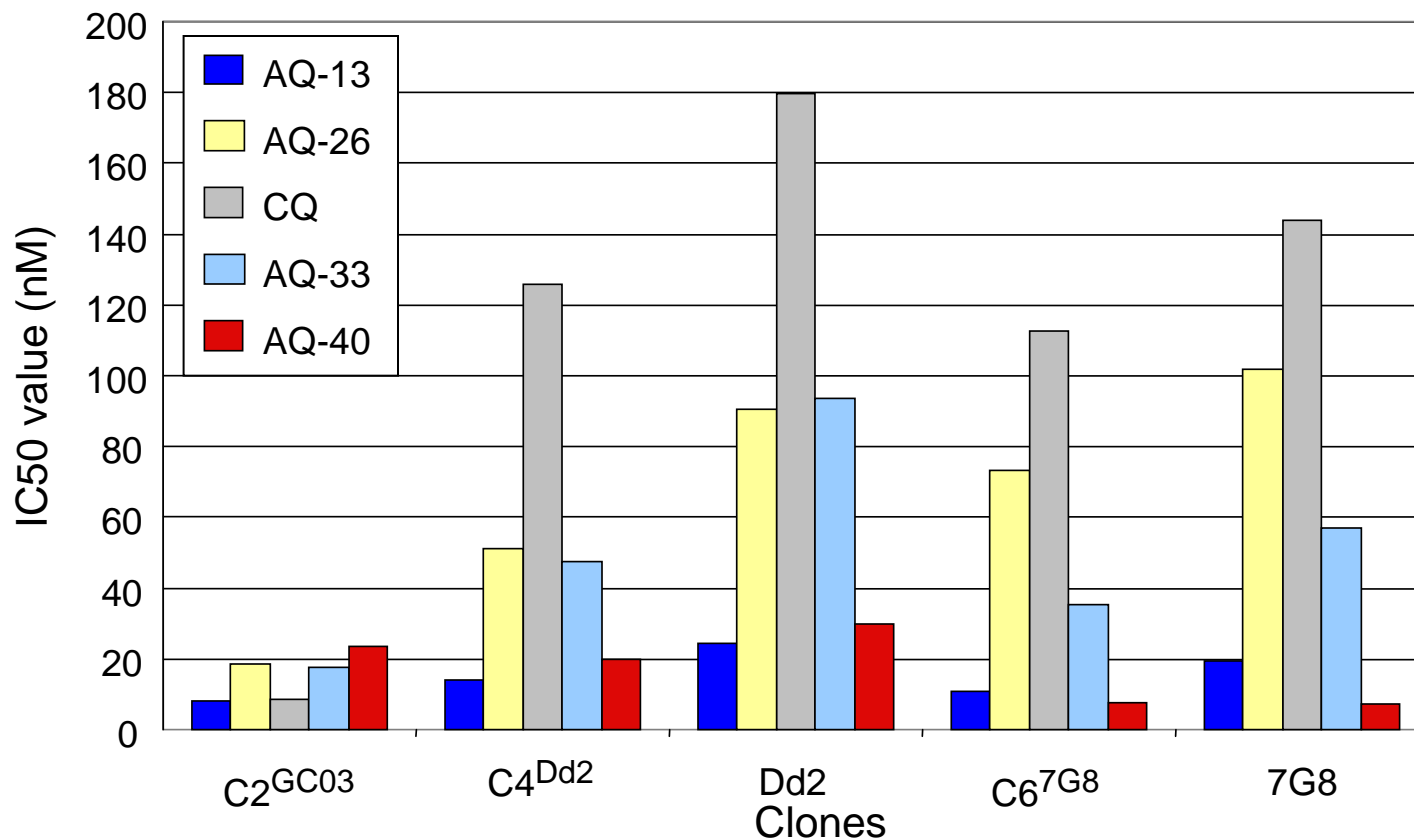


CQ-sensitive parasites with mutant *pfCRT* alleles acquire verapamil-reversible CQR phenotype



Recombinant mutant *pfCRT* clones: fulfill CQR criteria of higher IC₅₀ values, verapamil reversibility and reduced CQ accumulation

