



# LE PLASMODIUM EN 2016: BIOLOGIE, EPIDEMIOLOGIE, CLINIQUES, DIAGNOSTIC, TRAITEMENT ET PREVENTION

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*MEETING ORDRE DES MEDECINS MAI 21, 2016*

# MALI POPULATION DIVERSITY AND MALARIA

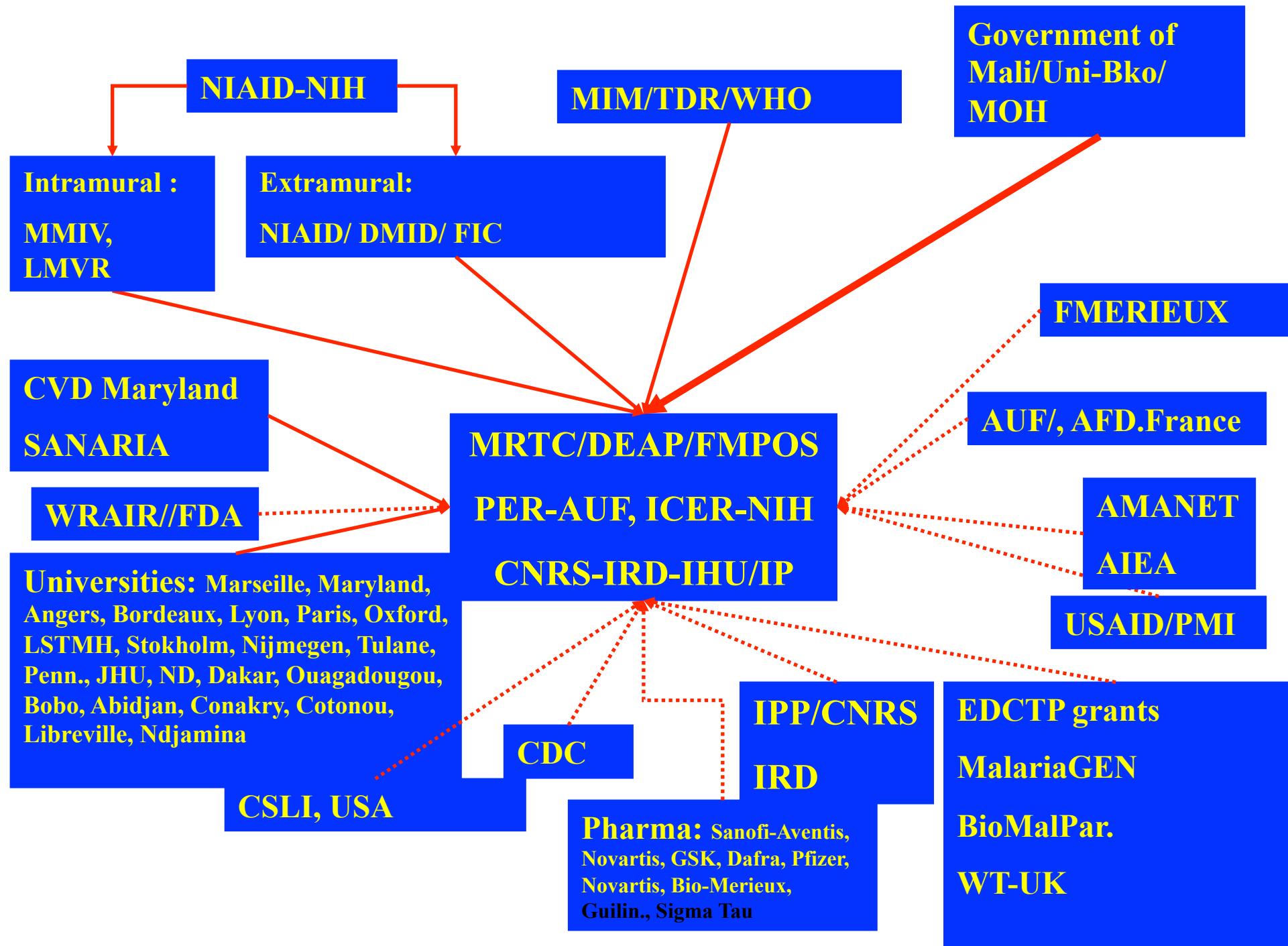


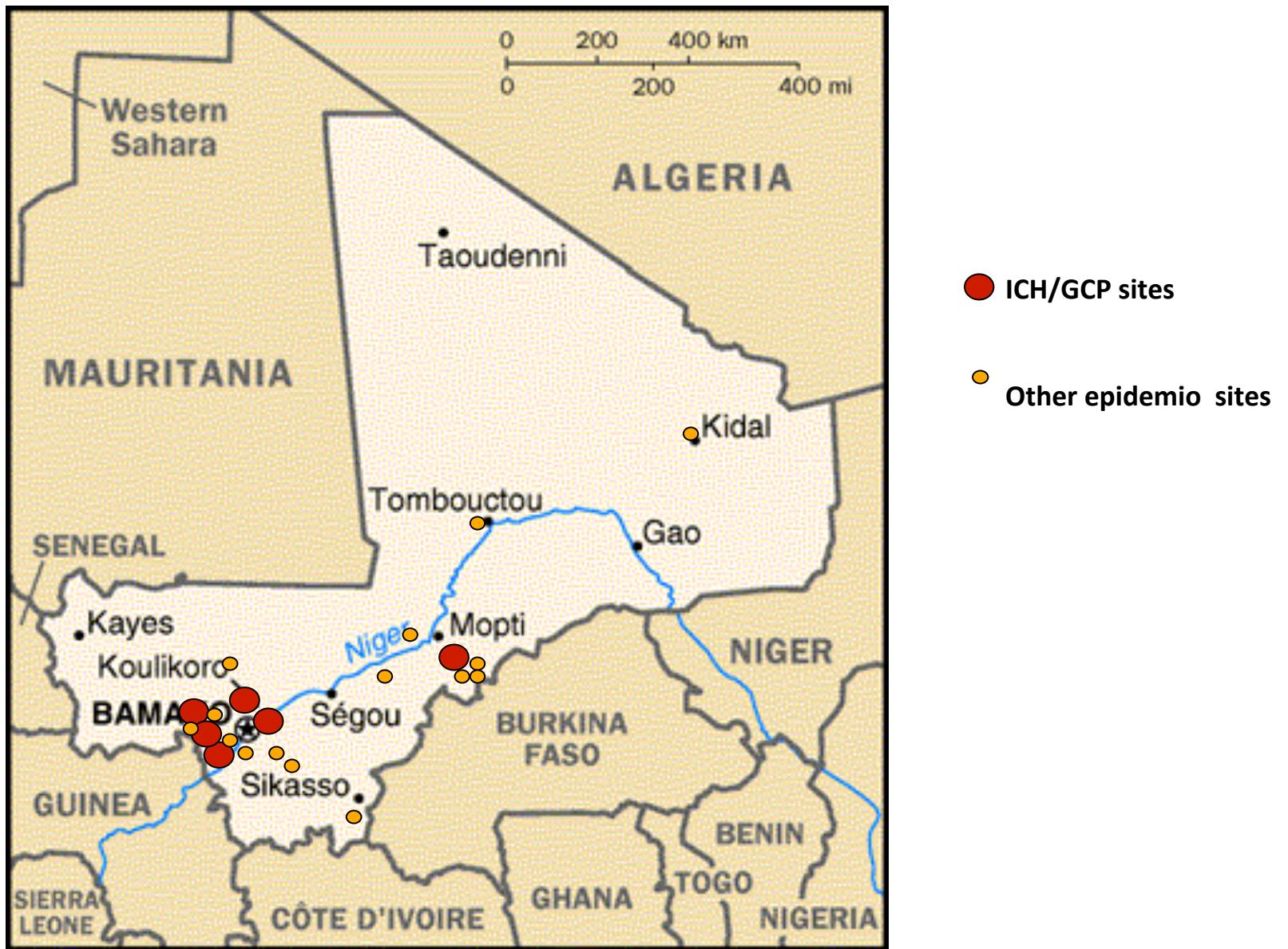


**Malaria = 3 sunami**

**Per year in Africa =  
INTERNATIONAL SOLIDARITY AND  
ETHICAL CONCERNS → NEW TOOLS**

- 1] DIAGNOSTIC**
- 2] TREATMENT**
- 3] VACCINES**

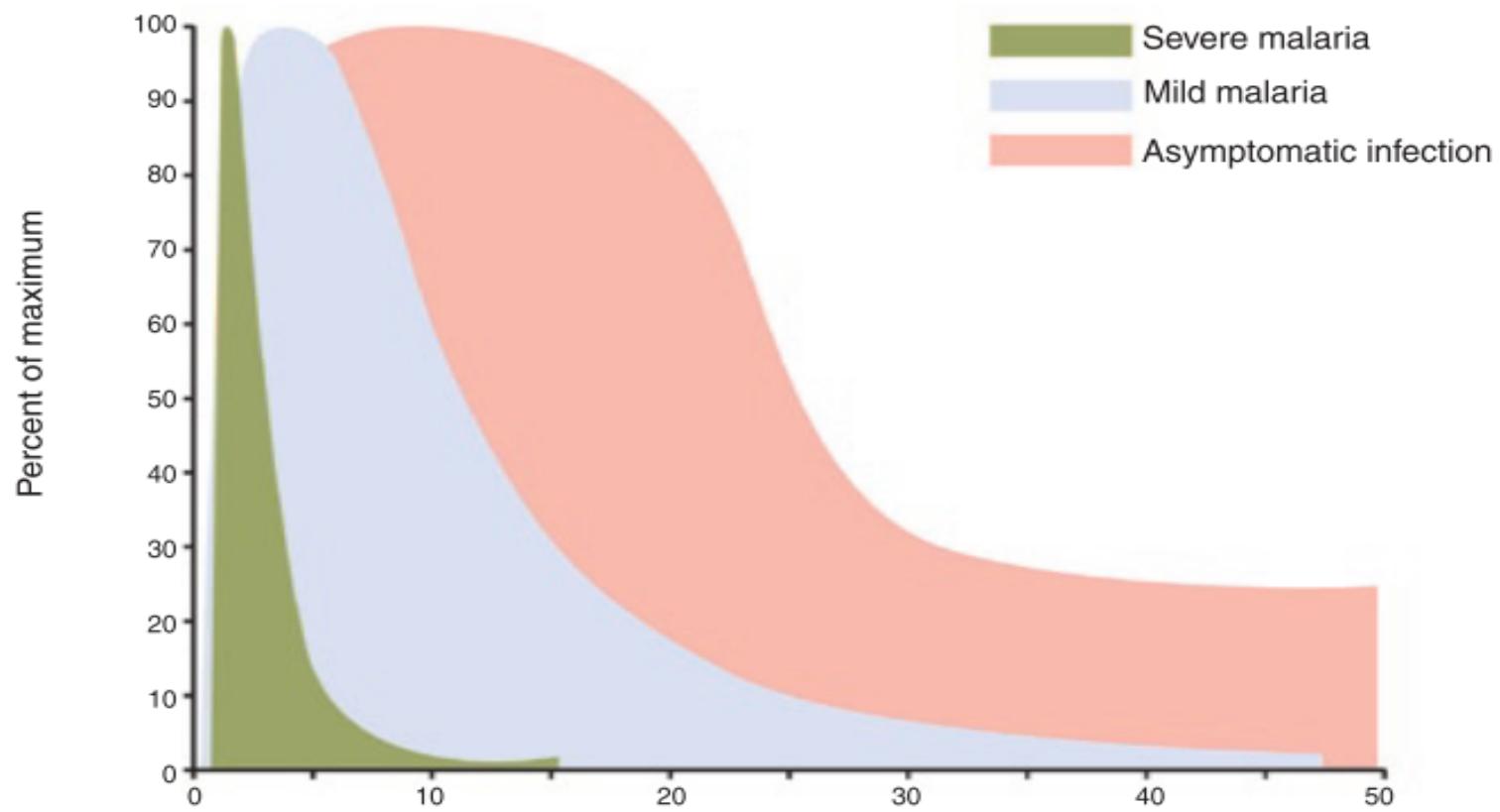




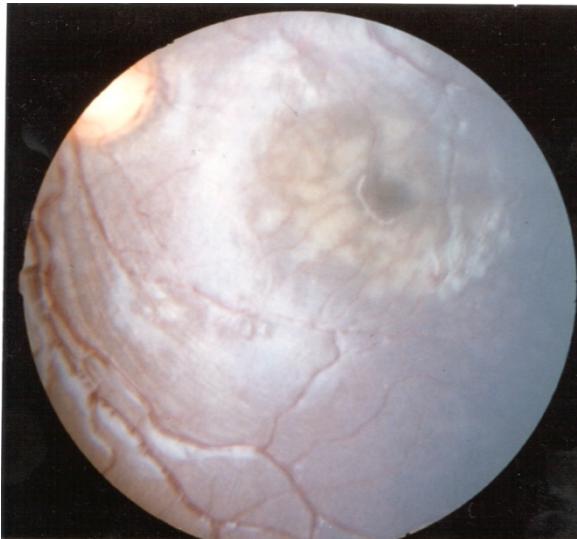
# MALARIA SPECIES IN HOMO SAPIENS

- 1] *Plasmodium falciparum* (*Africa*)
- 2] *Plasmodium malaria* (*Africa*)
- 3] *Plasmodium vivax* (*Africa*)
- 4] *Plasmodium ovale* (*Africa*)
  - *Plasmodium ovale wallikeri*
  - *Plasmodium ovale curtisi*
- 5] *Plasmodium knowlesi* (*SEA*)

