Drugs and resistance

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Mechanisms of resistance and implications for Multiple First-line Therapies

Some approaches to deterring resistance

Resistance: mechanisms and status

Drug	**Resistance mechanism**	**Current status of resistance**
SP	DHFR, DHPS mutations	High level resistance in Asia and S America "Moderate" resistance in Africa
Chloroquine	Conferred by Pfcrt mutations, modulated by Pfmdr1 mutations	Resistance nearly everywhere except Latin America, Middle East; Receding in Africa?
Quinine, Mefloquine, Lumefantrine	Pfmdr1 mutations, copy number, expression levels, other membrane transporters?	Modest levels in Asia, patchy elsewhere Mefloquine resistance reversed by combination with artemisinins
Amodiaquine	*Pfcrt, Pfmdr1 mutations?*	Limited data; increasing resistance in Africa?
Piperaquine	Unknown; conflicting data on cross resistance with other quinolines	Increased IC50s and treatment failures reported after widespread use in China ***No recent data from SE Asia***
Pyronaradine	Unknown	Unknown
Atovaquone/ proguanil	*Cytochrome B/DHFR mutations?*	Few reported cases in returned travelers
Artemisinins	*Pfmdr1 mutations, copy number, expression levels; PfATPase6 mutations? Non-heritable traits?*	Suspected tolerance/resistance in SE Asia

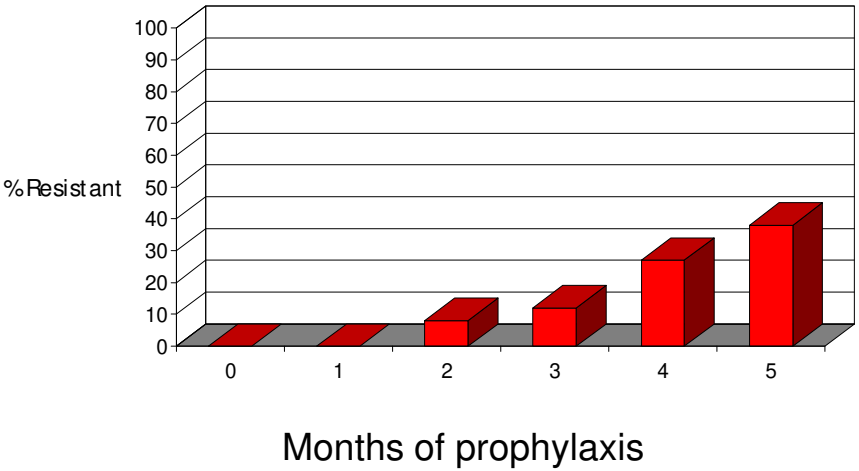
Resistance-conferring mutations

- Appearance
  - "Blink" on but fail to persist
- Emergence
  - Stable local persistence
- Dissemination
  - Geographic spread