Evidence for stereospecificity of PfCRT-mediated CQR



Diaminoalkane side chain analogs

Compound

Side chain

AQ-13

$(\text{CH}_2)_3$

AQ-26

$(\text{CH}_2)_4$

CQ

$(\text{CH}_2)_5$

AQ-33

$(\text{CH}_2)_6$

AQ-40

$(\text{CH}_2)_{12}$

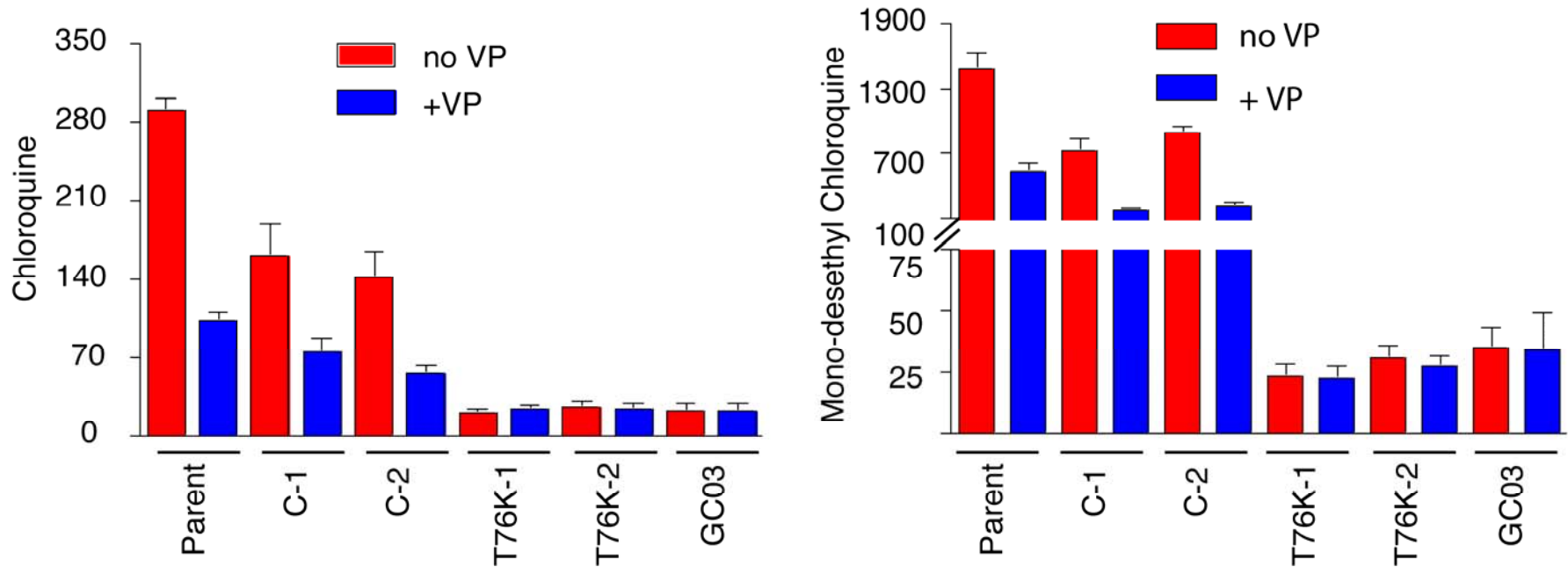
AQ-13 under clinical development

***pfcrt* mutations associated with *in vivo* CQ failure**

- **PfCRT K76T observed in CQ-treatment failures**
First assessment: 400 patients uncomplicated malaria,
K76T in all 60 CQ treatment failures (odds ratio 18.8)
- **Some patients cured despite K76T marker**
Host immunity (higher clearance rates in older children)?
Requirement for contribution of other genes?

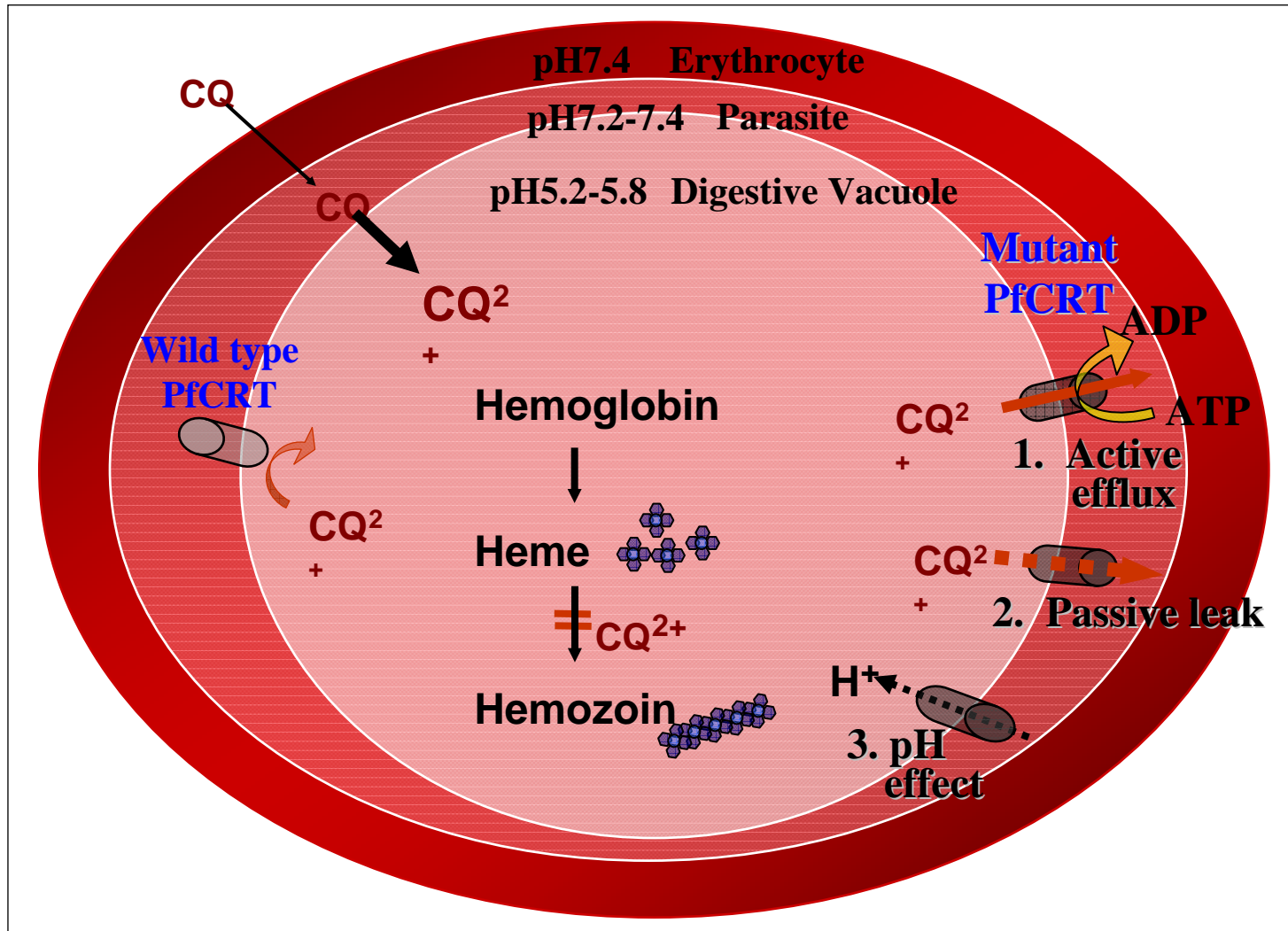
What is the role of K76T in CQ resistance?