# NATURAL HISTORY OF MALARIA IN ENDEMIC COUNTRIES: CHANGING EPIDEMIOLOGY IN AFRICA



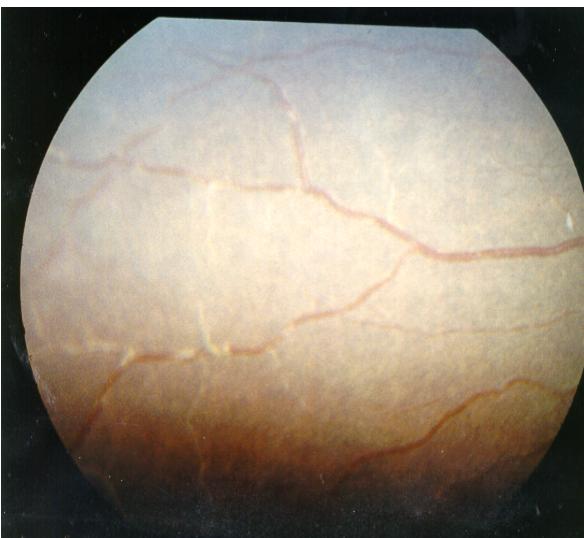
## The four ocular fundus features comprising the malaria retinopathy. In U5



(a) Whitening, shown here in the peri-macular area.



(b) White-centered hemorrhages, in a patient with whitening.



(c) Vessel changes. Note that the changes can be patchy



(d) Papilledema, more readily recognized with an indirect ophthalmoscope because a binocular, three-dimensional image can be attained.

# Malaria prevention Measures and the needs of molecular resistance surveillance

## Chemoprevention



IPTp: 3 to 4 doses os SP  
From the 2<sup>nd</sup> trimester

Monthly administration  
of SP+AQ during the  
transmission season

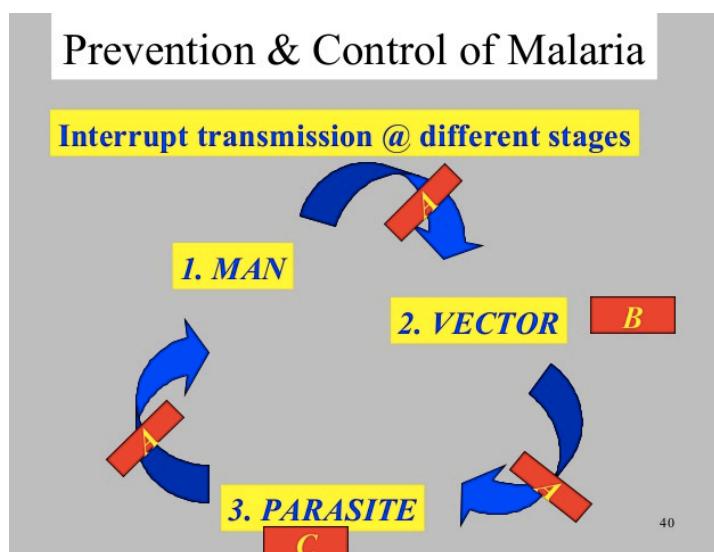
## Mechanical prevention



Continuous distribution  
Of LLINs through  
Antenatal and  
immunization services