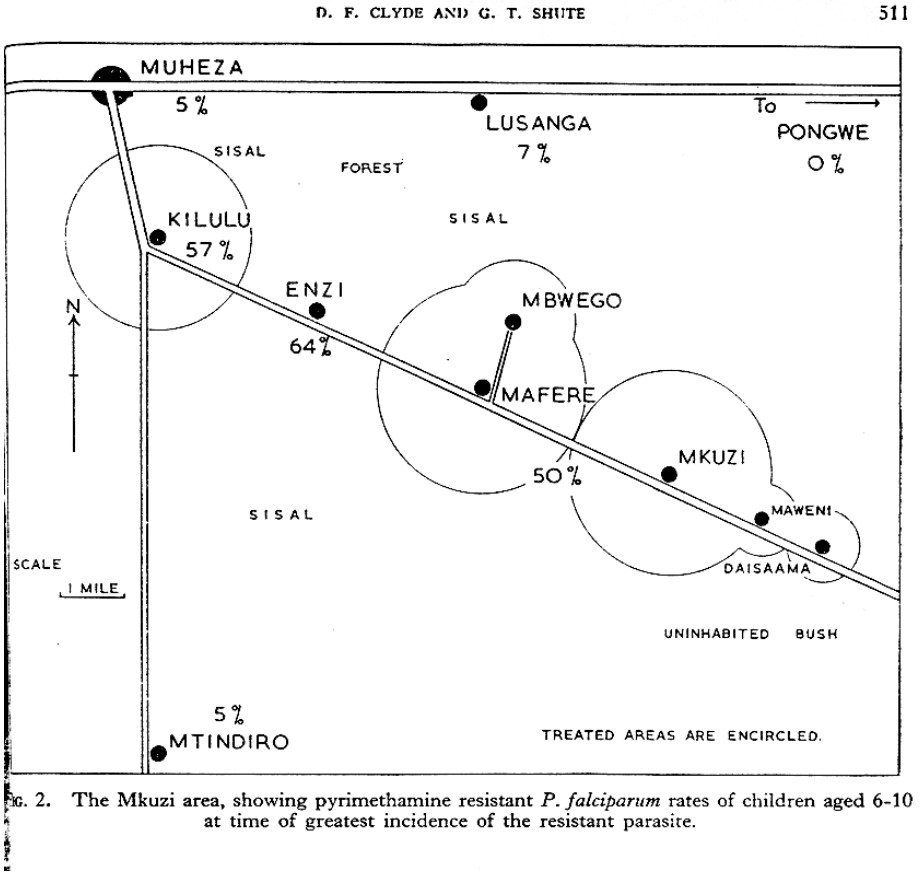
Dave Smith, Kruger, Yesterday

Rapid selection of antifolate resistance suggested frequent local emergence

Pyrimethamine resistance in response to prophylaxis

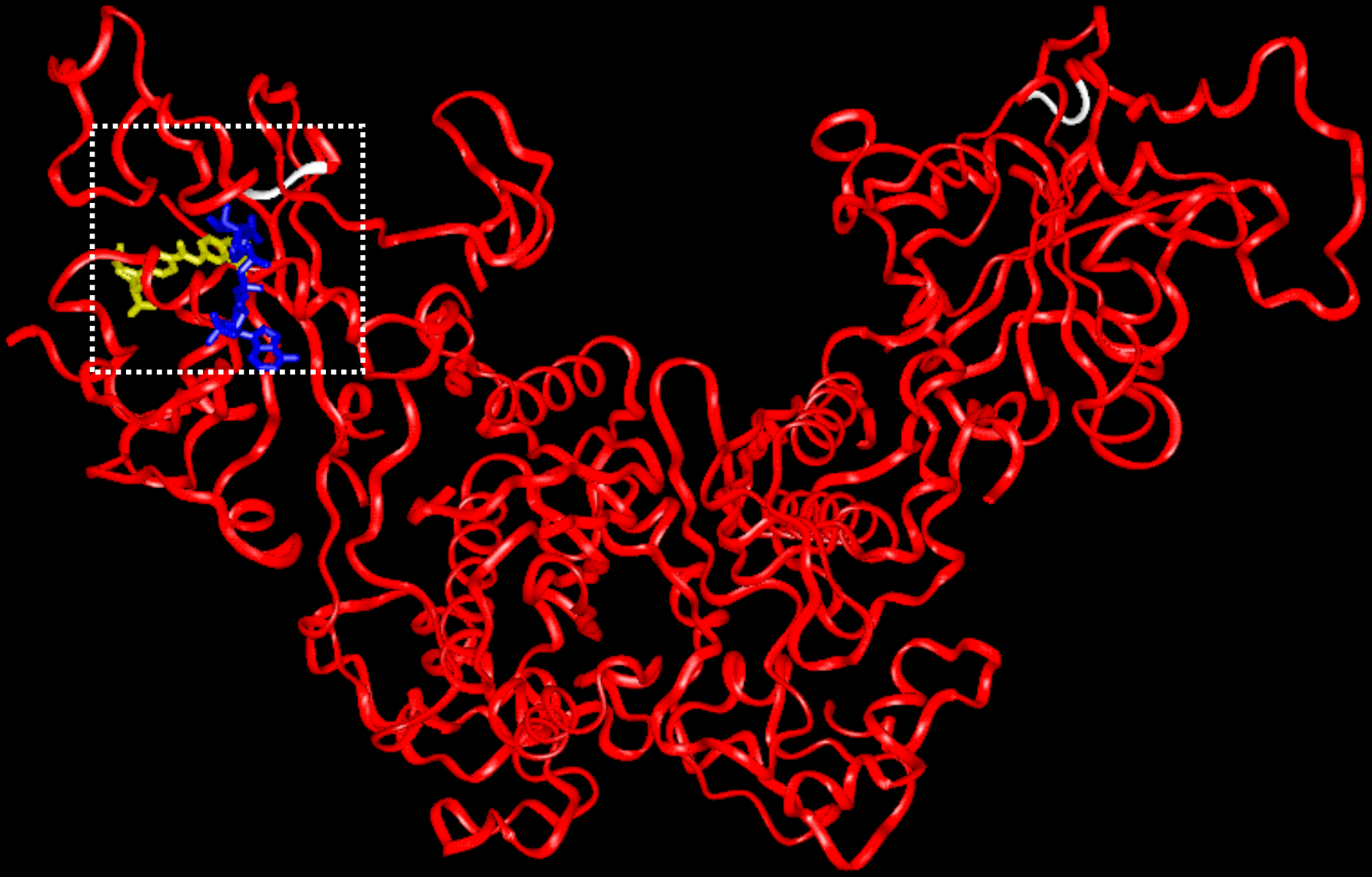


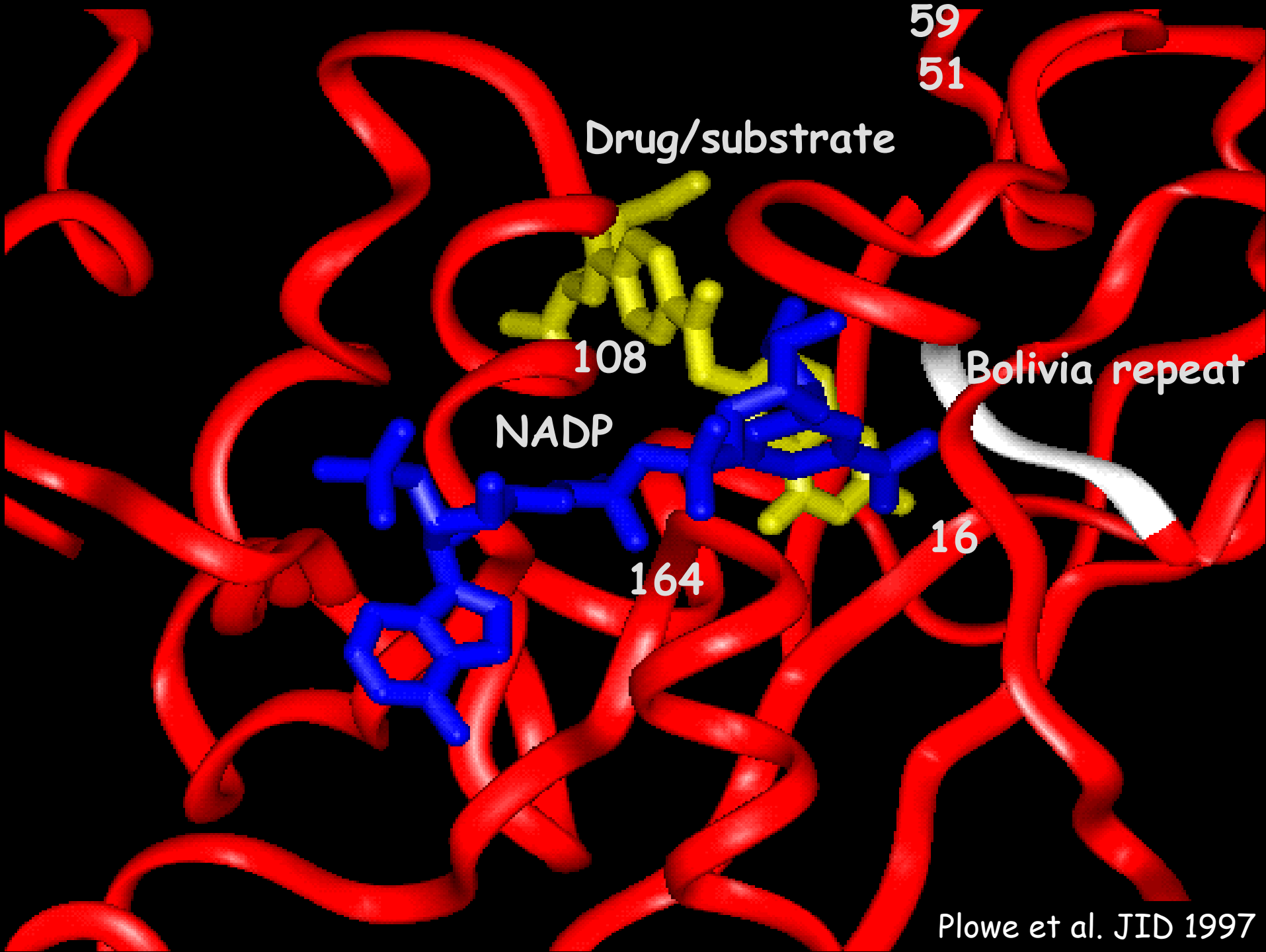
Clyde and Shute, TRSTMH 1954



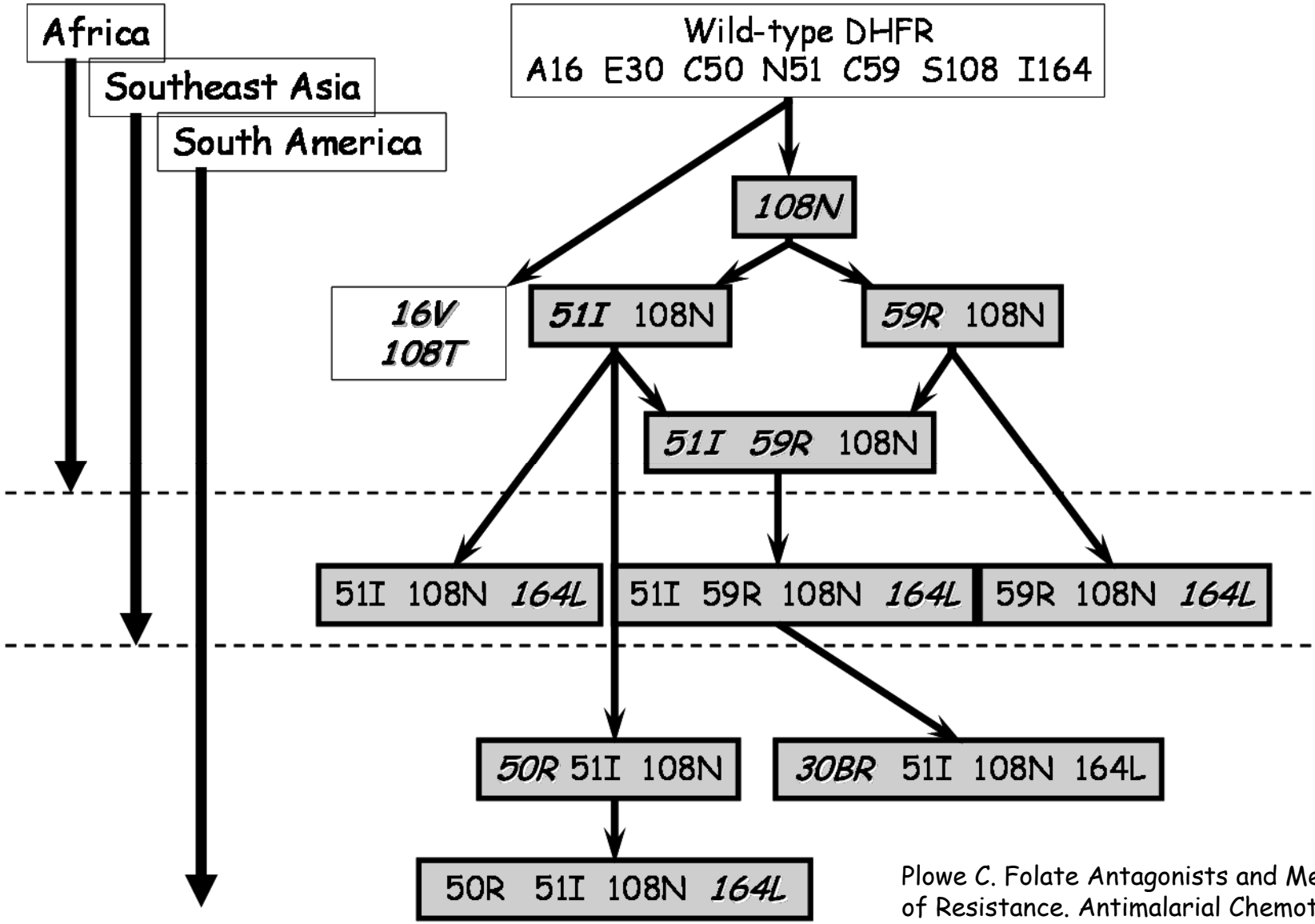
G. 2. The Mkuzi area, showing pyrimethamine resistant *P. falciparum* rates of children aged 6-10 at time of greatest incidence of the resistant parasite.

Pyrimethamine resistance is caused by mutations in *P. falciparum* dihydrofolate reductase (DHFR)



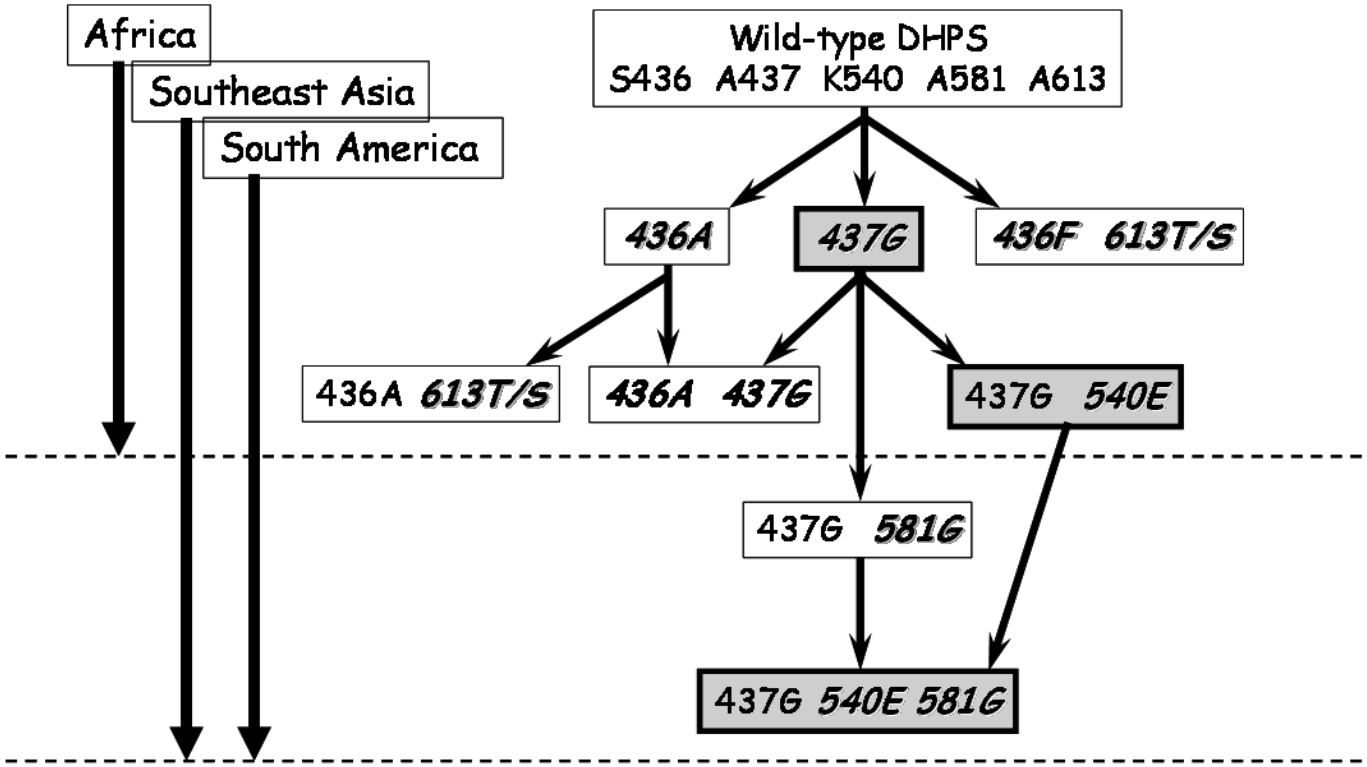

59
51
Drug/substrate
108
Bolivia repeat
NADP
16
164
Plowe et al. JID 1997

DHFR mutations emerge in a stepwise fashion



Plowe C. Folate Antagonists and Mechanisms of Resistance. Antimalarial Chemotherapy, Humana Press, 2001

Sulfadoxine resistance is caused by stepwise accumulation of mutations in *P. falciparum* dihydropteroate synthase (DHPS)



DHFR triple + DHPS double = SP resistant (Africa)

DHFR quadruple = SP resistant (Asia, S. America)

Resistance is not an all-or-none phenomenon

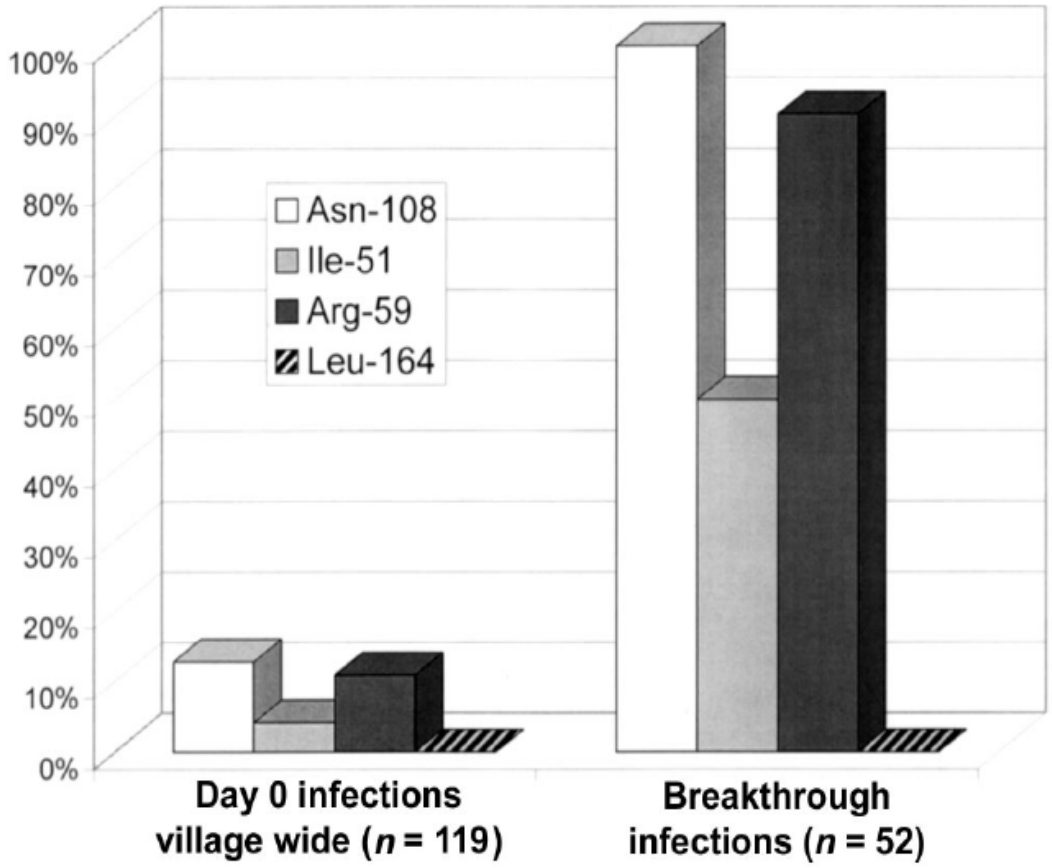
and mutations can both confer resistance and compensate for fitness loss

Strain	Amino acid position 16	51	59	108	164	Pyrimethamine IC50	Effect on Fitness*
3D7	A	N	C	S	I	1	--
HB3	A	N	C	N	I	331	↓
It.D12	A	I	C	N	I	755	↑
K1	A	N	R	N	I	1048	↓ ↓
Dd2	A	I	R	N	I	2371	↓ ↓ ↓
V1/S	A	I	R	N	L	22477	↑ ↑ ↑