Removal of K76T mutation leads to total loss of CQR (New and Old World strains)



K76T is essential for verapamil reversibility
Degree dictated by adjacent mutations
K76T also controls level of CQ accumulation

How does mutant PfCRT mediate CQR?



PfCRT implicated in mechanisms of drug efflux, altered pH, and altered redox regulation

SP - a failing treatment for CQR malaria

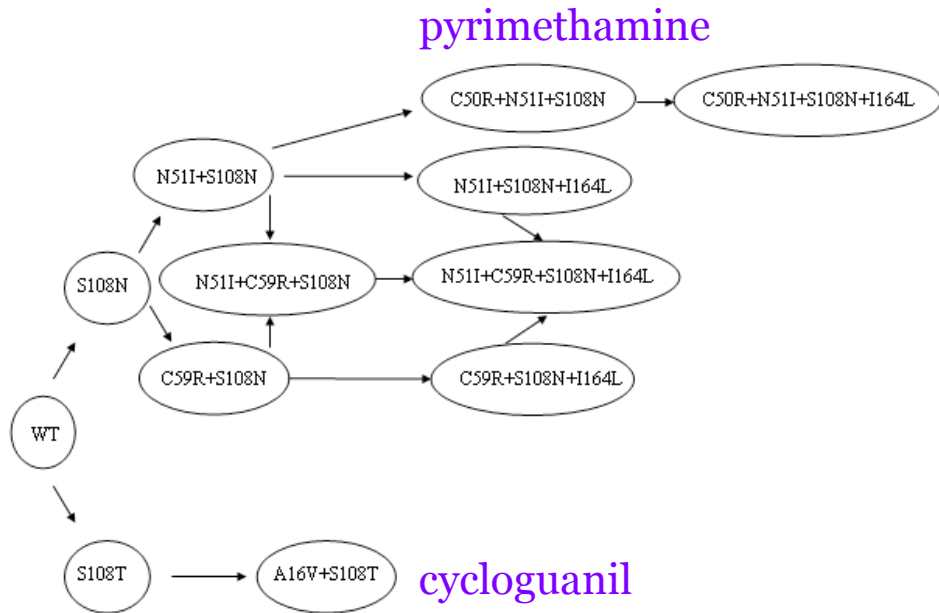
Sulfadoxine-Pyrimethamine (SP; Fansidar) - potent inhibitors of *P. falciparum* DHFR (dihydrofolate reductase) and DHPS (dihydro-pterolate synthase), sequential enzymes in folate biosynthesis. Comparable cost to CQ.

Cluster of 3 mutations in DHFR and 2 in DHPS predicts SP treatment failure. Failure can also result from quadruple DHFR mutant.

Mutations originated in Asia, migrated to Africa. Quadruple mutant very rare in Africa, will cause new antifolate LapDap to fail.