IRS



# Vaccins antipaludiques: Stades et Impact

## Pré-érythrocytique

Vaccins pour prévenir l'infection et impacter sur la maladie

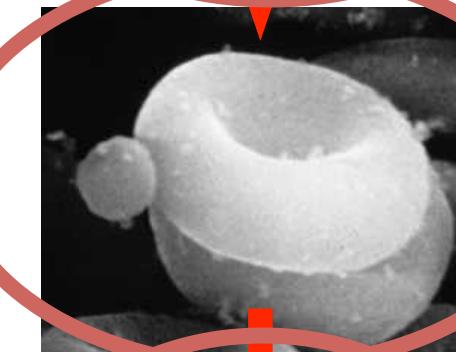
## Stade Sanguins

Vaccins pour éliminer la maladie

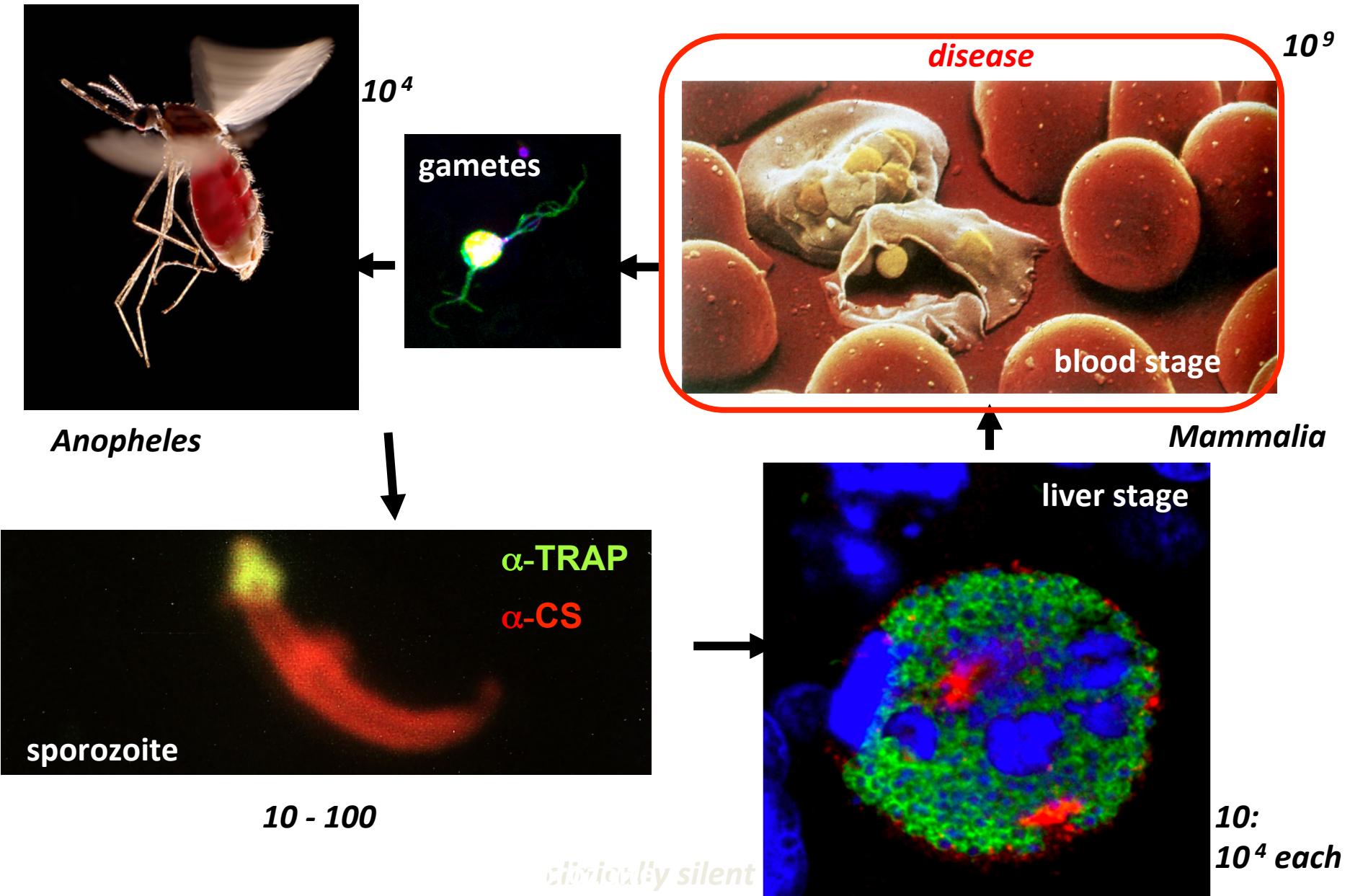
## Bloquant la Transmission

Vaccins pour prévenir la transmission

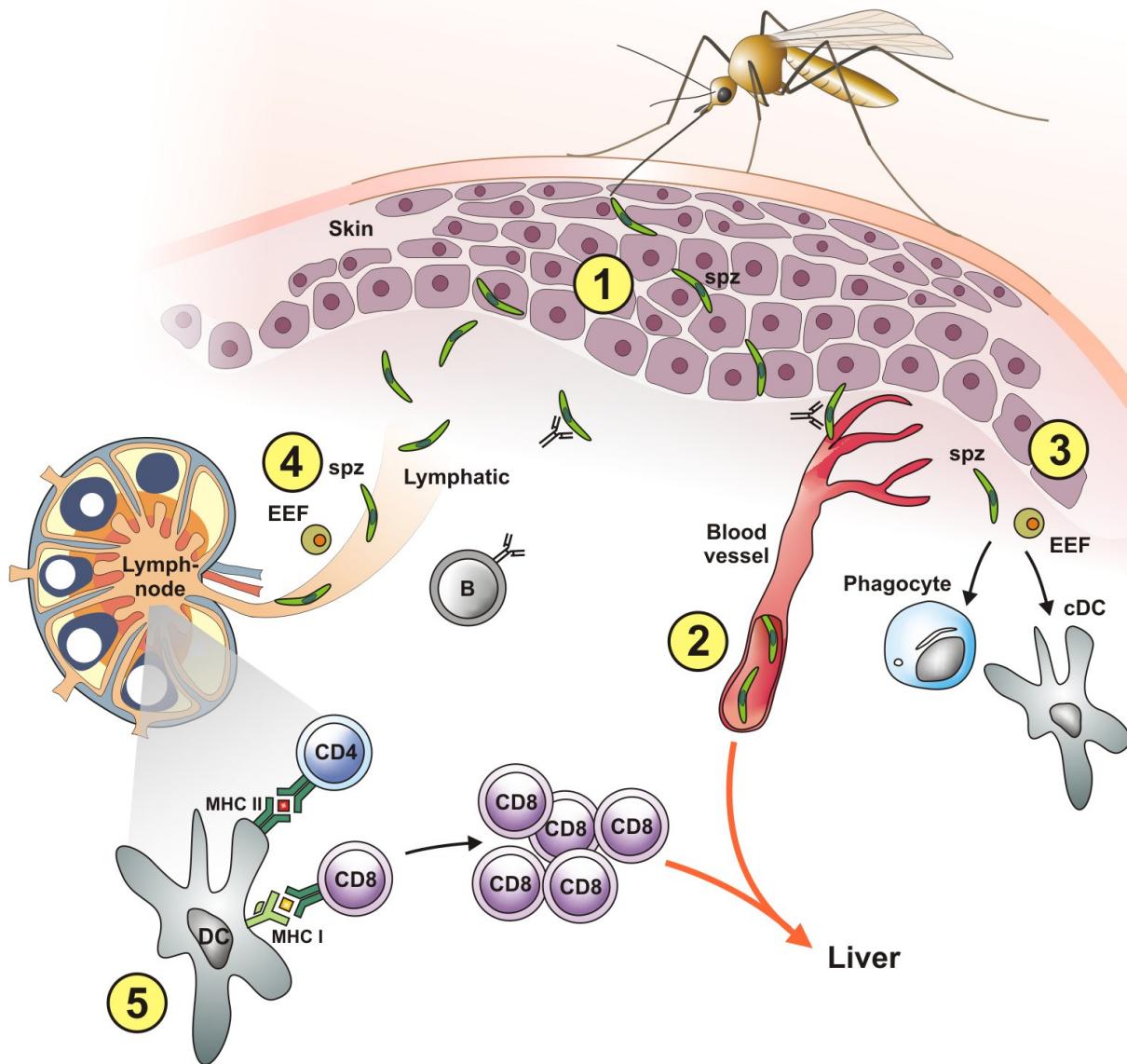
Vaccins qui interrompent la Transmission du Paludisme