*Relative to previous mutation
Sirawaraporn et al. PNAS 1997

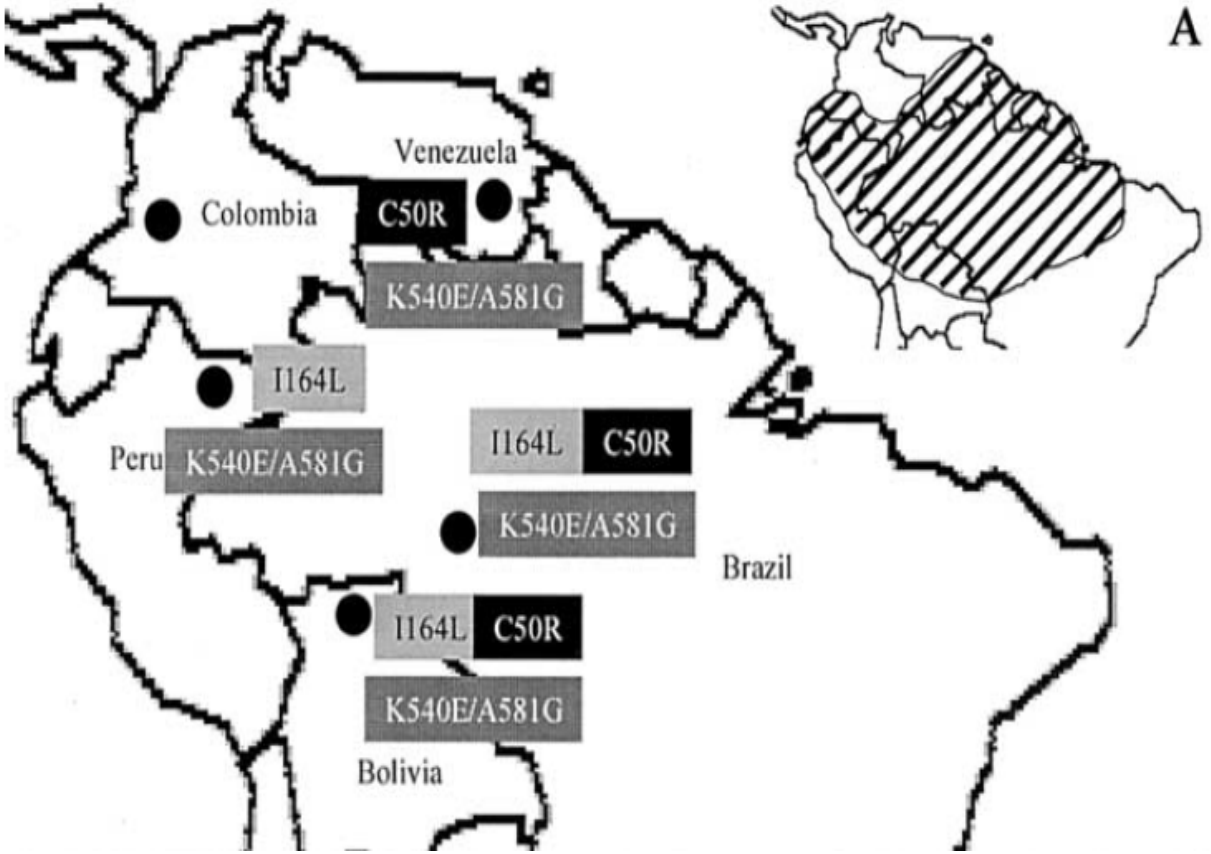
Iyer et al. Lancet 2001

Selection of DHFR mutations during 6 weeks of pyrimethamine prophylaxis



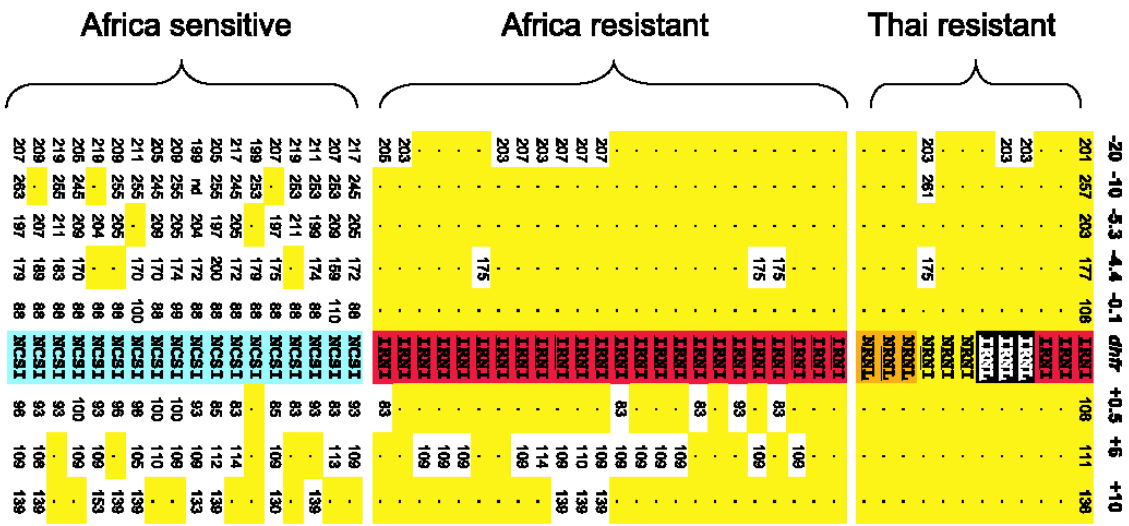
Doumbo et al. JID 2000

High-level antifolate resistance spread in a genetic sweep across the Amazon region



Cortese et al., JID 2002

"Moderate" pyrimethamine resistance (DHFR triple mutant) disseminated in a single genetic sweep



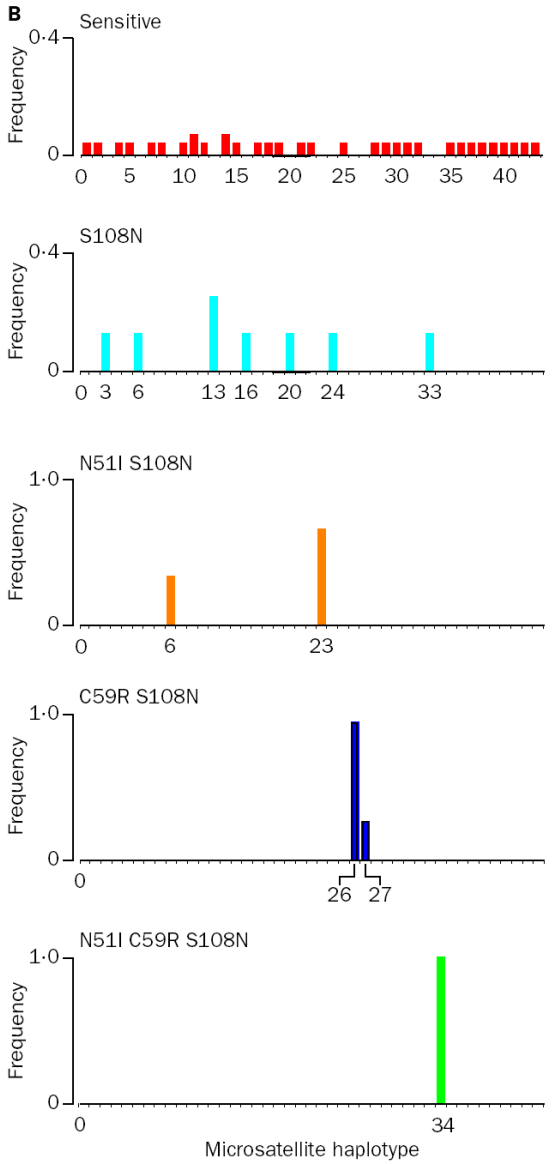


Figure 2: ***dhfr* in 1995 and 1999 in Ingwavuma district, KwaZulu-Natal, South Africa**
(A) Changes in frequency of allelic haplotypes. Curves were drawn by computation of frequency changes based on relative fitness values. (B) Allele associated microsatellite polymorphism in the flanking region.

Roper et al., Lancet 2004, Science 2004

Inexorable, contiguous spread of chloroquine resistance from limited foci suggested rare, complex genetic event

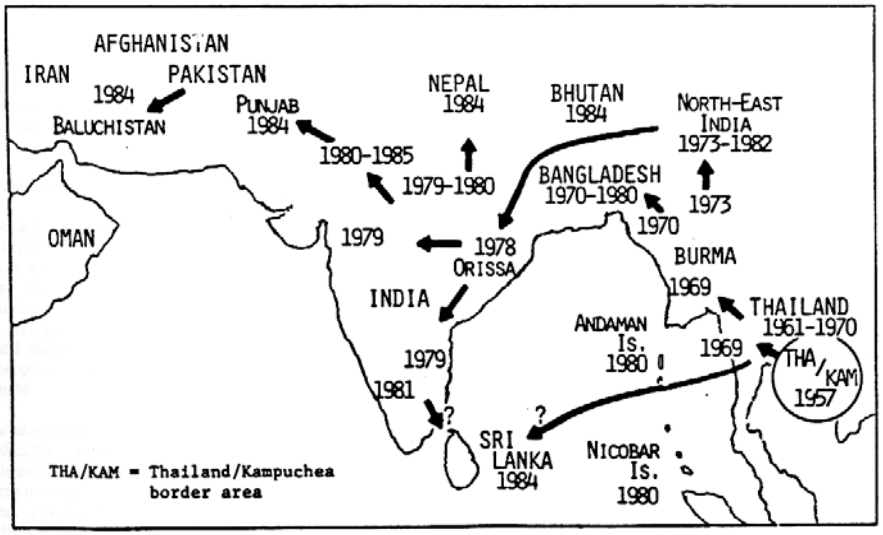


FIG. 4 Chronological spread of chloroquine-resistant falciparum malaria westwards in Asia.

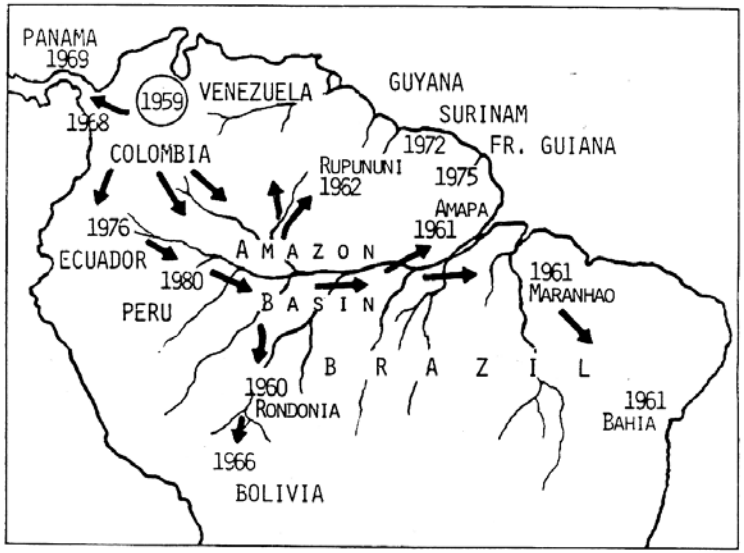
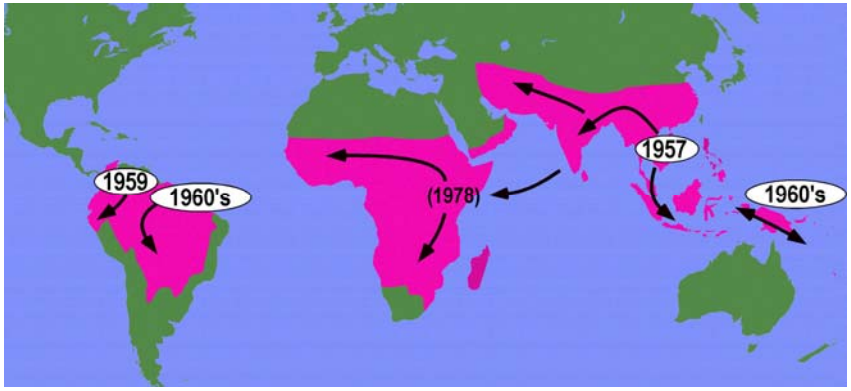
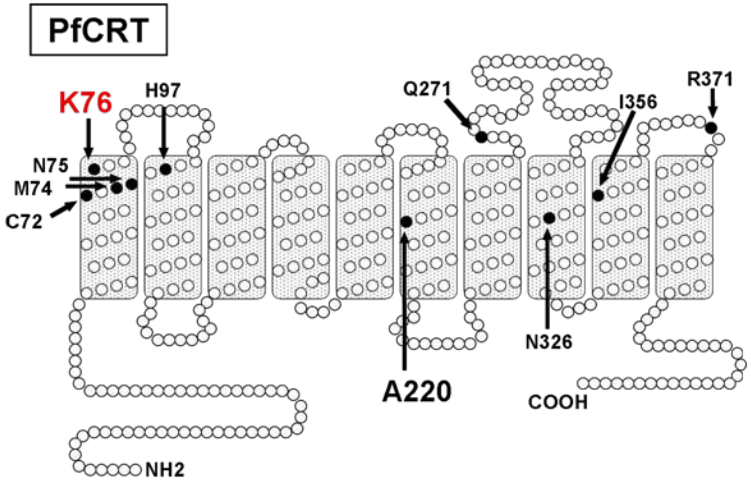


Figure 1 Chronological spread of chloroquine-resistant falciparum malaria in the American region.