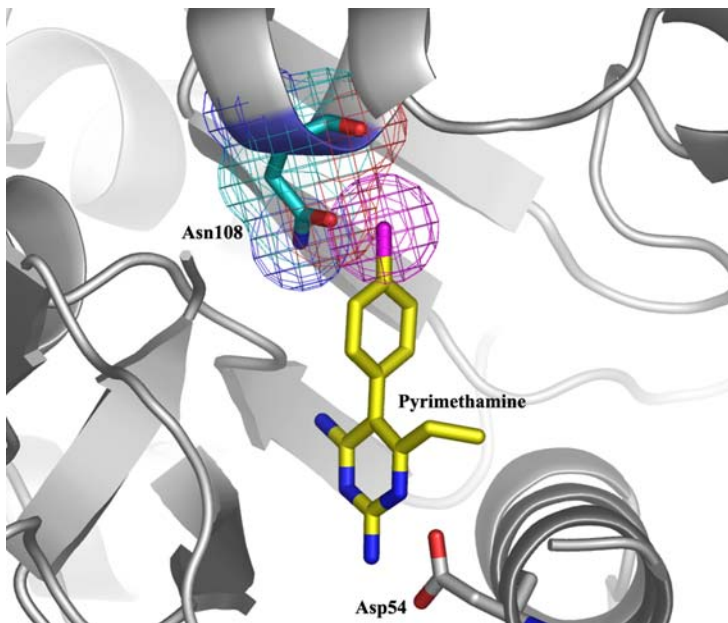
Bacterial resistance to trimethoprim-sulfamethoxazole appears to select for SP-resistant alleles in *P. falciparum*.

Evolution of and structural insights into mutant DHFR



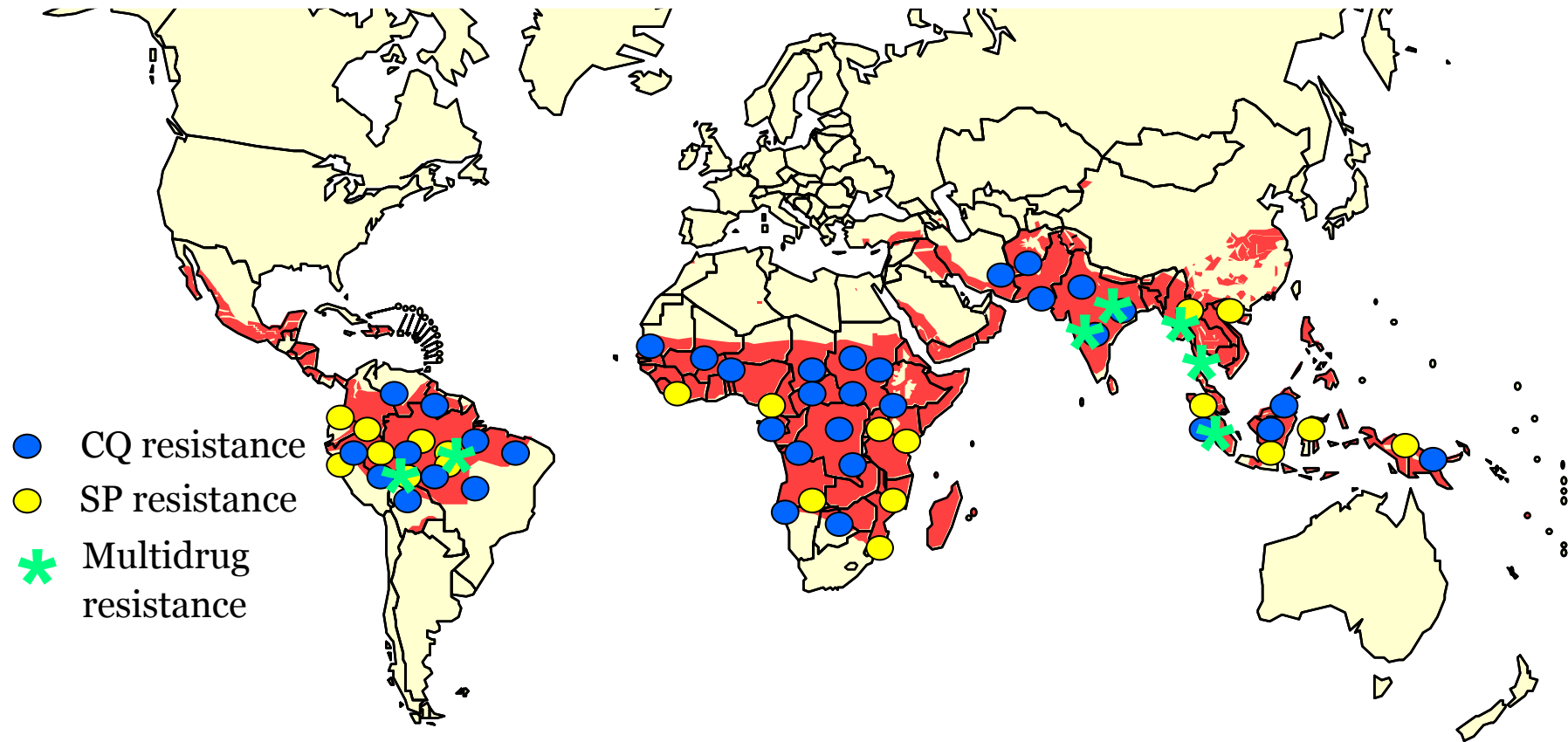
Evolution of *dhfr* mutations observed in field isolates.

Patterns represent balance between fitness costs of mutations and degrees of resistance.



Crystal structure of DHFR bound to pyrimethamine. Aids development of novel DHFR inhibitors that can bind mutant enzymes.