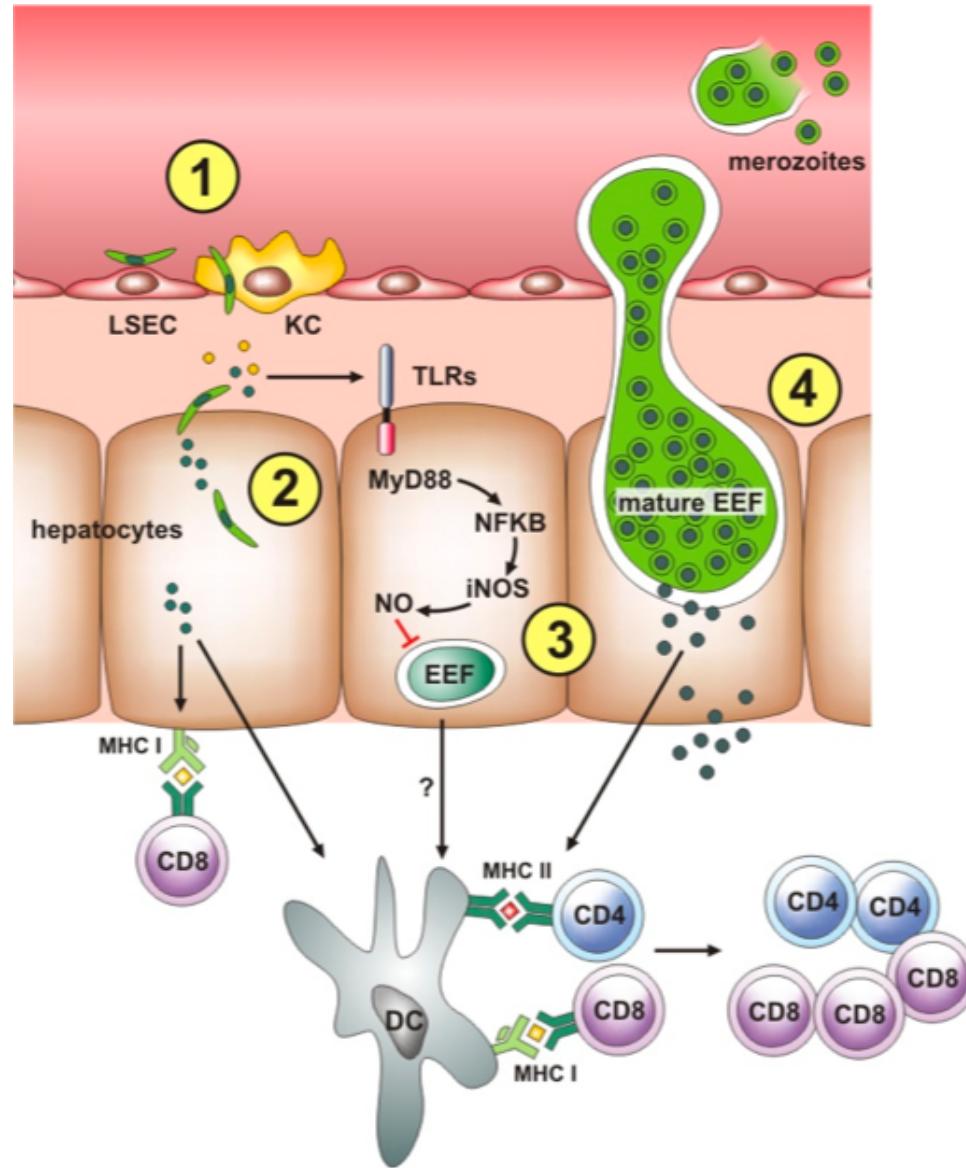
# The *Plasmodium* life cycle



## *What new in Plasmodium biology, transmission and building of natural immunity in vertebral host*



# *Plasmodium* liver stages: induction of immune responses

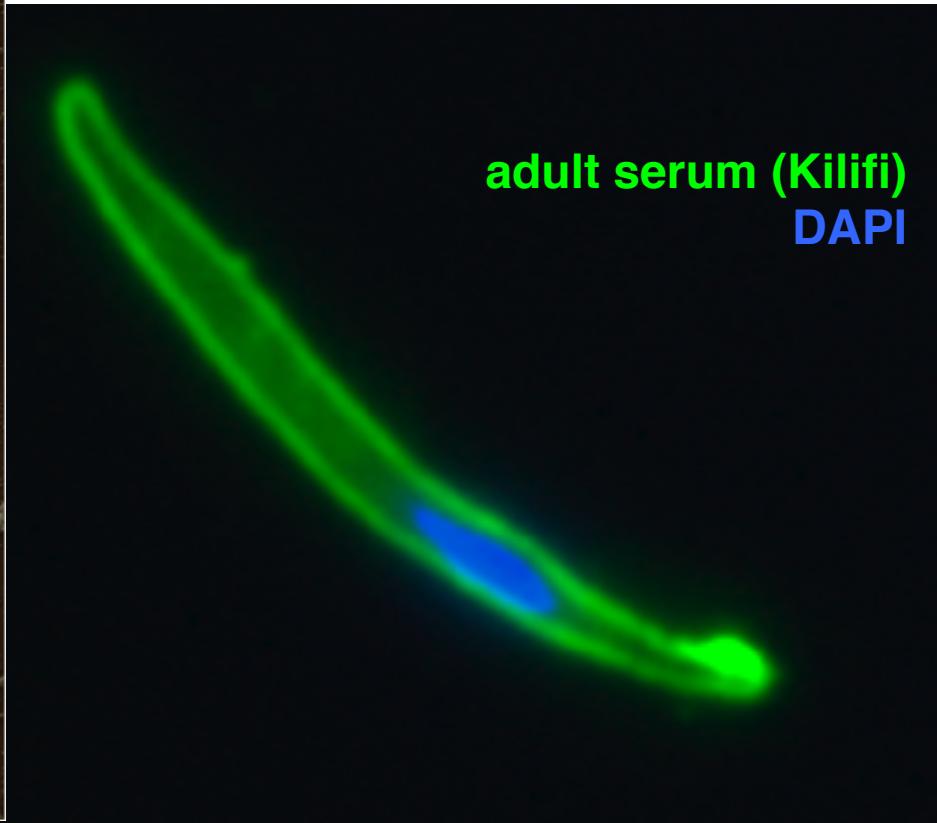


Hafalla et al., 2011

# Phase Ib Trial in Adults of PfSPZ-Sanaria DIV Whole Sporozoite vaccine candidate in Mali: 2014-2016



*Pb* sporozoite

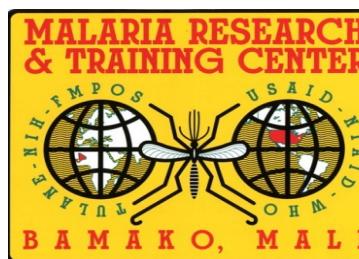


*Pf* sporozoite

# **Assessment of Safety and Immunogenicity of Intravenous Immunization with radiation attenuated *Plasmodium falciparum* NF54 Sporozoites (PfSPZ Vaccine) in Healthy Malian Adults in Africa**

**Principal Investigators:** Dr Mahamadou S. Sissoko, MRTC, USTTB, Mali  
Dr Michael Walther, LMIV/NIAID/NIH, USA

**Senior Investigators:** Ogobara Doumbo, MRTC, USTTB, Bamako, Mali  
Patrick Duffy, LMIV/NIAID/NIH, USA



&



**LABORATORY OF  
MALARIA**  
Immunology &  
Vaccinology

*IND Sponsor: Sanaria Inc.*

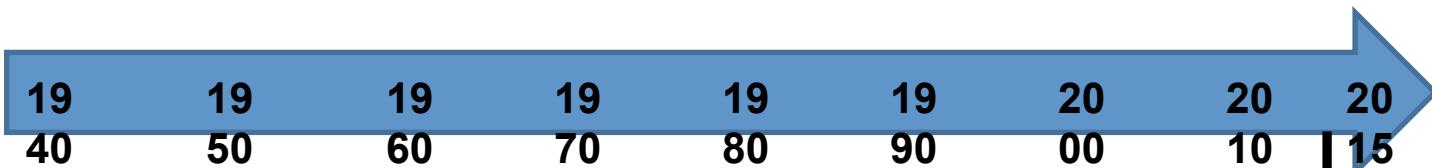
# Background

## Irradiated Sporozoites via Mosquito Bite

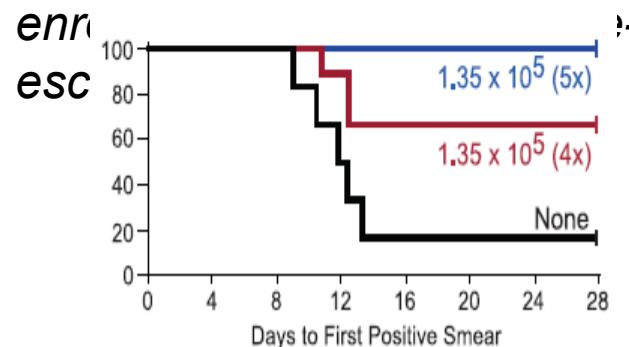
- Development had not been previously pursued due to:
  - Considered technically impractical or impossible
  - Considered unnecessary since modern subunit vaccines would solve the problem



# Clinical Trials: PfSPZ DVI Trial



*Open-label evaluation of the safety, tolerability, immunogenicity and protective efficacy against CHMI of the PfSPZ vaccine at successively higher dosages. Subjects are enrolled sequentially.*