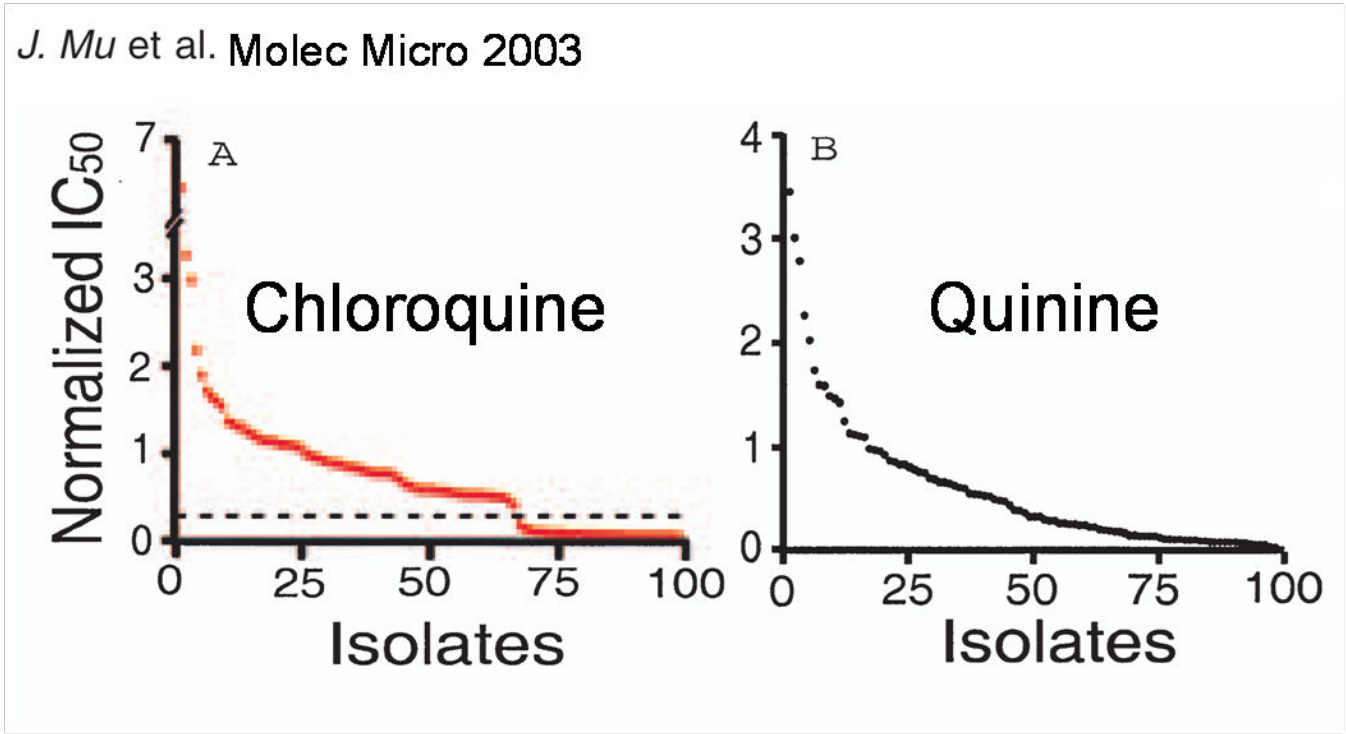


Chloroquine resistance is conferred by a single mutation that must occur in a milieu of presumably compensatory mutations

	PfCRT position & encoded amino acid									
Parasite type & origin	72	74	75	*76*	97	220	271	326	356	371
Chloroquine sensitive										
"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
Chloroquine resistant										
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 P1	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

Wellems & Plowe 2001: Fidock et al. 2000, Chen et al. 2001

Resistance can be categorical, continuous, or both

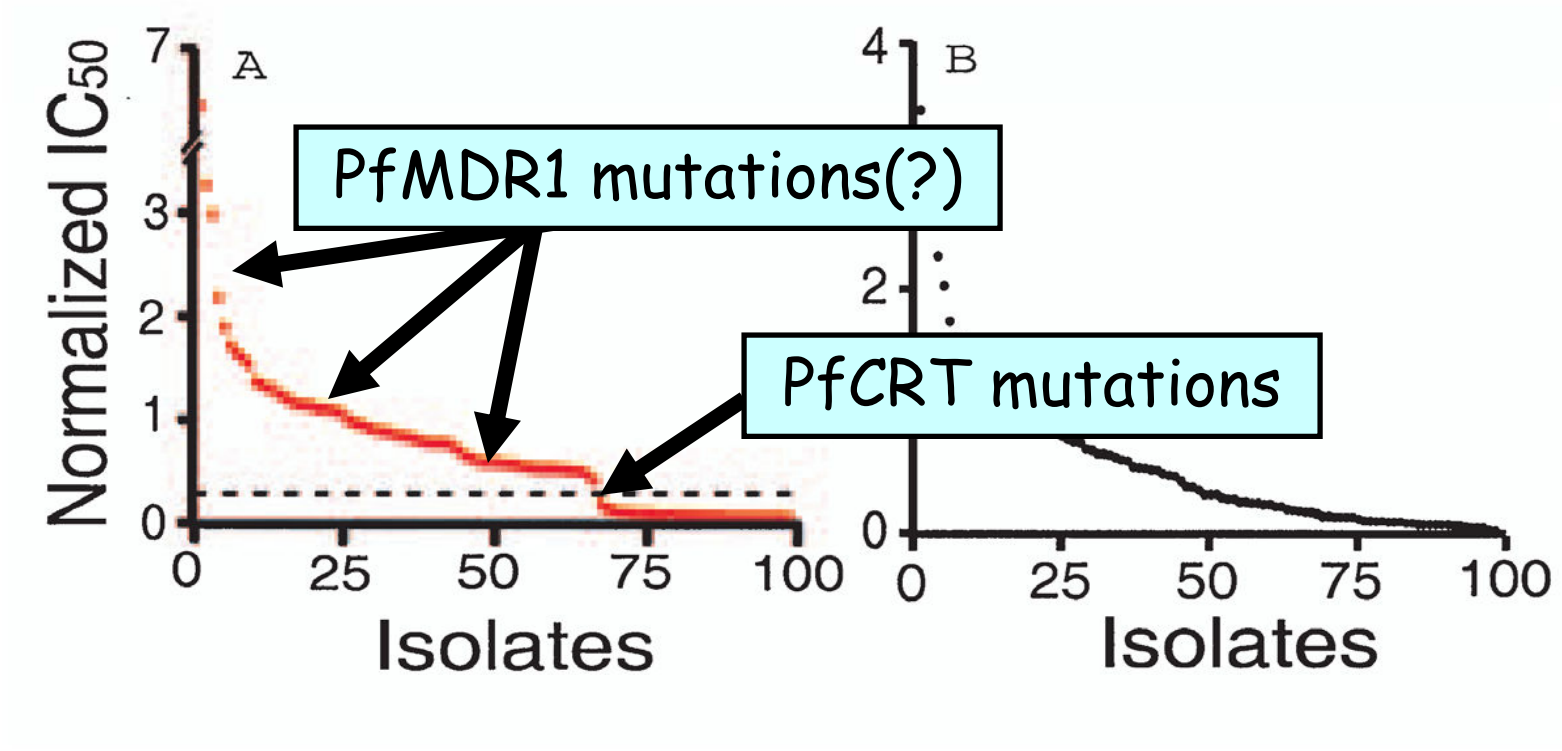


Implications for mechanisms, and for appearance, emergence, and dissemination

- Multigenic resistance easily broken up by recombination

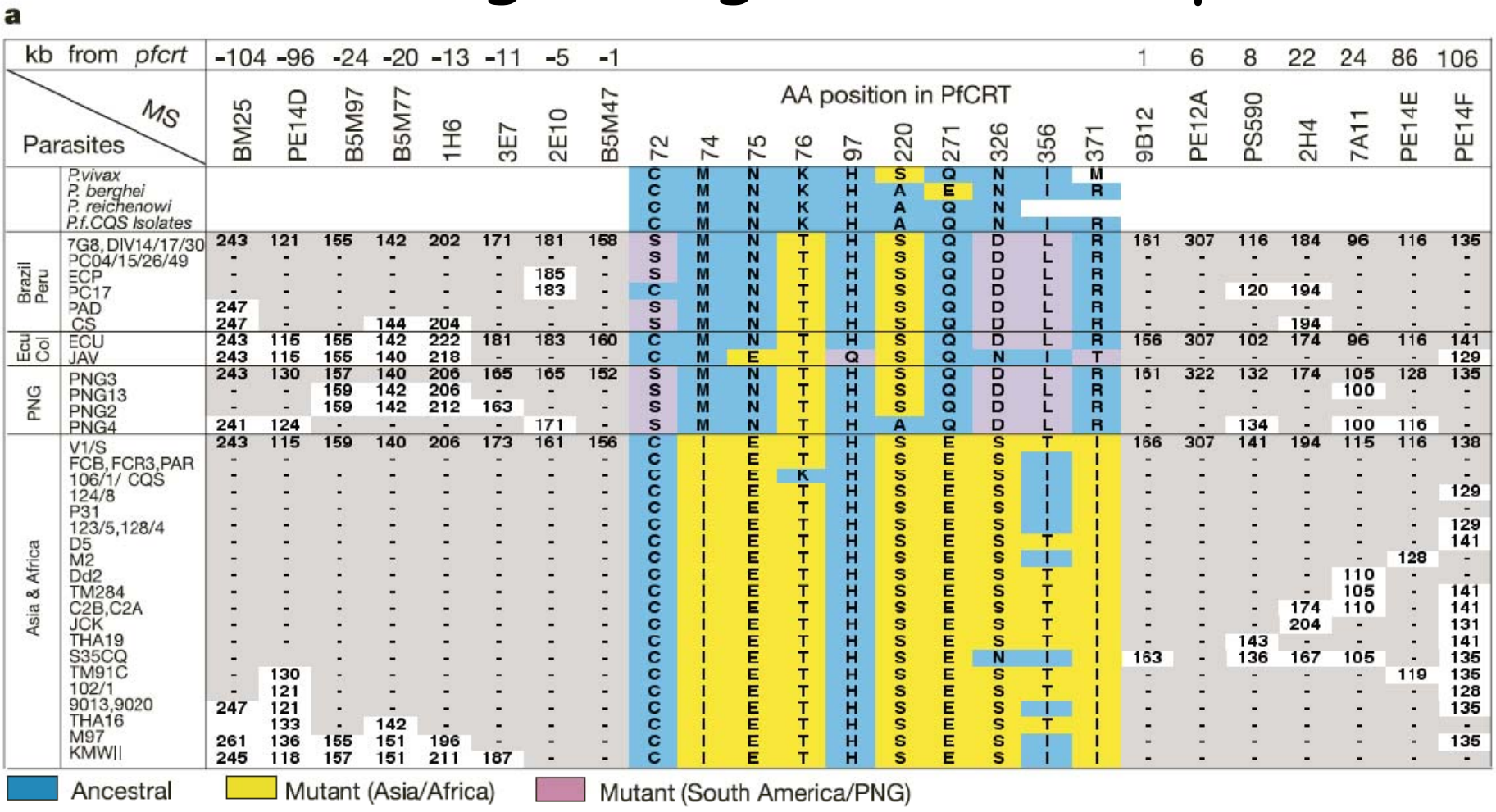
Resistance can be categorical, continuous, or both

J. Mu et al. Molec Micro 2003



Implications for mechanisms, and for appearance, emergence, and dissemination

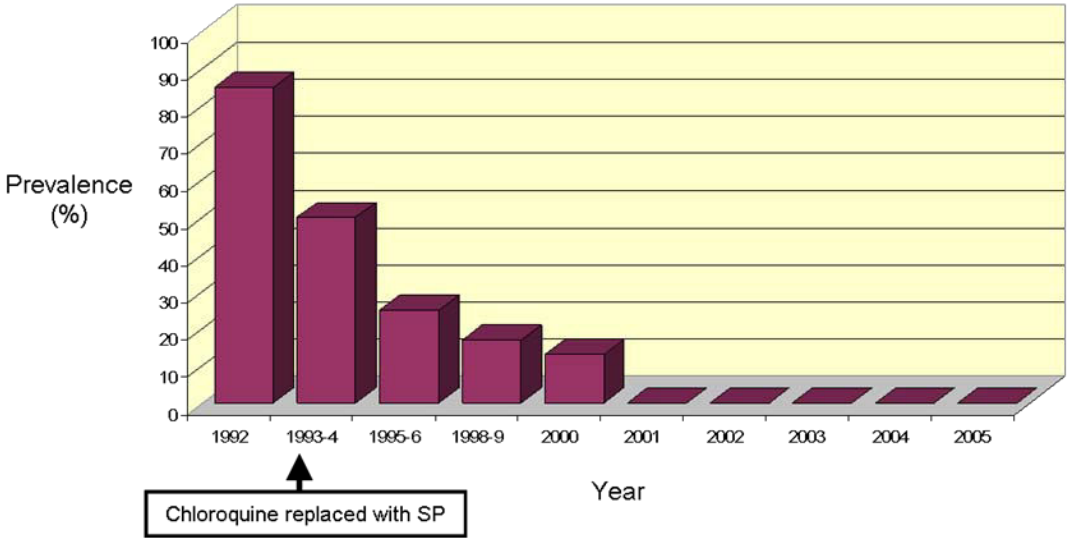
Chloroquine resistance spread in wide regional genetic sweeps



Wootton et al. Nature 2002

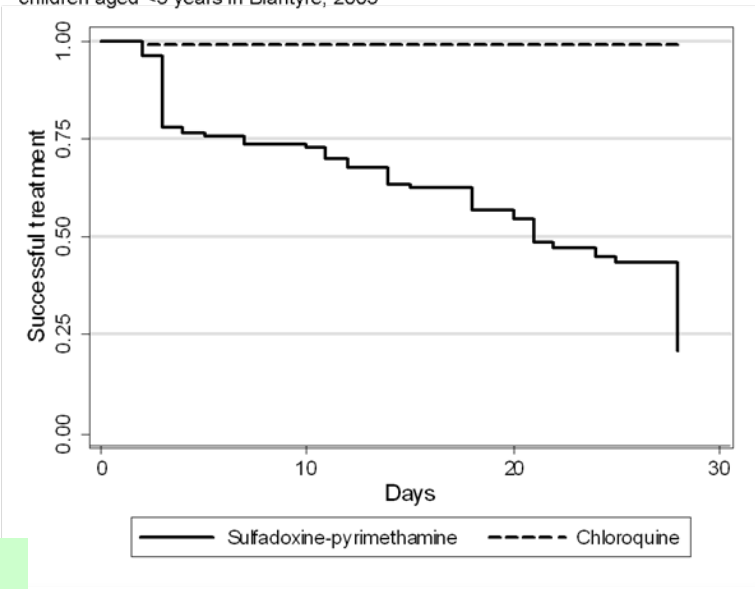
Chloroquine sensitivity returned rapidly after cessation of chloroquine use in Malawi

Figure 1. Prevalence of PfCRT chloroquine resistance marker in Blantyre, Malawi 1992-2005



Kublin et al., JID 2003

Figure 2. Time to treatment failure in clinical trial of chloroquine vs. sulfadoxine-pyrimethamine efficacy for treatment of uncomplicated falciparum malaria in children aged <5 years in Blantyre, 2005



This was not predicted based on mathematical and in vitro models

Laufer et al., NEJM 2006

Will resistance to other drugs do the same thing?