Spread of *P. falciparum* drug resistance



Treatment of CQ and SP-resistant malaria:

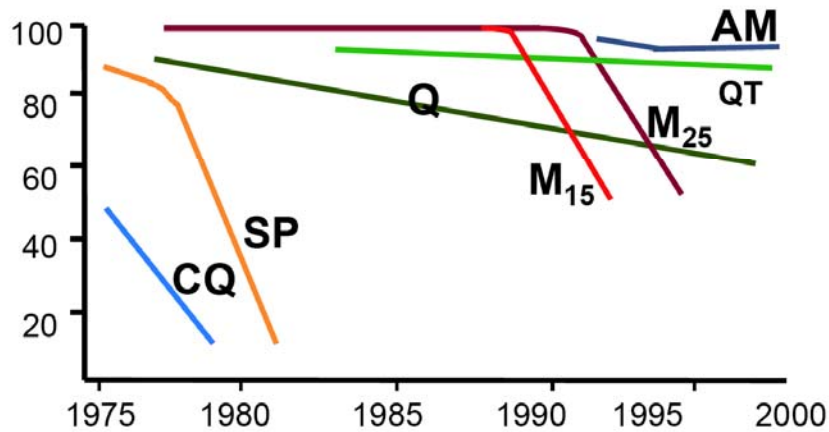
Quinine
Amodiaquine

Mefloquine
Artemisinin-based combinations

Cost and efficacy issues associated with treating CQ and SP-resistant malaria

The example of Thailand

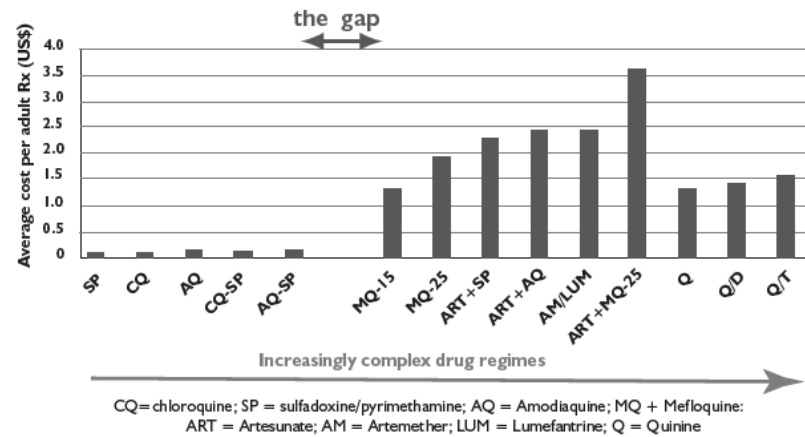
Clinical and Parasitological Cure rate in western Thailand
Lancet 2000; 356: 297 - 302



CQ, chloroquine; SP, sulfadoxine-pyrimethamine;
 Q, quinine; M15, low-dose mefloquine; M25, high-dose mefloquine; QT, quinine+tetracycline;
 AM, artemether+lumefantrine

Multidrug resistant strains prevalent, effectively treated by quinine-tetracycline or artemether-lumefantrine

Increasing cost of antimalarials

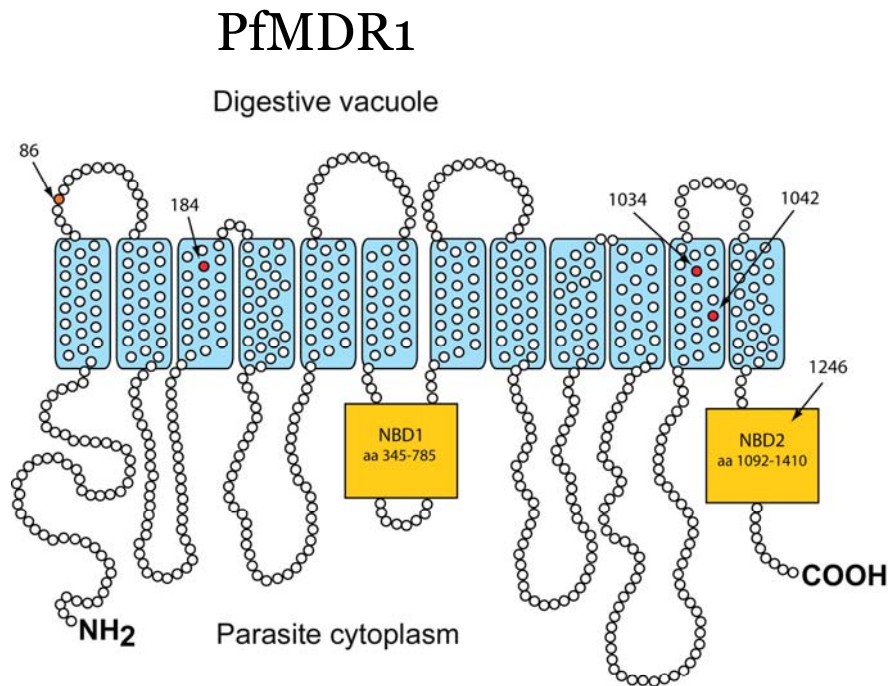


CQ= chloroquine; SP = sulfadoxine/pyrimethamine; AQ = Amodiaquine; MQ + Mefloquine;
 ART = Artesunate; AM = Artemether; LUM = Lumefantrine; Q = Quinine

Source: WHO, 2002

Drugs effective against MDR malaria are ≥ 10 times more expensive than CQ or SP

Involvement of PfMDR1 in resistance to mefloquine and quinine



Association found between increased *pfmdr1* copy number and increased risk of mefloquine treatment resistance.