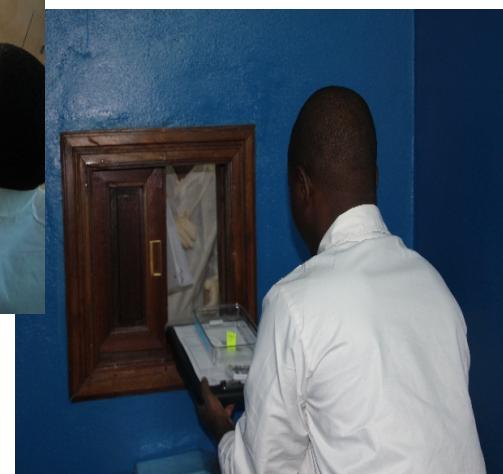
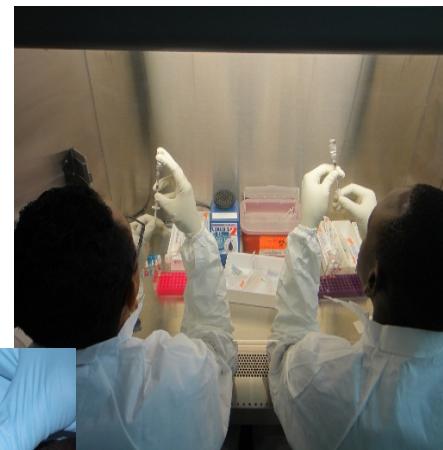


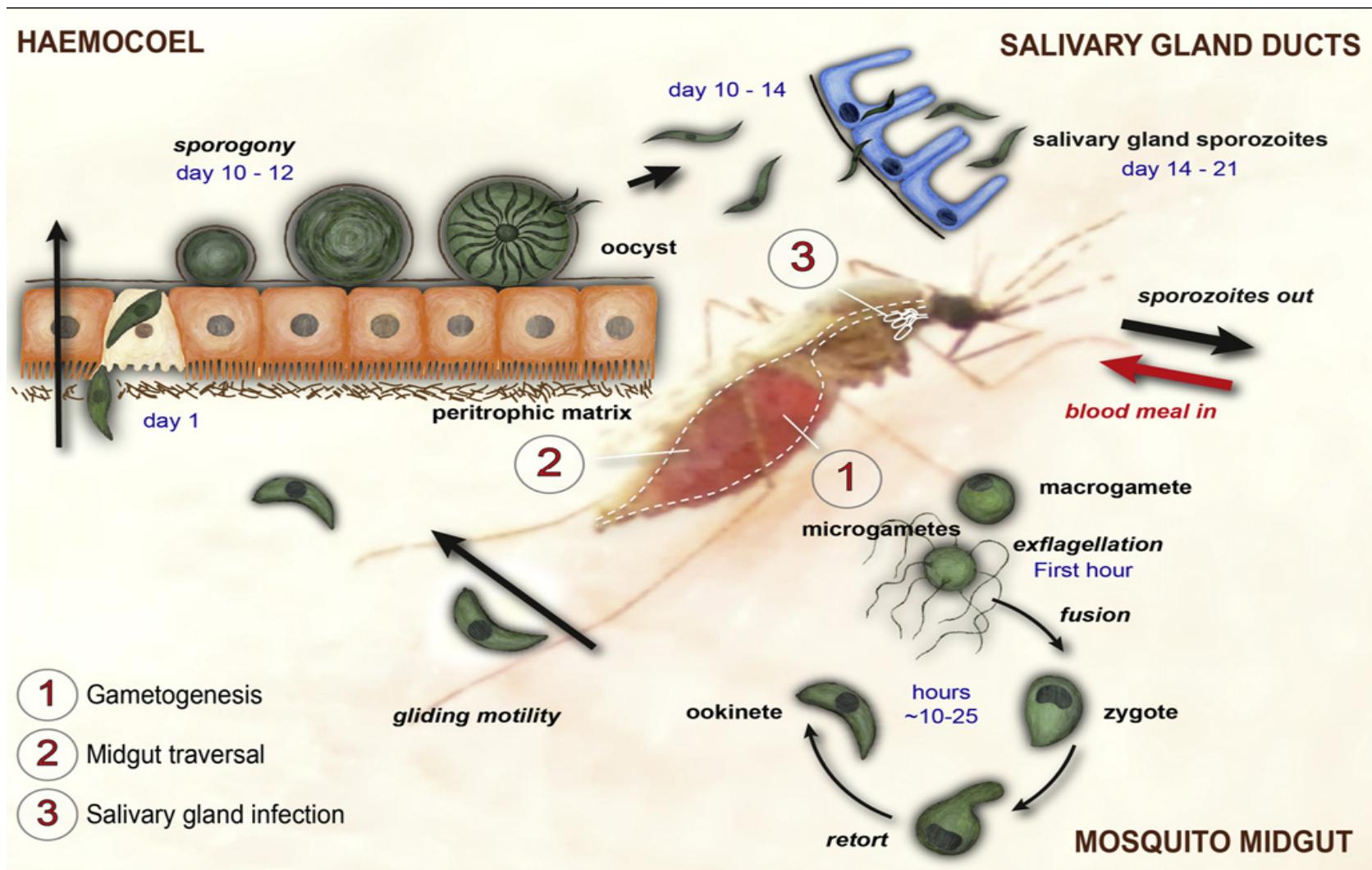
Seder et al. Science. 2013

Vaccination Dose	CHMI # Inj.	# of Parasite* Subjects	Parasite Free	Vaccine Efficacy
None		3D7 6	1	
1.35 x 10 <sup>5</sup>	4	3D7 9	6	60%
1.35 x 10 <sup>5</sup>	5	3D7 6	6	100%

# PfSPZ Vaccine in Mali: Natural Transmission

- Pilot Safety Group – completed in March 2014
- Main Study Group – Vaccination #5 of 5 completed in July 2014
- 502 vaccinations administered
- PhaseIb: in 2016





# *Direct Skin Feeds and TBV in Mali*

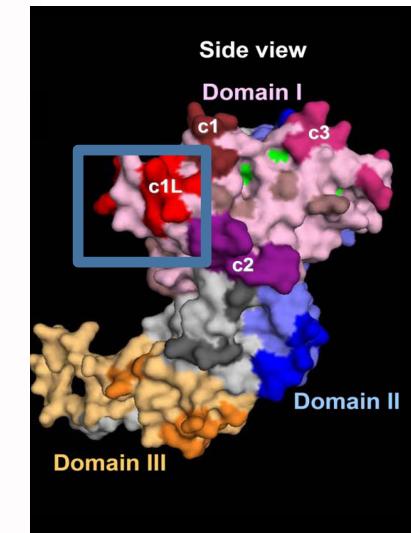
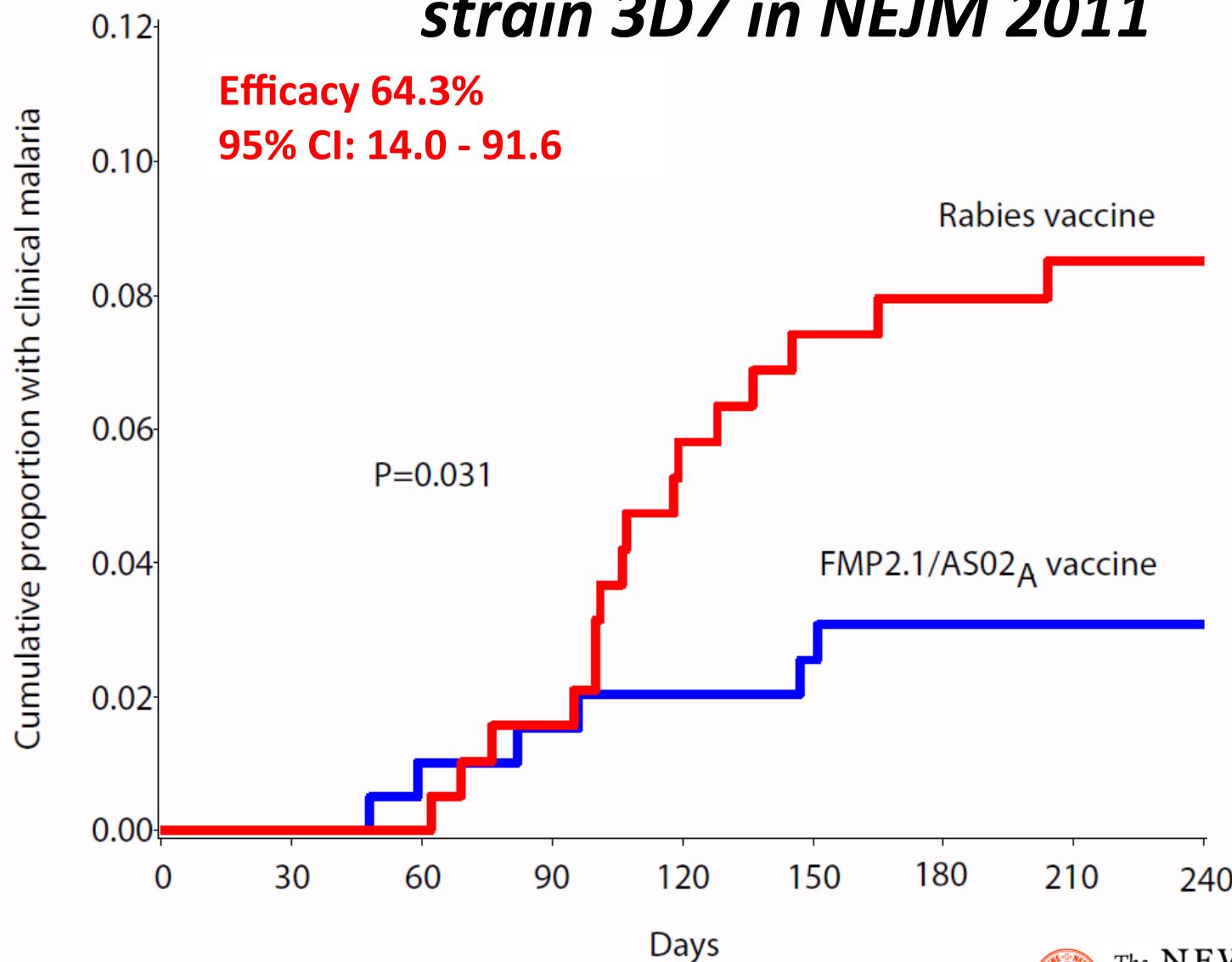