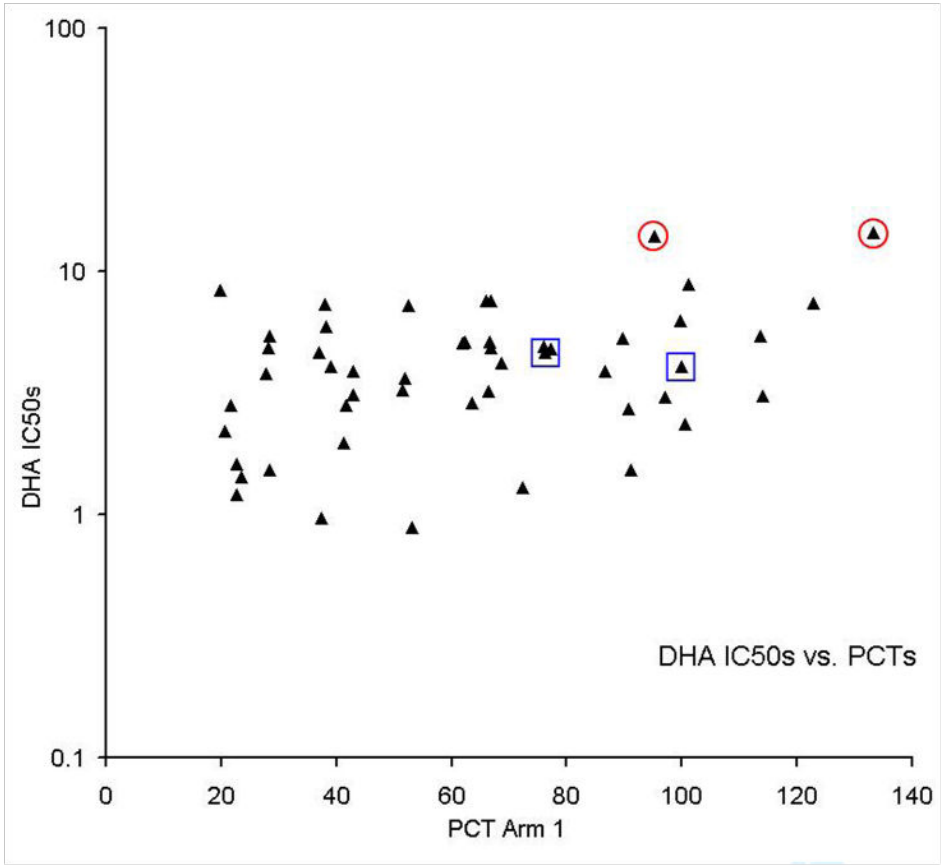
- It should not be assumed that other resistance mechanisms have a similar fitness cost, or <u>any</u> fitness cost, after evolution of compensatory mutations
  - *E. coli* model suggests resistance will remain fixed
- SP resistance has stayed fixed in SE Asia and South America
  - Due to low transmission? No fitness cost? Compensatory mutations? Ongoing antifolate pressure?

Microsatellite typing: Expansion of diverse sensitive parasites

Table 1: Microsatellite markers flanking PfCRT, the molecular marker for chloroquine resistance

	Year	-55	-29	-10.8	-4.4	-2.8	*pfcrt* 76	+0.6	+10.4	+39.6
Resistant	1992	137	147	175	228	178	T	149	200	193
	1992	135	147	175	228	178	T	149	190	193
	1994	139	147	175	228	178	T	149	200	193
	1995	135	149	175	228	178	T	149	200	193
	1995	145	147	175	228	178	T	149	190	193
	1997	135	147	175	228	178	T	149	200	203
	1997	137	147	175	228	178	T	149	200	193
	1997	135	147	175	228	178	T	149	200	185
Susceptible	1993	139	147	185	228	176	K	155	193	193
	1995	135	147	175	228	174	K	153	200	193
	1995	135	147	181	228	172	K	145	200	193
	1997	135	150	177	228	186	K	147	200	203
	1997	137	149	173	220	170	K	147	187	193
	1997	135	147	177	232	184	K	143	200	185
	2005	135	147	171	230	178	K	139	202	195
	2005	139	149	183	228	186	K	167	187	203
	2005	131	147	193	228	182	K	143	196	193
	2005	137	149	173	228	184	K	147	197	195

Artemisinin resistance in western Cambodia?



H. Noedl, M. Fukuda et al., submitted 2008

- In 2006, 60 falciparum malaria cases treated with 7 days of artesunate 4 mg/kg
- Four treatment failures between days 21-28
- Two met all criteria for resistance
- Not associated with mutations in candidate genes PfMDR1, PfATPase6
- Microsatellite typing confirms recrudescence

Population structure and gene flow

- Microsatellite typing of parasites from Western Cambodia and Eastern Thailand
- Population structure: Do the parasites at the two sites represent two distinct populations?
- Fst = (total heterozygosity minus mean heterozygosity within subpopulations)/total heterozygosity
- Measure of genetic differentiation between populations
  - Fst = 0.021, p=0.0166 ± 0.0039 (1000 permutations)
  - Some differentiation between sites, but a lot of gene flow
  - Implications for containment
- If malaria is an island, parasites can island-hop
- Therefore start malaria eradication here!

Mechanisms of resistance and implications for Multiple First-line Therapies

Some approaches to deterring resistance

Chloroquine in Malawi as a model for combination partner drugs

- High efficacy but resistance likely to return
- Easy to detect and quantify returning resistance:
  - PfCRT T76 molecular resistance marker
- Develop approaches to prevent resistance to all partner drugs (not just artemisinins)

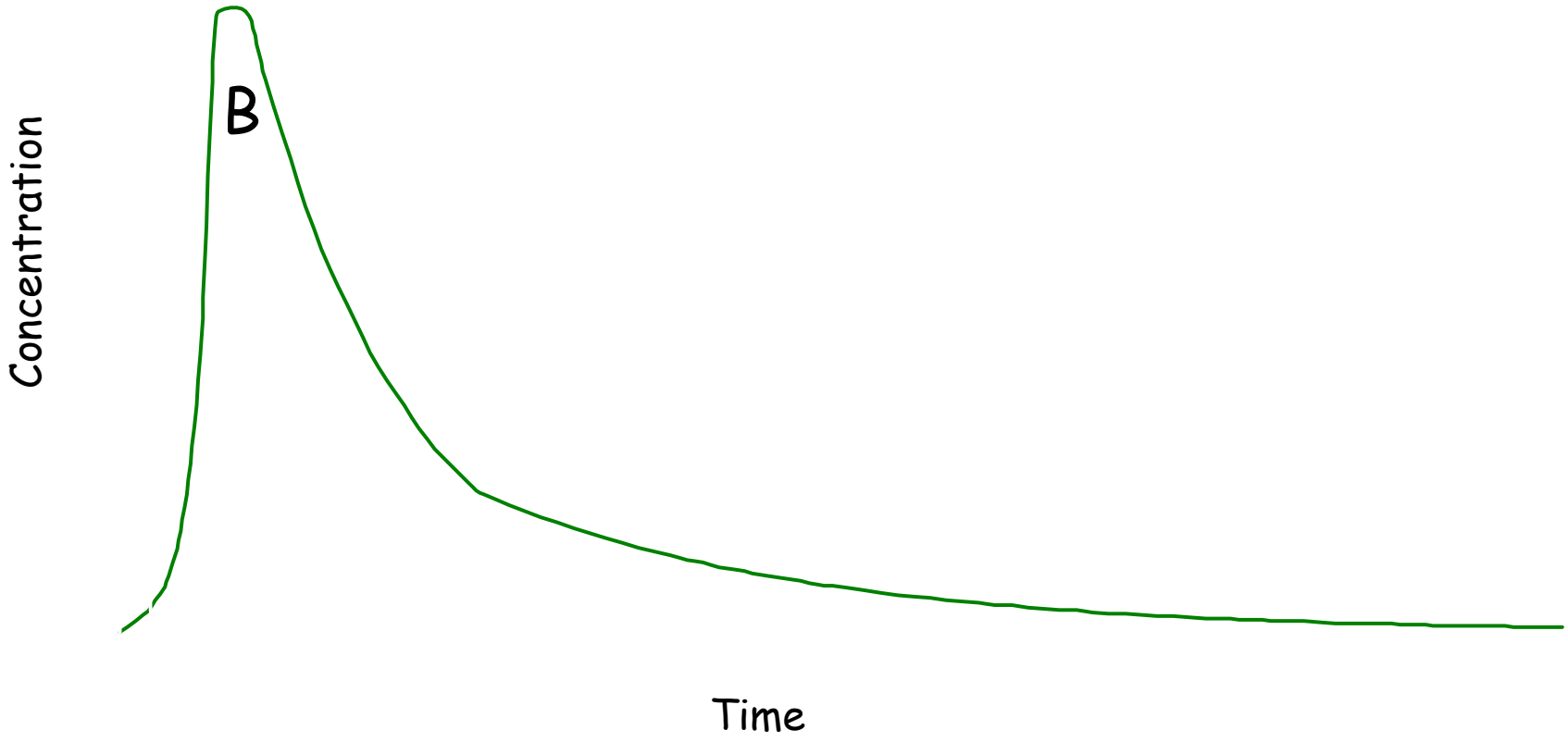
Longitudinal trial of chloroquine combinations

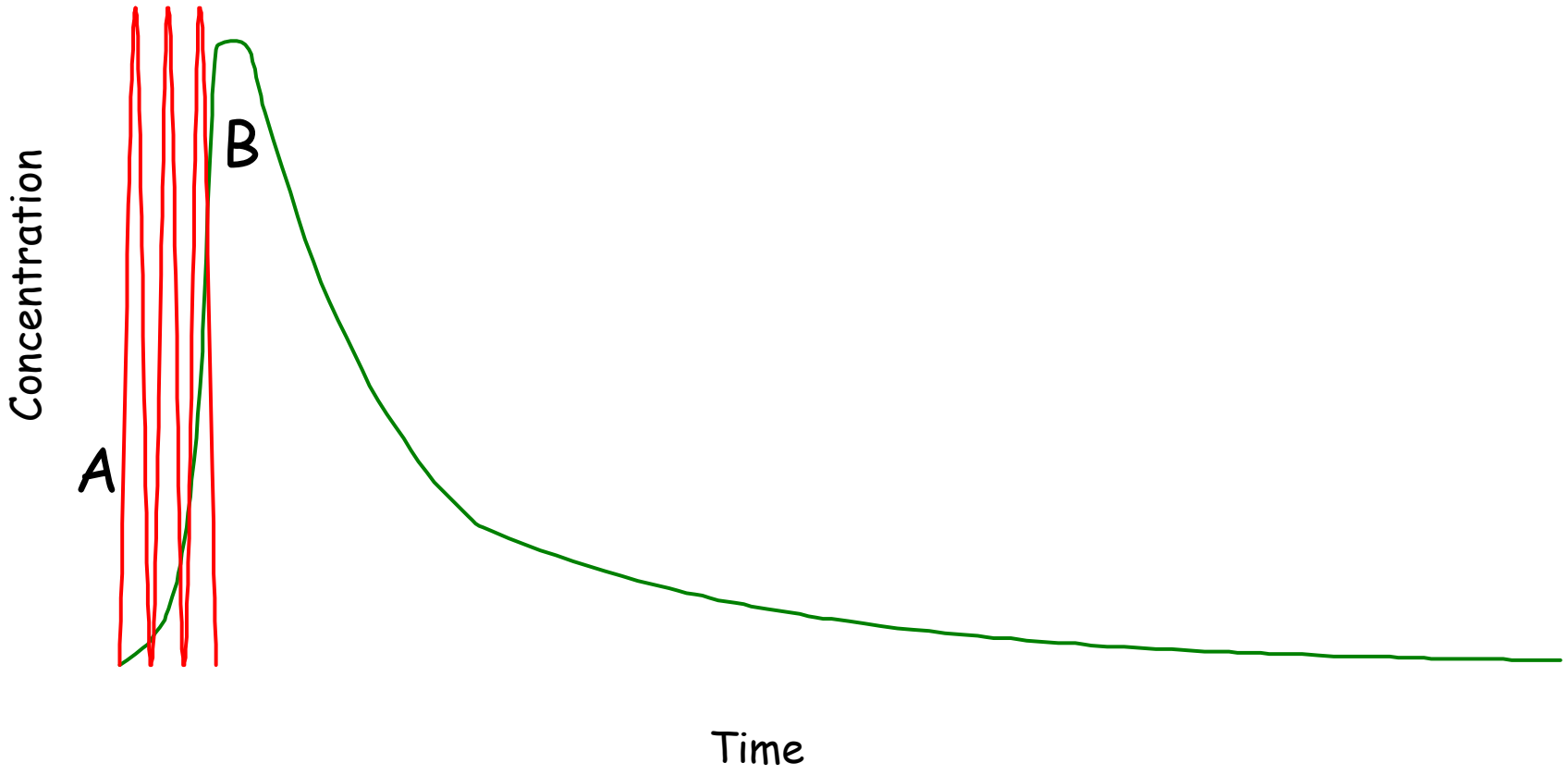
- Children with acute malaria randomized to
  - CQ alone
  - CQ + artesunate
  - CQ + azithromycin
  - CQ + atovaquone/proguanil
- Treated with same combination for every malaria episode for 1 year
  - Compare incidence of clinical malaria episodes
  - Compare ability of partner drugs to deter (re)emergence of resistance
  - Define "selective window*" for chloroquine