Confirmed *in vitro* through derivation of mutants carrying different copy numbers. Appears to involve altered transport across digestive vacuole membrane.

3' point mutations also affect *in vitro* responses to mefloquine and quinine.

pfmdr1 variants identified in *P. falciparum* field isolates and laboratory-adapted lines.

Region	Reference line (origin)	PfMDR1 position and amino acid					Copy number
		86	184	1034	1042	1246	
All	Wild type (3D7, Netherlands)	N	Y	S	N	D	1
Asia/Africa	FCB (Southeast Asia)	N	Y	S	N	D	≥2
	K1 (Thailand)	Y	Y	S	N	D	1
South America	7G8 (Brazil)	N	F	C	D	Y	1

Gray shading indicates residues that differ from the wild type allele.

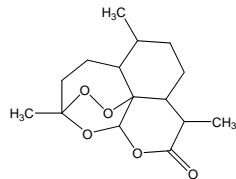
Artemisinin-based combination therapies

Based on artemisinin - extremely potent endoperoxide, kills *P. falciparum* in low nanomolar range, effective *in vivo*, reduces transmission

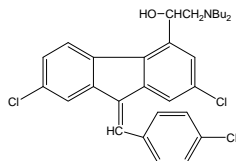
Short half-life of derivatives -> combining with other antimalarials (lumefantrine, piperazine, pyronaridine, amodiaquine)

Combinations rapidly becoming first-line antimalarials worldwide

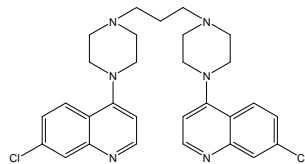
Concerns: toxicity, high cost (\$2 per treatment regime)



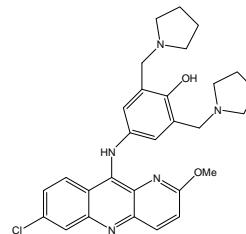
artemisinin



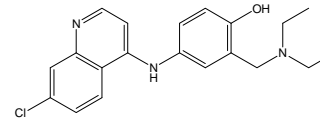
lumefantrine



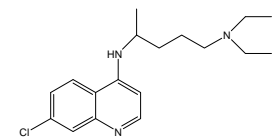
piperazine



pyronaridine



amodiaquine

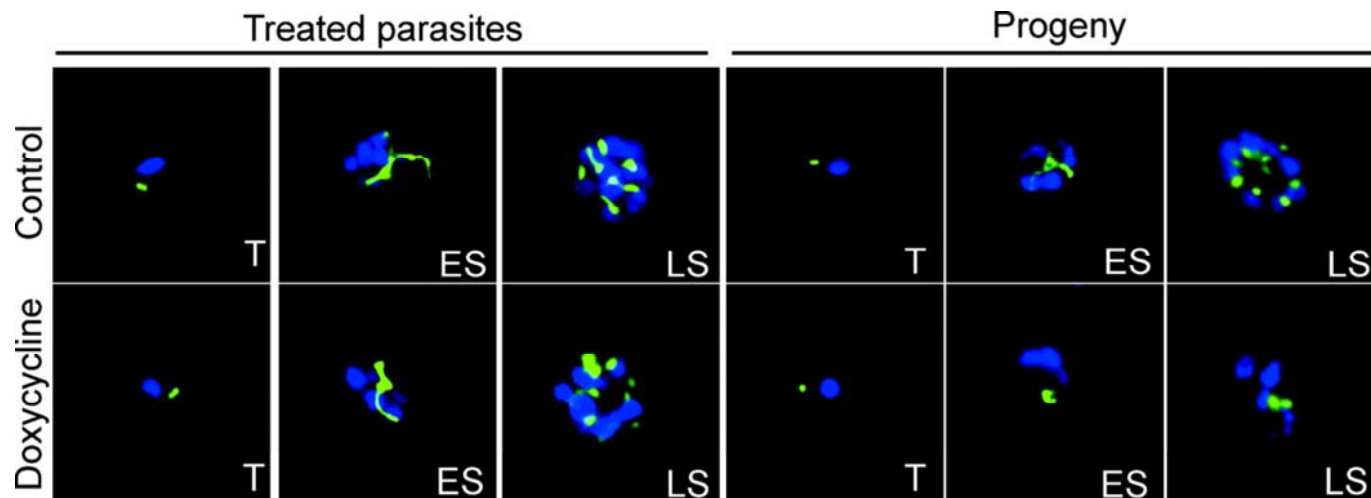


chloroquine

Is there a place for antibiotics in treating malaria?