- Have been conducted in Mali since the early 1990's
- Mosquitoes reared in Bamako
  - Laboratory colony of *A. gambiae* established from a local catch in 2008 are used
- Mosquitoes are transported to the site for feeds
- 2 feeding pints with 30 pre-starved mosquitoes/pint
- Placed on subjects for 15-20 minutes
- Topical antihistamine/antipruritics offered following feeds to subjects
- Only one DSF related AE in one subject (Grade 2 erythema) has been reported in over 2,000 DSF

**Coulibaly, M et al. Symposium 126: Malaria Transmission:  
How Will We Assess Vaccines for Elimination and  
Eradication? ASTMH2015**

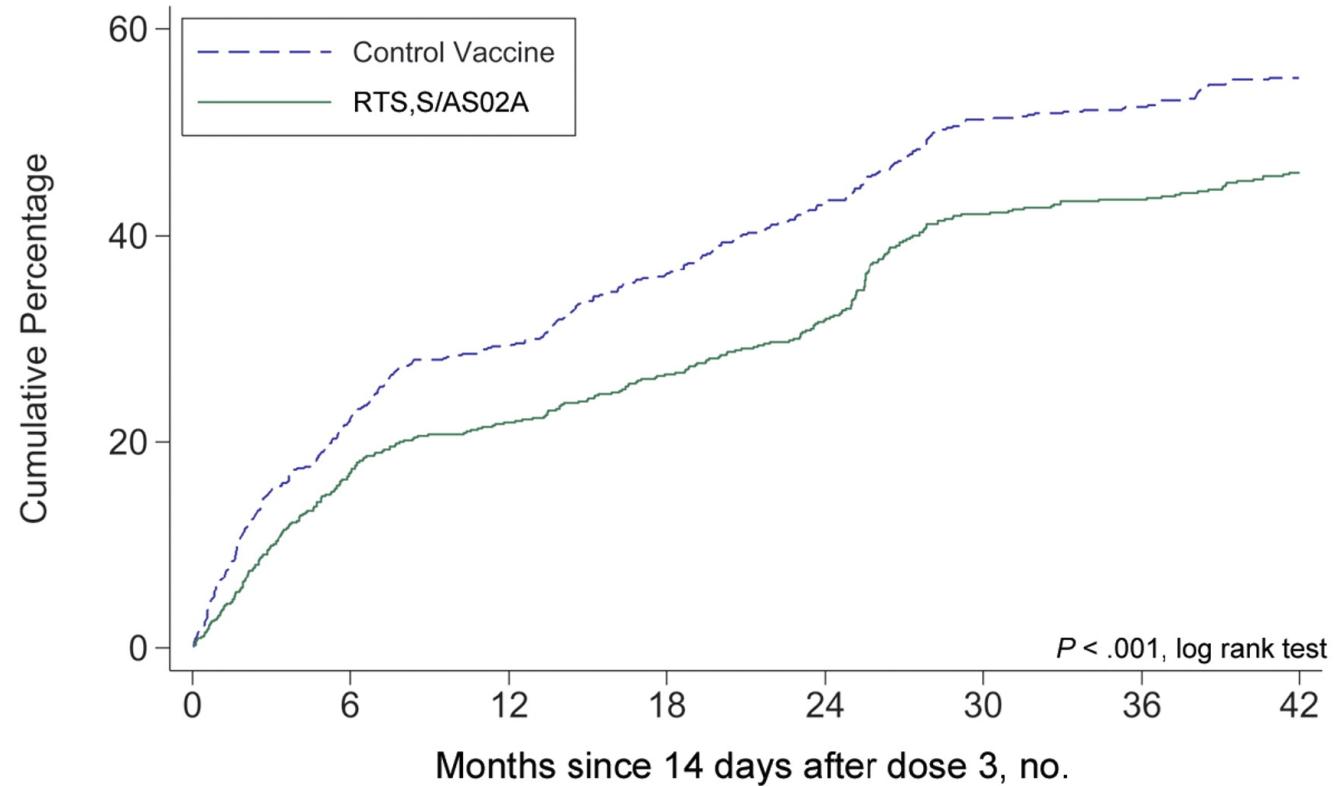
# *Malaria Vaccine Molecular endpoint : Strain-specific efficacy against clinical malaria matching vaccine*

*strain 3D7 in NEJM 2011*



# RTS,S (VE=≈50% (EMA 58, WHO 2015)

Kaplan-Meier curves for the cumulative proportion of children with ≥1 episode of clinical malaria.



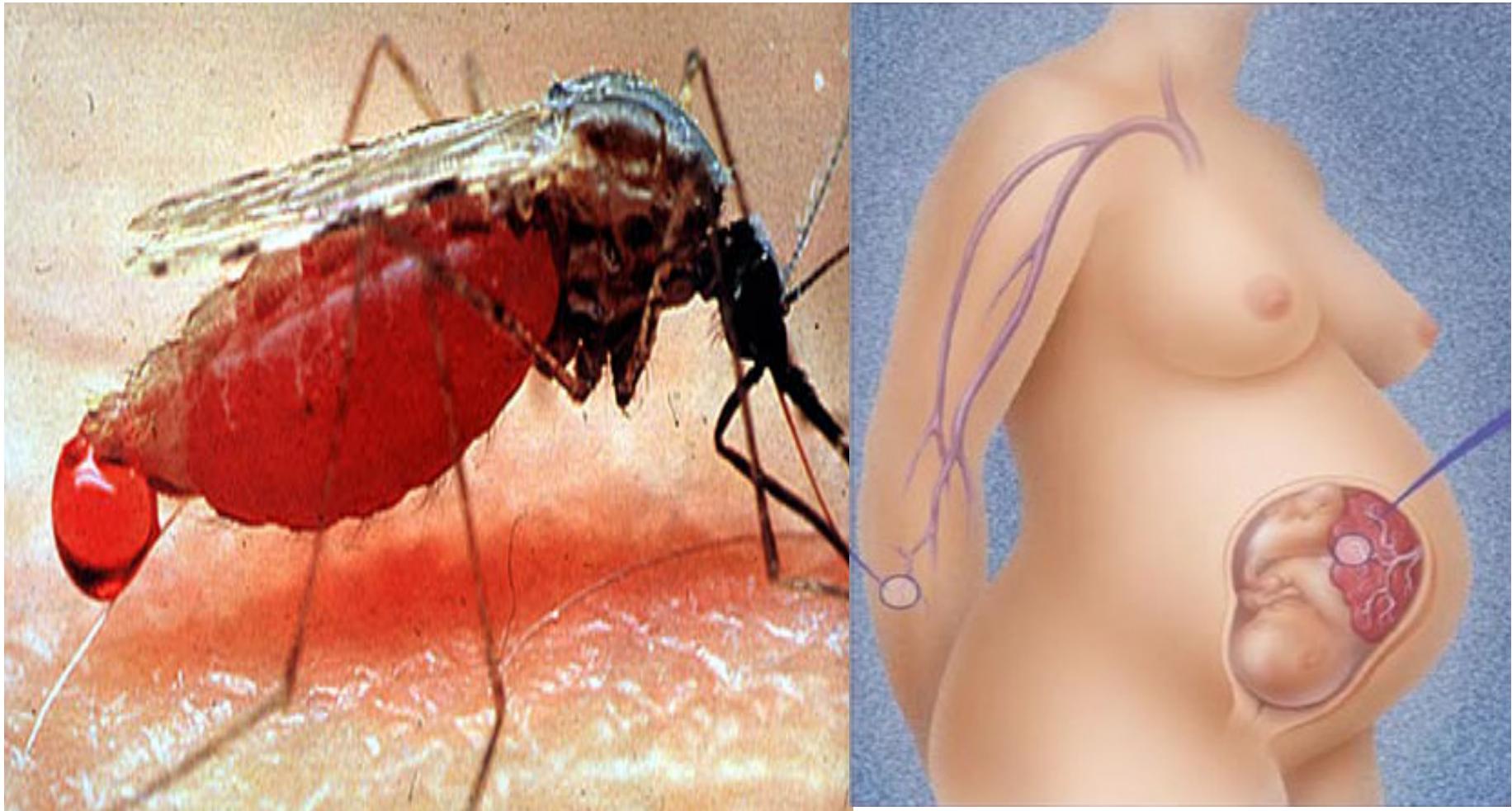
Efficacy 30-60%  
against clinical  
disease and  
infection