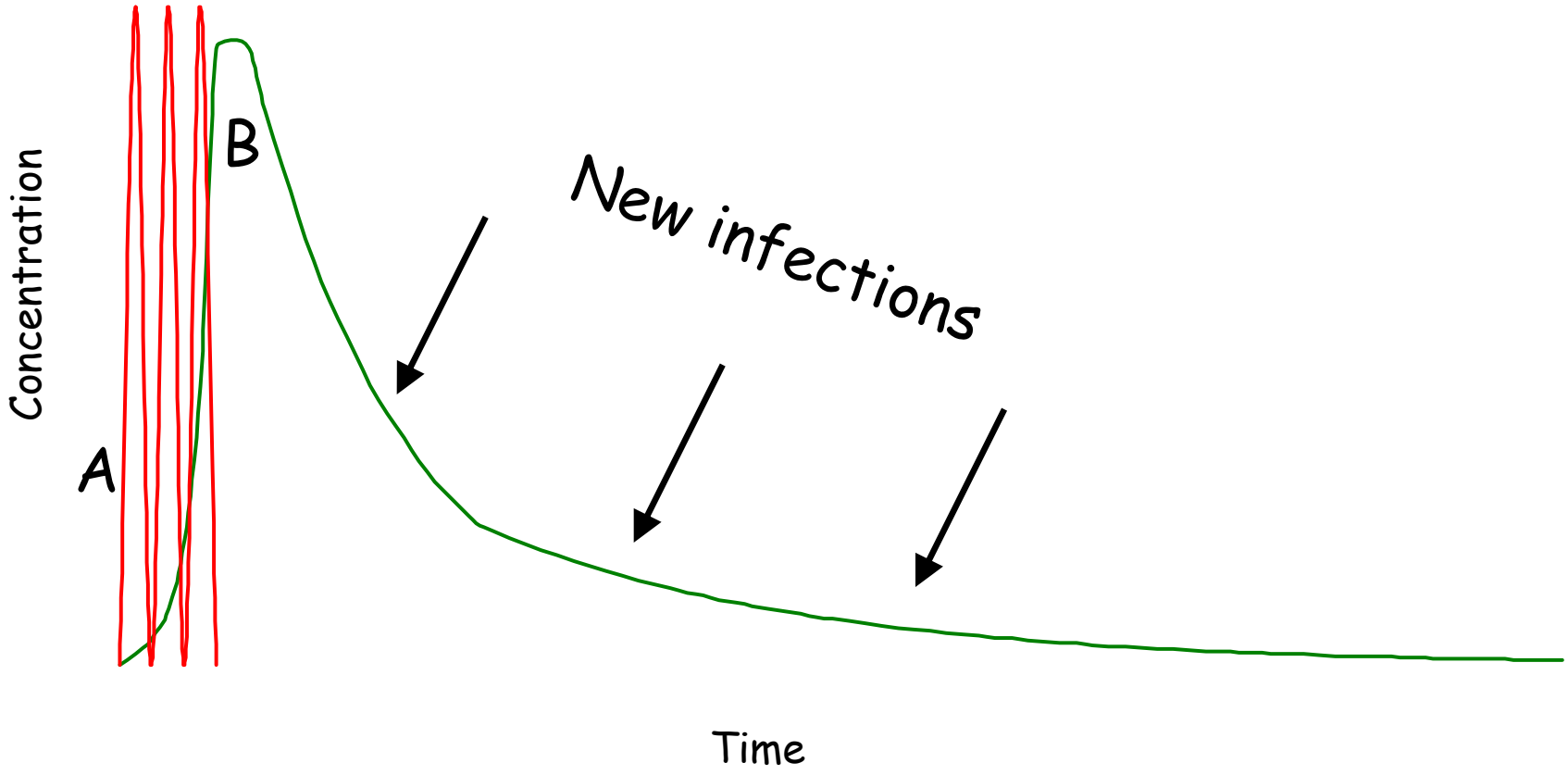
*Hastings & Watkins Trends Parasitol 2006

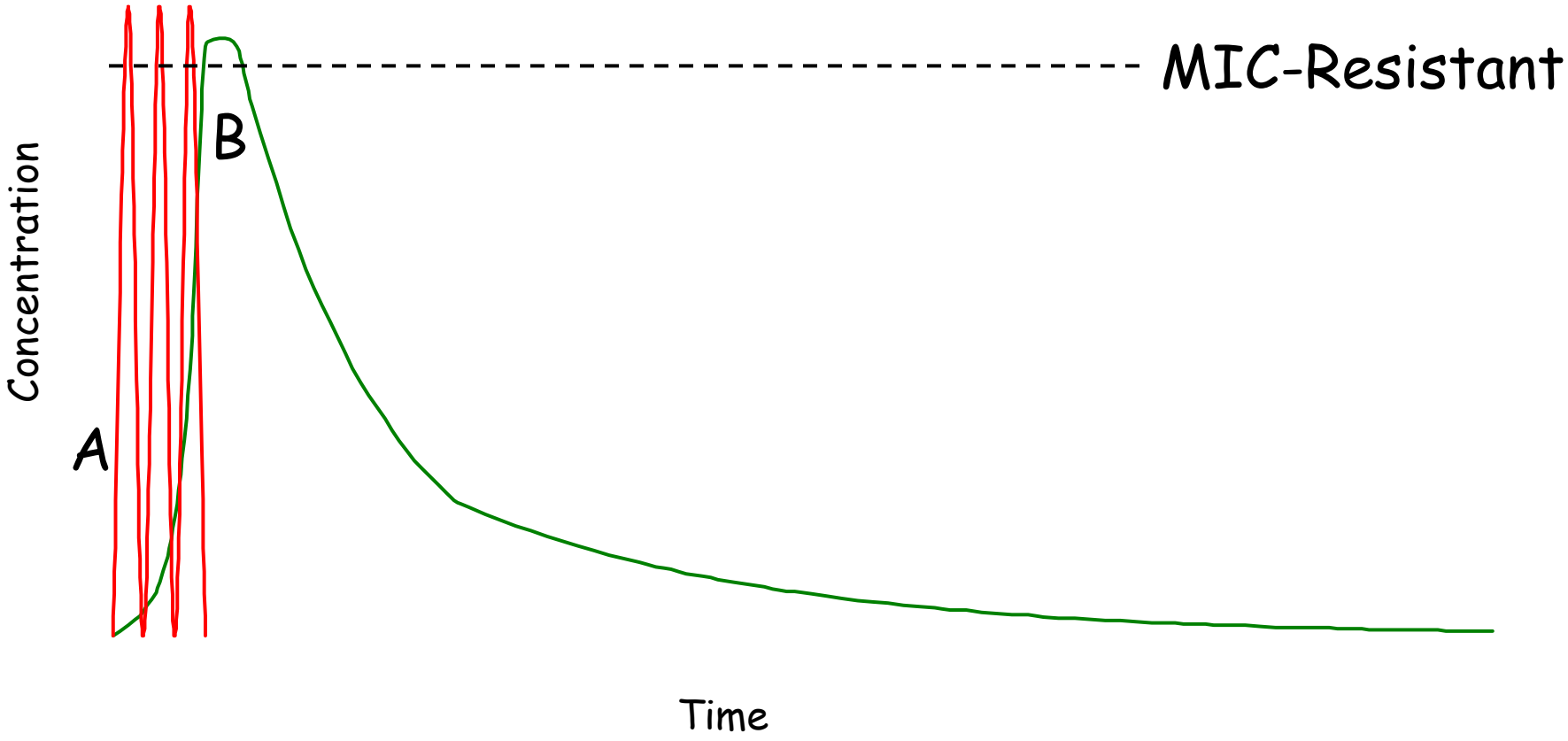
Acknowledgments:

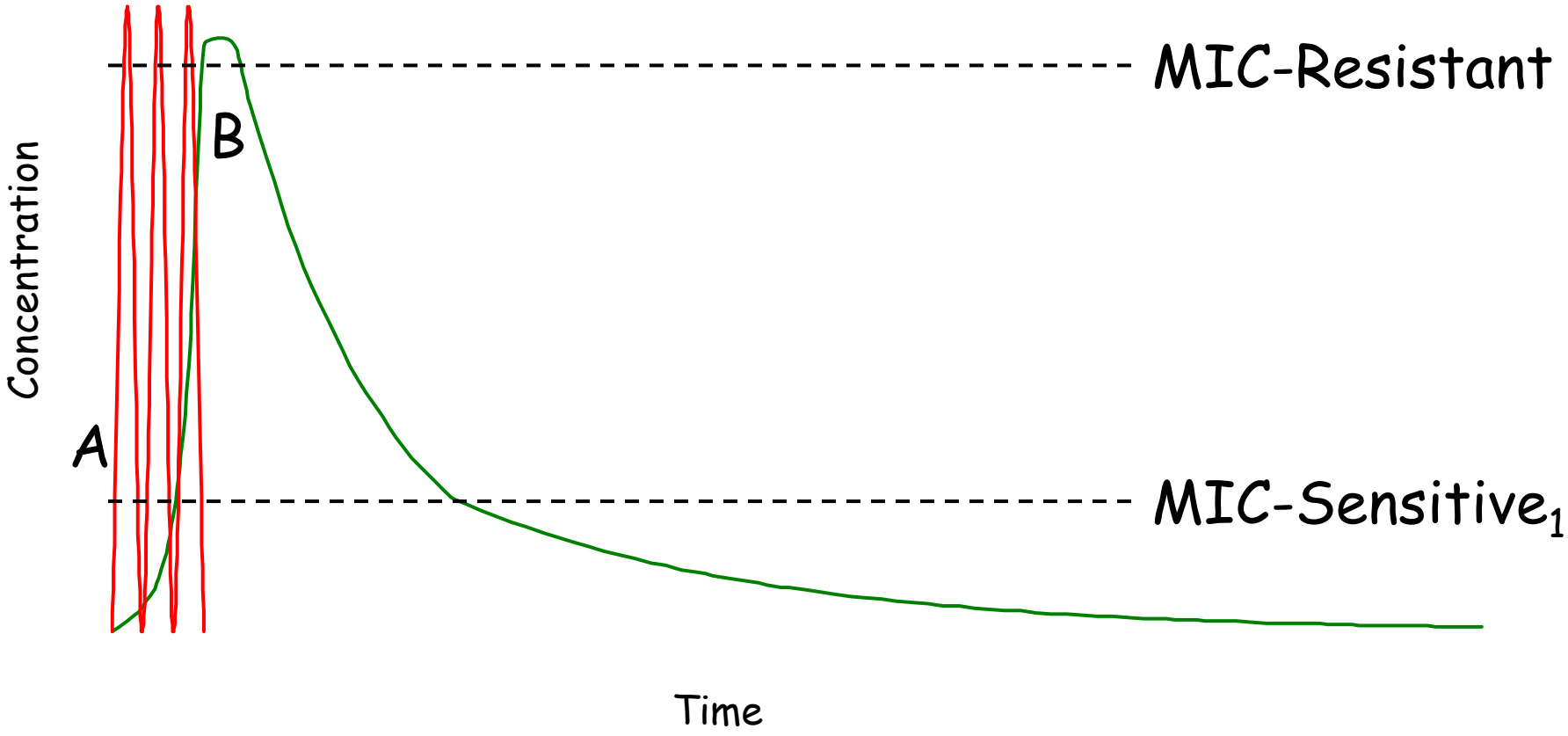
- Ian Hastings
- Bill Watkins
- Nick White