Tetracycline and doxycycline used with quinine to treat CQ-resistant malarial infections in older children and adults. Not recommended for children < 8 years of age and pregnant women.

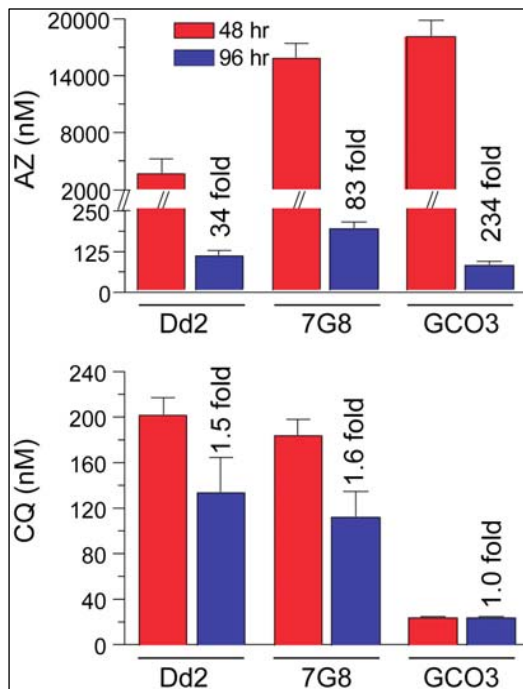
Shown to target *Plasmodium* apicoplast - of cyanobacterial origin. Only kill progeny of drug-treated parasites (“delayed death”).



A possible use for azithromycin (AZ)?

Macrolide, proven safety young children & pregnant women, long lasting
AZ+CQ 97% efficacy in curing *P. falciparum* malaria in India
AZ+quinine 92% efficacy against MDR malaria in Thailand
AZ monotherapy 88% efficacy against *P. vivax* malaria in India

In bacteria - targets 50S ribosome comprising large subunit
ribosomal RNA and ribosomal proteins



AZ manifests 30-230 fold increase in potency during second generation of *P. falciparum* exposure to drug *in vitro*

Dunne 2005 *J. Infect. Dis.*; Dunne 2005 *Am. J. Trop. Med. Hyg.*; Noedl 2006 *Clin. Infect. Dis.*

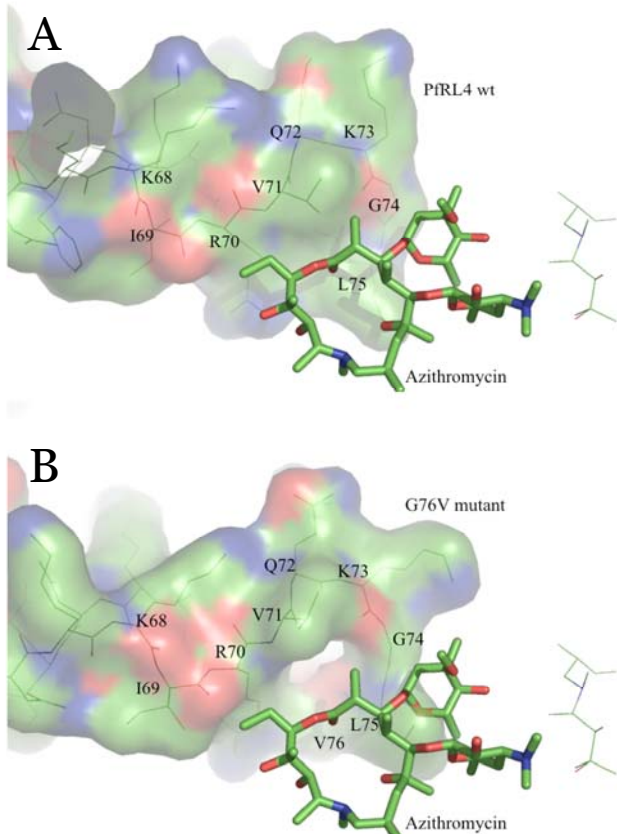
AZ resistance rapidly acquired *in vitro*

AZ pressure: resistant parasites selected in 21 days

Simultaneous mutations in 23S rRNA and ribosomal L4 gene

L4 mutation confers AZ resistance in *Streptococcus pneumoniae*

Directly implicates apicoplast as target of AZ



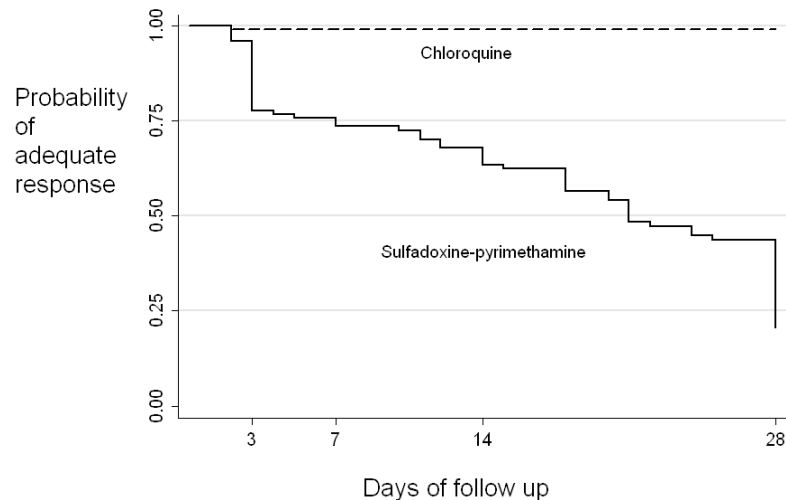
Structural models of (A) wild-type and (B) the G76V mutant PfRpl4 with AZ. These models, derived using the *Deinococcus radiodurans* crystal structure of protein Rpl4 as a template, reveal a steric clash between the side chain of 76V in the G76V mutant and AZ, consistent with this mutation conferring AZ resistance.