## No. at risk

Control	745	570	512	428	377	324	312	272
RTS,S	745	599	564	496	449	382	373	324

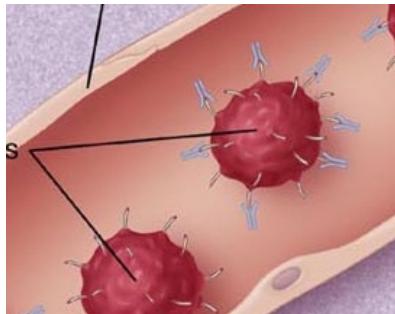
**1] *Plasmodium falciparum* –*Homo sapiens*: 6000-8000 years of co-evolution and co-adaptation**

**2] IN UTERO BURDEN OF MALARIA IN AFRICA: IMPACT ON  
INTELLECTUAL DEVELOPMENT, SCHOOL ATTENDENCY AND NATION  
WIDE CREATIVITY**

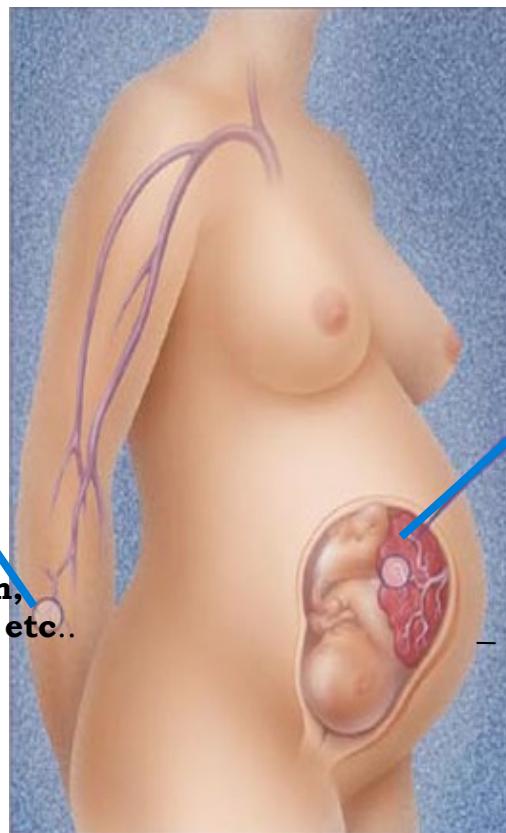


## BIOLOGY OF PREGNANCY ASSOCIATED MALARIA

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CD36, ICAM-1, E-Selectin,  
VECAM, PECAM-1/CD31 etc..

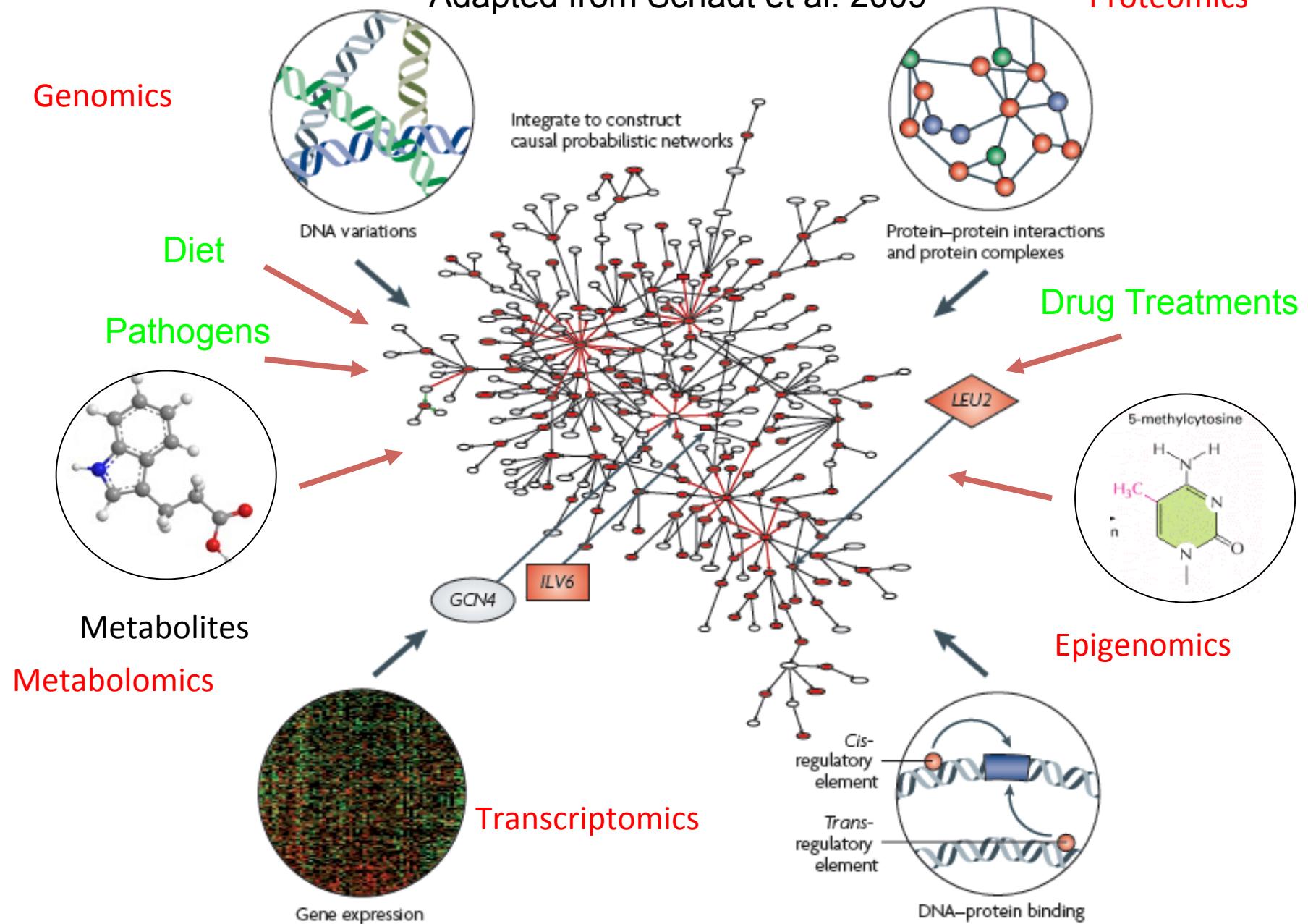


- Receptors on placenta**
- CSA +++
  - Hyaluronic Acid
  - Fc IgG
  - others receptors ?

*Fried & Duffy, 1996, 1998, 2000 and others from field  
to labs and toward PAM vaccines candidates, Phasela,b*

# Systems Biology Approach and Big Data

Adapted from Schadt et al. 2009



# **HOMO SAPIENS EST UN ECOSYSTEME ET UNE CHIMERE GENETIQUE**

- 1] POUR UNE CELLULE DITE HUMAINE
- 2] 1000 VIRUS
- 3] 100 BACTERIES
- 4] 10 ARCHAEA
  - MAINTIENT DE L'HOMEOSTASIE ET MALADIE?