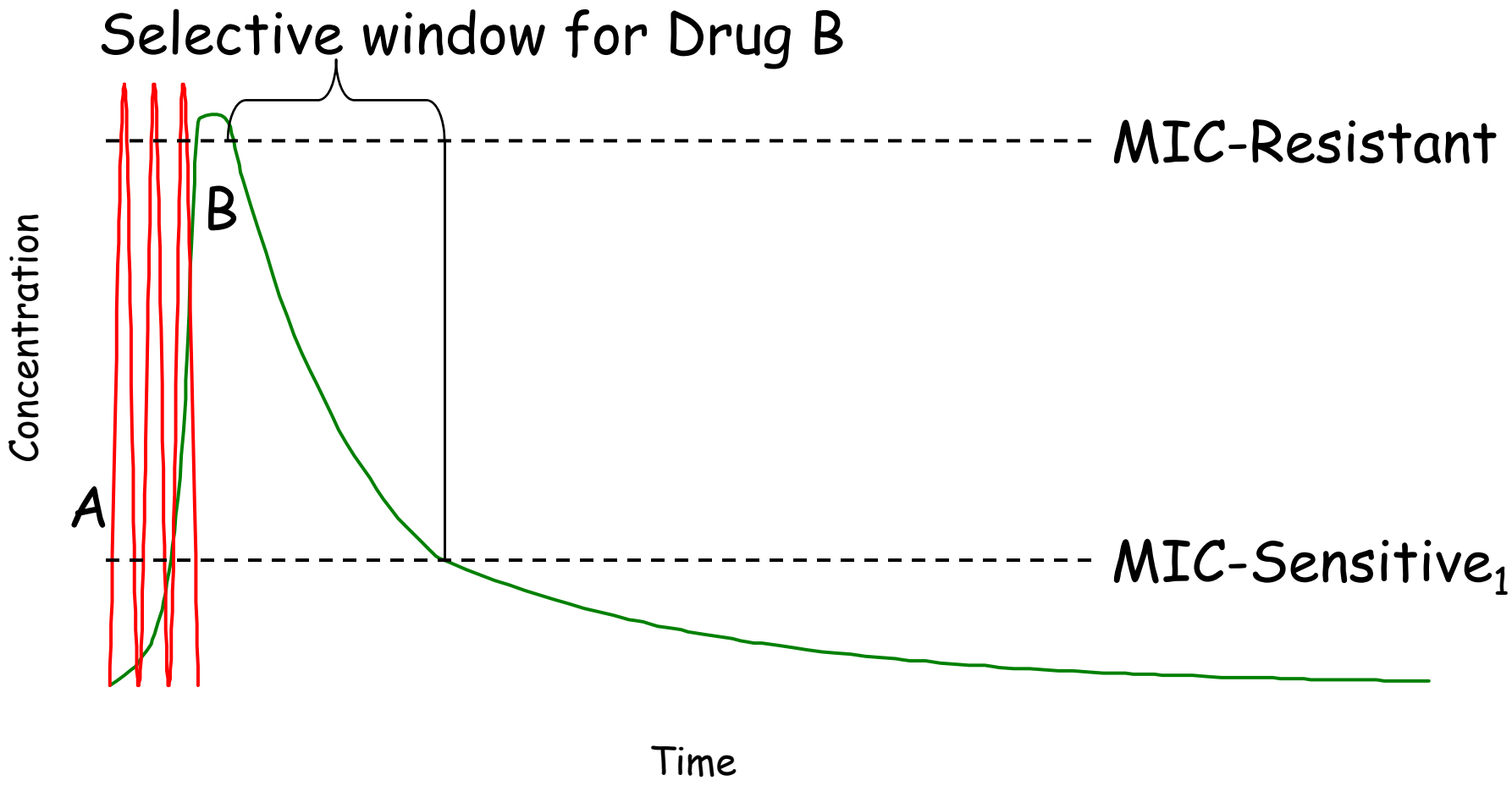


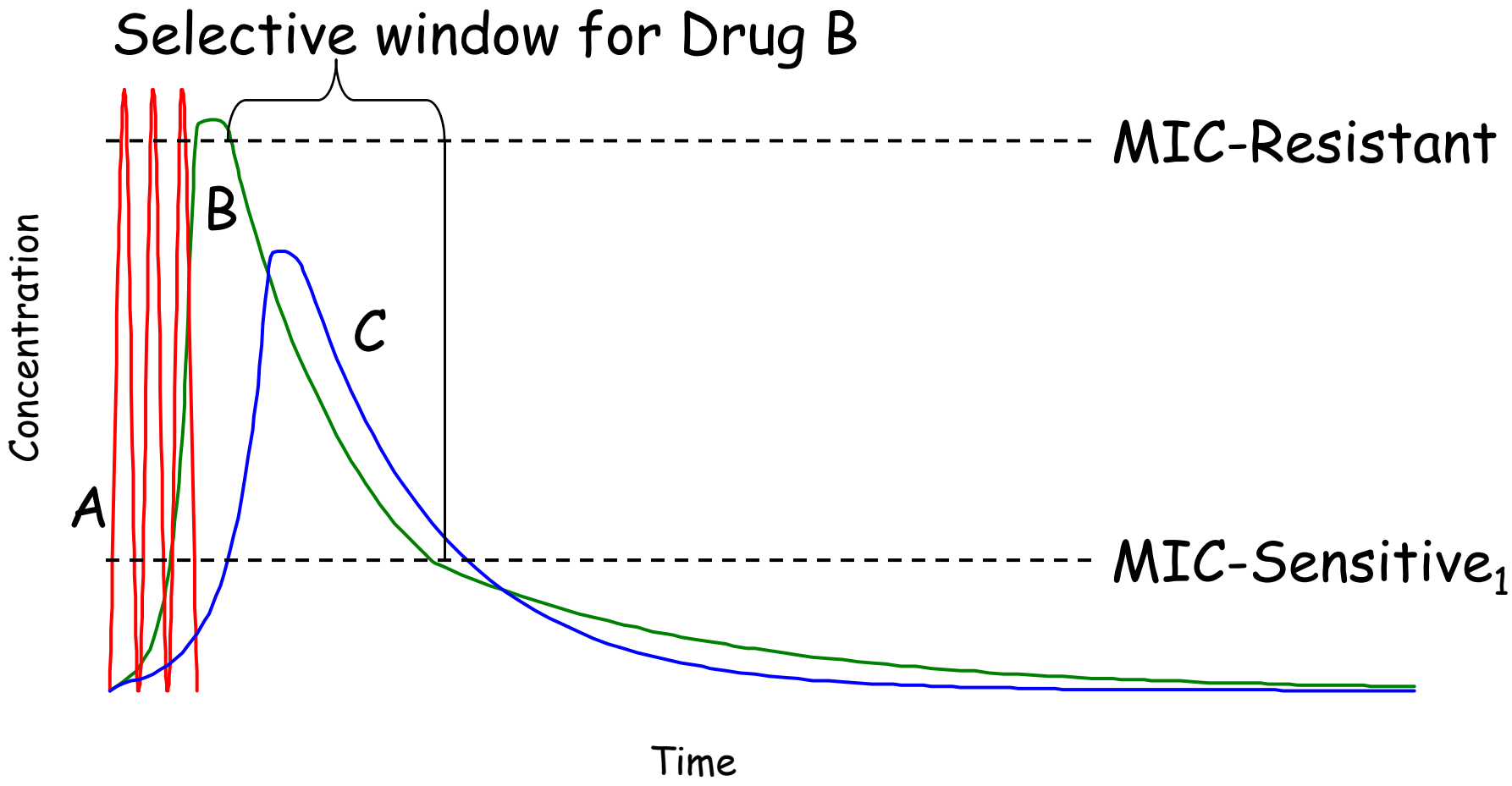

Concentration
A
B
Time

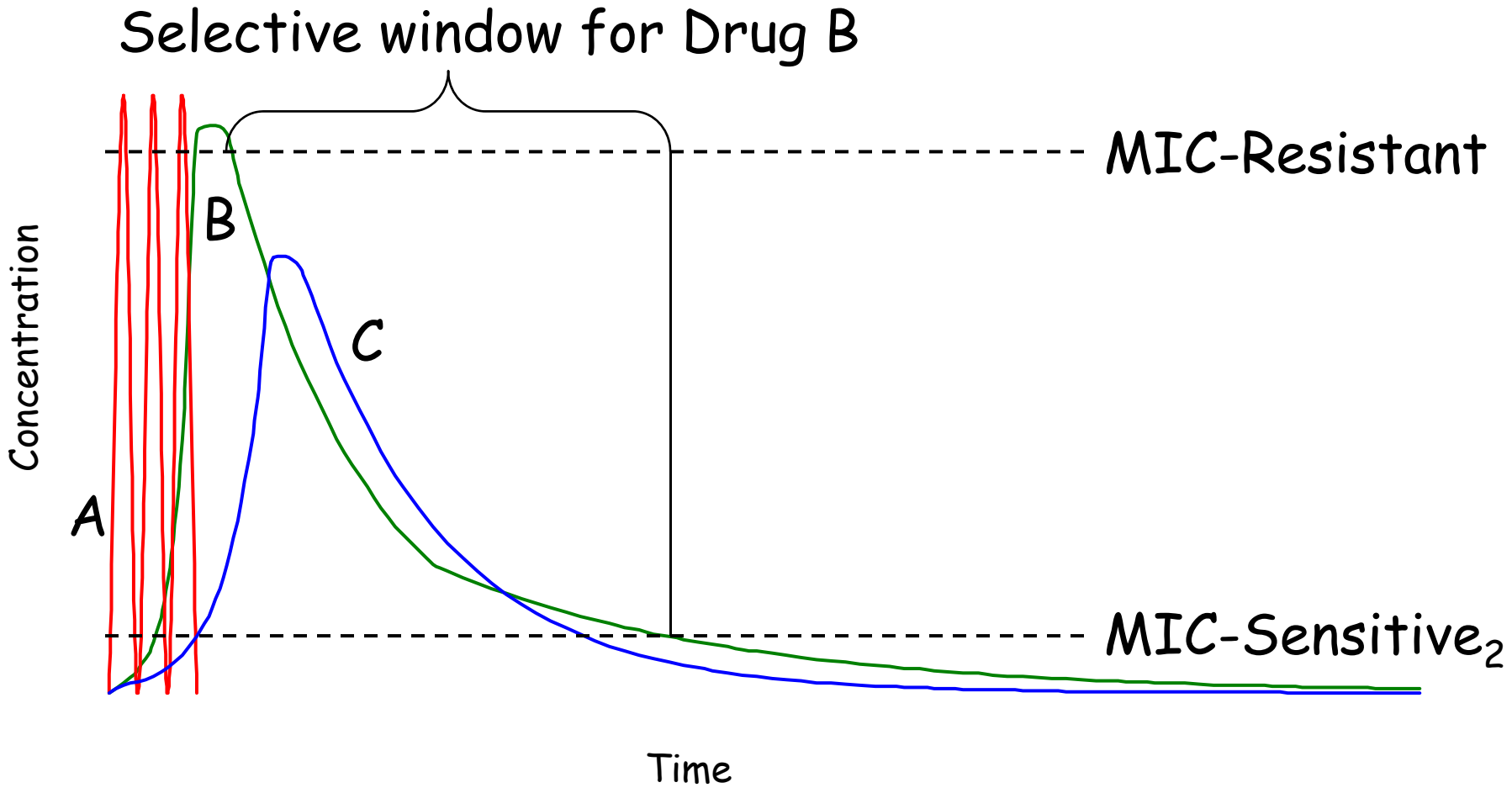

Concentration
A
B
New infections
Time

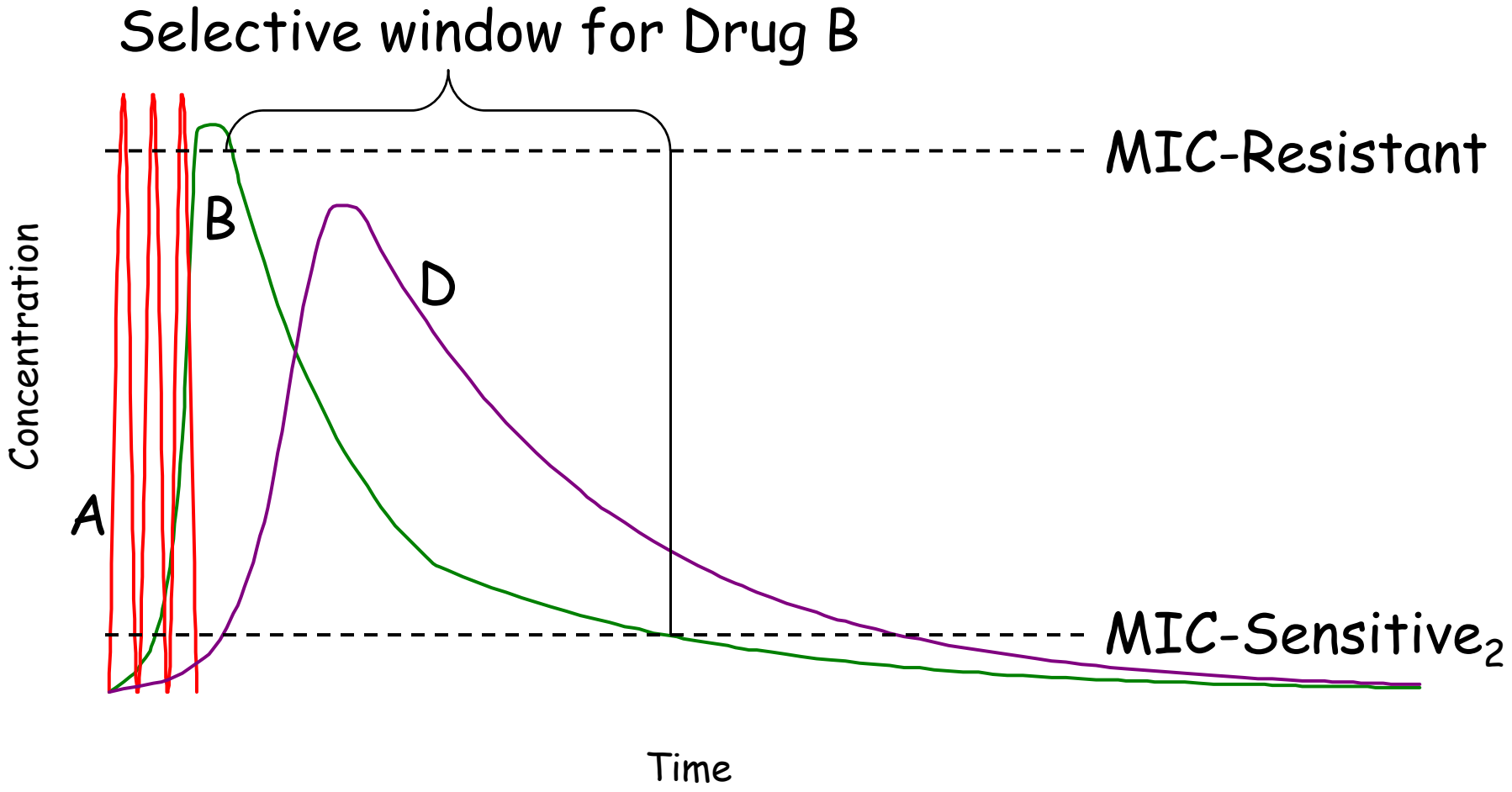

MIC-Resistant
B
A
Concentration
Time


MIC-Resistant
B
Concentration
A
MIC-Sensitive1
Time


Selective window for Drug B
MIC-Resistant
B
A
MIC-Sensitive1
Concentration
Time


Selective window for Drug B
MIC-Resistant
MIC-Sensitive1
Concentration
Time
A
B
C


Selective window for Drug B
MIC-Resistant
MIC-Sensitive2
A
B
C
Concentration
Time

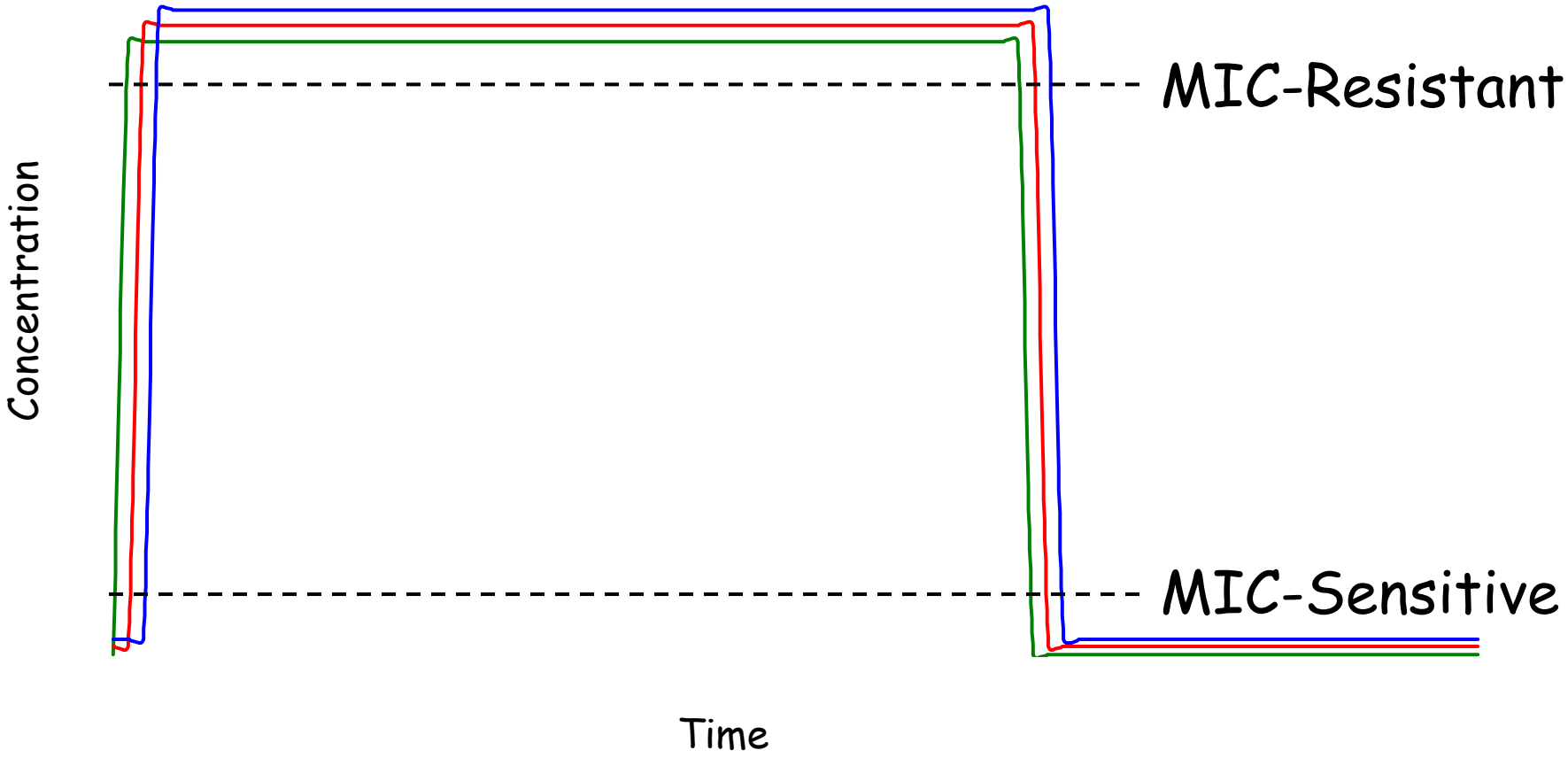

Selective window for Drug B
MIC-Resistant
MIC-Sensitive2
B
D
A
Concentration
Time

Selective windows

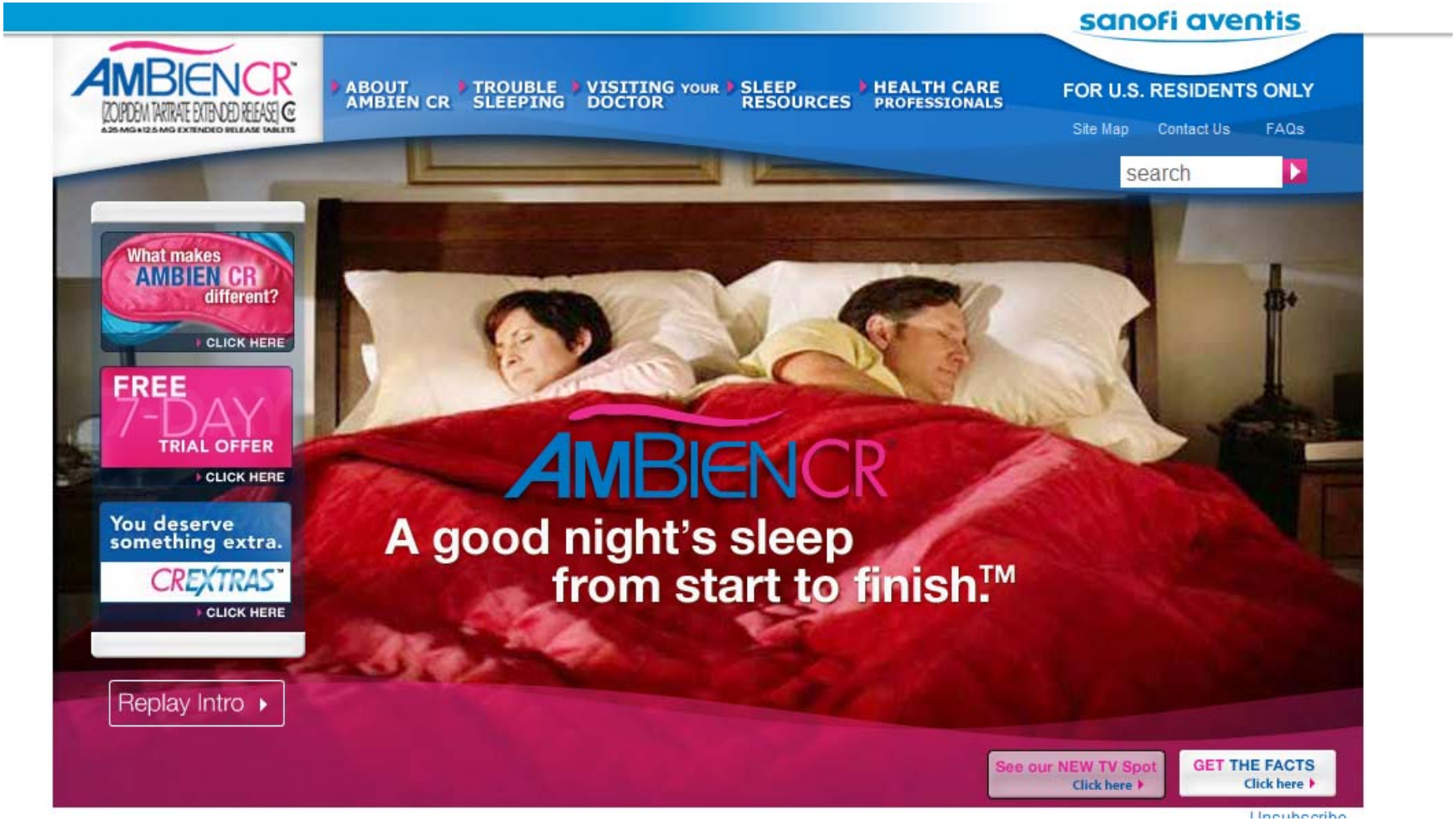
- Defined only for 2 antimalarial drugs
  - Pyrimethamine: 52 days (Watkins & Mosobo 1993)
  - Lumefantrine: 30 days (Hastings & Ward 2005)
- To design combinations with long useful therapeutic lives, need to know selective windows, MICs and durations of action
- Include pharmcokinetic, pharmacodynamic and resistance measures in clinical efficacy trials

The ideal antimalarial drug combination

- Combine drugs with different mechanisms to deter resistance
- Rapid onset to resolve illness
- Long action to allow single dosing and prevent new illness
- Rapid elimination to prevent selection of resistance



Is malaria as important as insomnia?



Controlled-release antimalarials?

- Both rapidly and slowly released components
  - Rapid release to ensure cure