A case for selective reintroduction of chloroquine?

Malawi - first African country to switch from CQ - in 1993

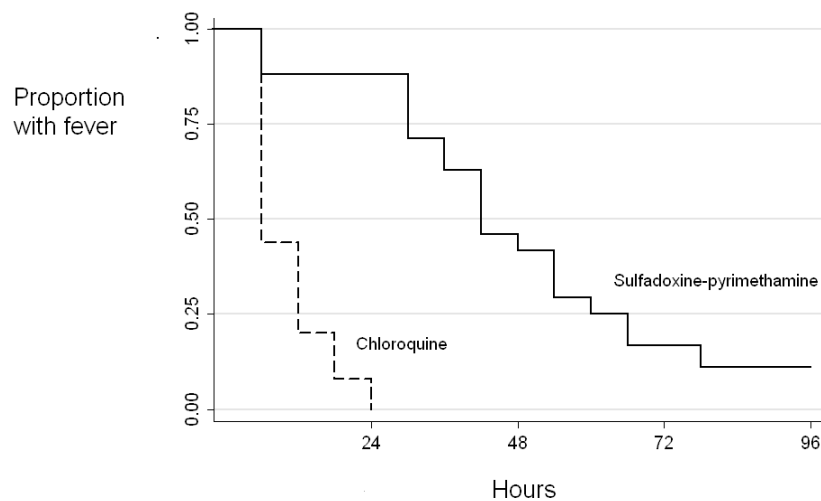
Prevalence of mutant *pfcr*t decreased in 2001 down to undetectable levels

Clinical trial to test efficacy of CQ vs. sulfadoxine-pyrimethamine



Enrolled 105 children in each arm (CQ vs SP), mean age 3 years
CQ: 80 completed study; 1 treatment failure (early)
SP: 87 completed study; 71 treatment failures
0% prevalence of PfCRT K76T mutation

Reduced fitness leads to loss of CQ-resistant parasites in absence of drug pressure.
Concept of drug cycling - reintroduce CQ with other antimalarial after sustained absence of drug?



New areas in antimalarial drug development

- New artemisinin-based combinations (artesunate-amodiaquine, artesunate-lumefantrine, dihydroartemisinin-piperaquine)
- Synthetic endoperoxides to replace artemisinin
- New antifolates (LapDap) to treat SP-resistant infections
- CQ analogs to overcome resistance mechanism
- Inhibitors of choline and purine metabolism
- Protease inhibitors (problem of enzymatic redundancy)
- High throughput screens (including search for delayed death inhibitors)



CONCLUSIONS

First-line antimalarials, chloroquine & sulfadoxine-pyrimethamine, have failed. Replacement drugs have cross-resistance and/or toxicity concerns

Most promising: artemisinin-based combination therapies. Being heavily subsidized. Supply and toxicity issues

Resistance determinants identified for several drugs, proving very useful for surveillance and for drug analoging and mechanistic studies. PfCRT identified as cause of CQR. PfMDR1 predictive of mefloquine failure.

Traditional high throughput screening approaches not particularly successful, but multitude of parasite molecular targets.

Groundswell of interest from industry, funding bodies, academia.

Malaria control can become a reality if new drugs do not fail from overt toxicity and if funding bodies can support worldwide implementation.

Acknowledgments

Columbia University

Amar bir Singh Sidhu

Eric Ekland

Rich Eastman

Stephanie Valderramos

Ines Petersen

Juan-Carlos Valderramos

Louis Nkrumah

Lakshmanan Viswanathan

Marcus Lee

Lise Musset

Min Yu

Rebecca Muhle

Sophie Adjalley

Lise Musset

Dominik Verdier

David Johnson

Lab Parasitic Diseases, NIAID, NIH

Thomas Wellems, Xin-Zhuan Su

U. Maryland, MD

Chris Plowe, Abdoulaye Djimdé

Pfizer Inc.

Drew Lewis, Michael Dunne

GlaxoSmithKline

Jose-Francisco Garcia-Bustos

Frederico Gomez

Columbia U. Dept. Medicine

Anne-Catrin Uhlemann

Columbia U. Genome Center

Thomas Mayer, James Rothman

Funding Agencies

NIAID/NIH, Pfizer

Burroughs Wellcome Fund

Medicines for Malaria Venture

Ellison Medical Foundation

Speaker's Fund