  - Slow release for single dosing and avoid subtherapeutic selective concentrations
  - Used with popular sleep aids
- "Repository" formulations (Peters 1970)
  - Depot injection of cycloguanil provided 6+ months protection against experimental challenge with vivax and falciparum (Contacos et al. 1966)
- Liposomal artesunate (Gabriels & Plaizier-Vercammen 2003)
- Too expensive? In the context of malaria eradication, maybe not…

Summary

- Resistance evolves through various and unpredictable mechanisms
  - Frequent local tolerance → rare resistance → global spread
  - Rare resistance → global spread → secondary modulation
- Malaria "C" and "E" may foster emergence, dissemination and persistence of resistance and this should be anticipated
  - Africa: the new Asia?
- Combinations should protect non-artemisinin partners against resistance
  - Matching half-lives is too simplistic
  - Need to define selective windows and MICs
- Novel pharmacologial approaches are needed to deter resistance

In your folder: Nyunt MM and Plowe CV. Pharmacologic advances in the global control and treatment of malaria: Combination therapy and resistance. *Clinical Pharmacology & Therapeutics* 82(15):601-605, 2007.

Thanks:

University of Maryland
- Miriam Laufer
- Shannon Takala
- Phil Thesing
- Chuka Didigu
- Licheng Zhao

Johns Hopkins
- Myaing Nyunt

Blantyre Malaria Project
- Terrie Taylor
- Fraction Dzinjalamala

AFRIMS
- Harald Noedl
- Mark Fukuda

NIAID
Doris Duke Charitable Foundation
Howard Hughes Medical Institute
My parents