We are composed of several species:

- Eucaryotic
- Bacterial
- Archaea

As adults our microbial census exceeds the total number of our own human cells

- By about 10 fold

The largest collection of microbes resides within the intestine

- With  $10^{13-14}$  cells!!!!
- Several hundreds of species
- «The GUT MICROBIOTA»

**90 % microbes**

**100 % Human ?**



**10 % human cells**



FONDATION MERIEUX

# THE HUMAN

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

**25  
SPECIES**

in the stomach include:

- *Helicobacter pylori*
- *Streptococcus thermophilus*

**500-  
1,000  
SPECIES**

in the intestines include:

- *Lactobacillus casei*
- *Lactobacillus reuteri*
- *Lactobacillus gasseri*
- *Escherichia coli*
- *Bacteroides fragilis*
- *Bacteroides thetaiotaomicron*
- *Lactobacillus rhamnosus*
- *Clostridium difficile*

# MICROBIOME

**600+  
SPECIES**

in the mouth, pharynx and respiratory system include:

- *Streptococcus viridans*
- *Neisseria sicca*
- *Candida albicans*
- *Streptococcus salivarius*

**1,000  
SPECIES**

in the skin include:

- *Pityrosporum ovale*
- *Staphylococcus epidermidis*
- *Corynebacterium jeikeium*
- *Trichosporon*
- *Staphylococcus haemolyticus*

**60  
SPECIES**

in the urogenital tract include:

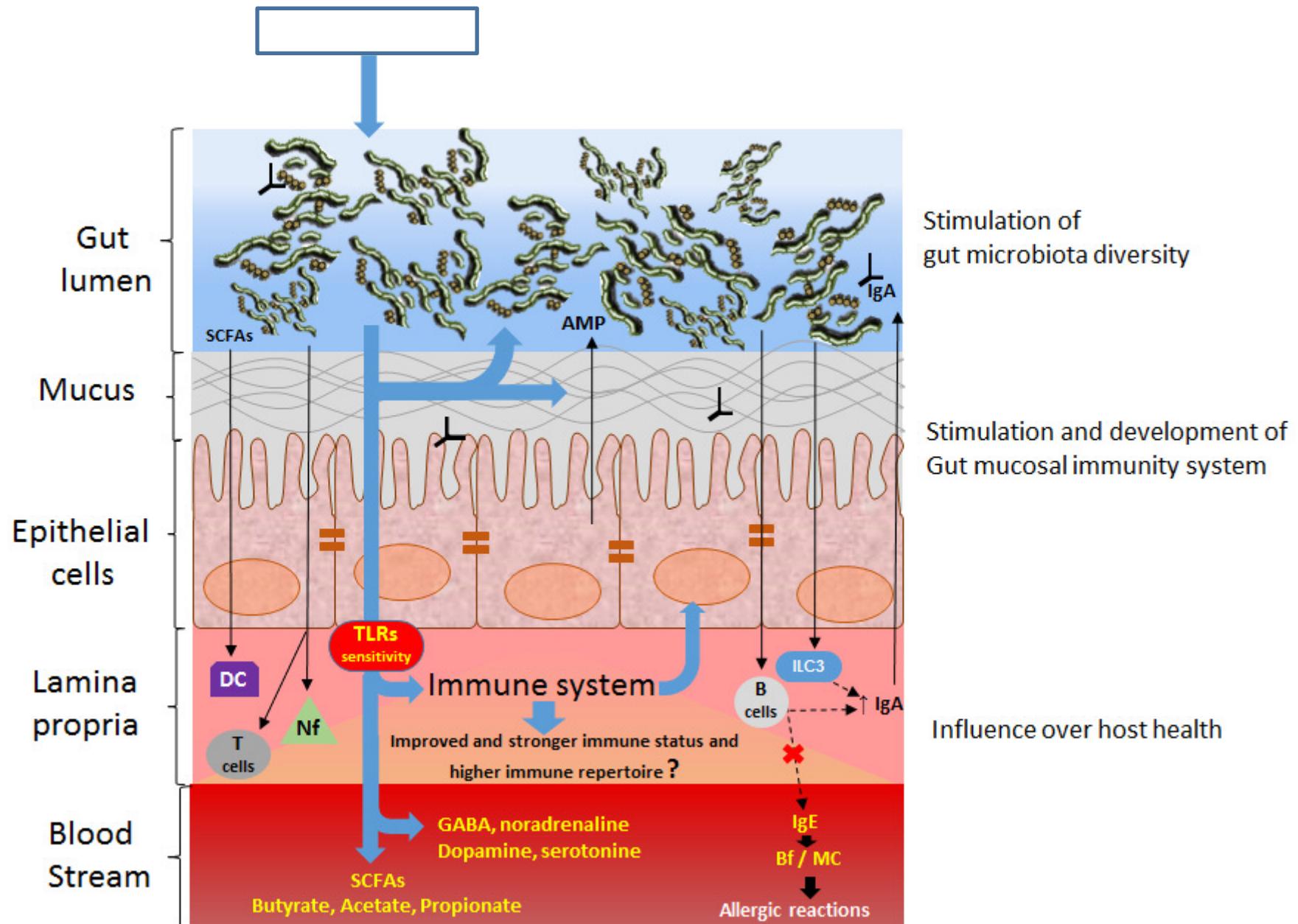
- *Ureaplasma parvum*
- *Corynebacterium aurimucosum*



# Diseases associated with microbiome composition

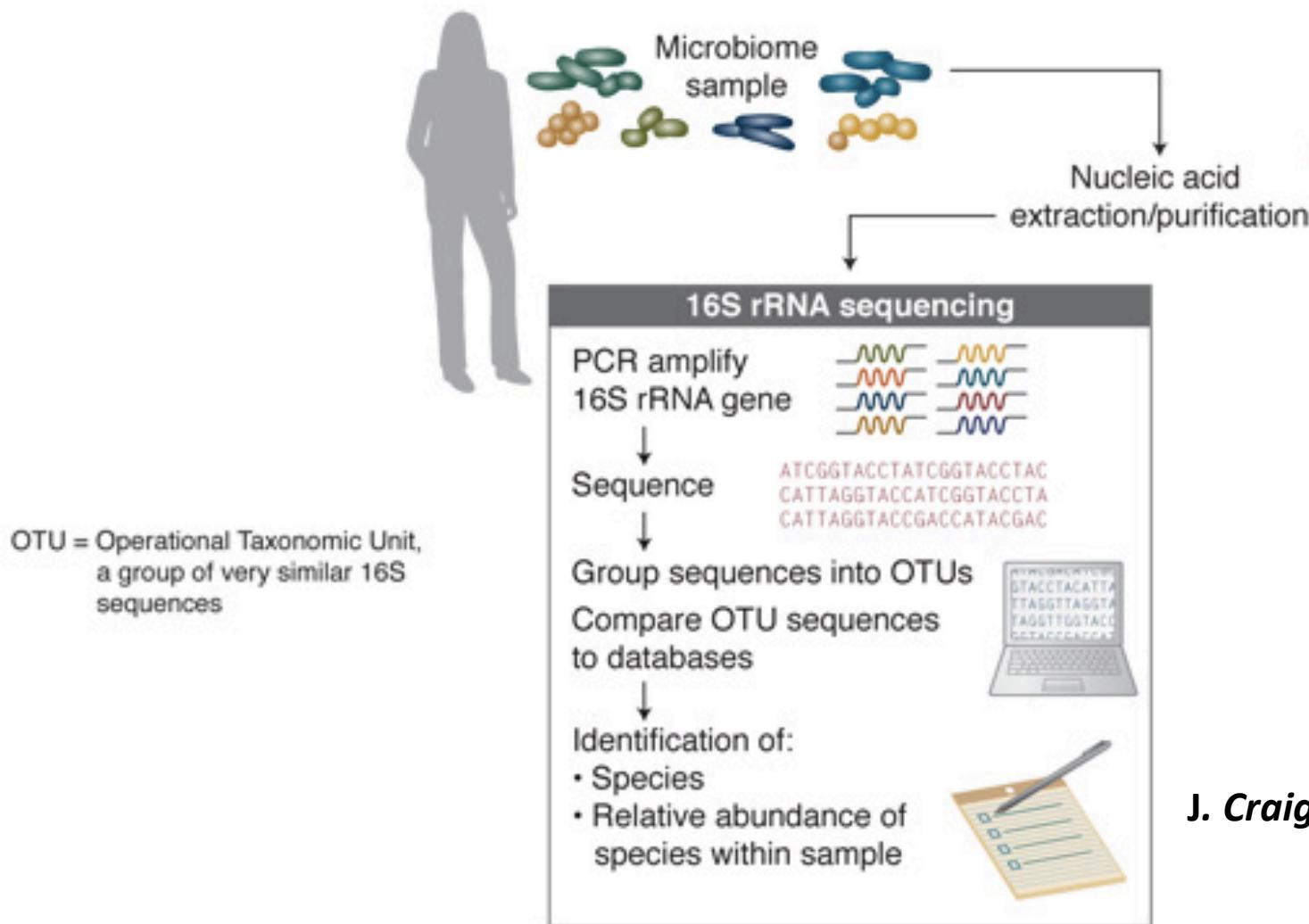
- Antibiotic-associated diarrhea
- Asthma/allergies
- Autoimmune diseases
- Cancer
- Dental cavities
- Depression and anxiety
- Diabetes
- Gastric ulcers
- Cardiovascular disease
- Inflammatory bowel diseases
- Malnutrition
- Obesity

***Malaria?***

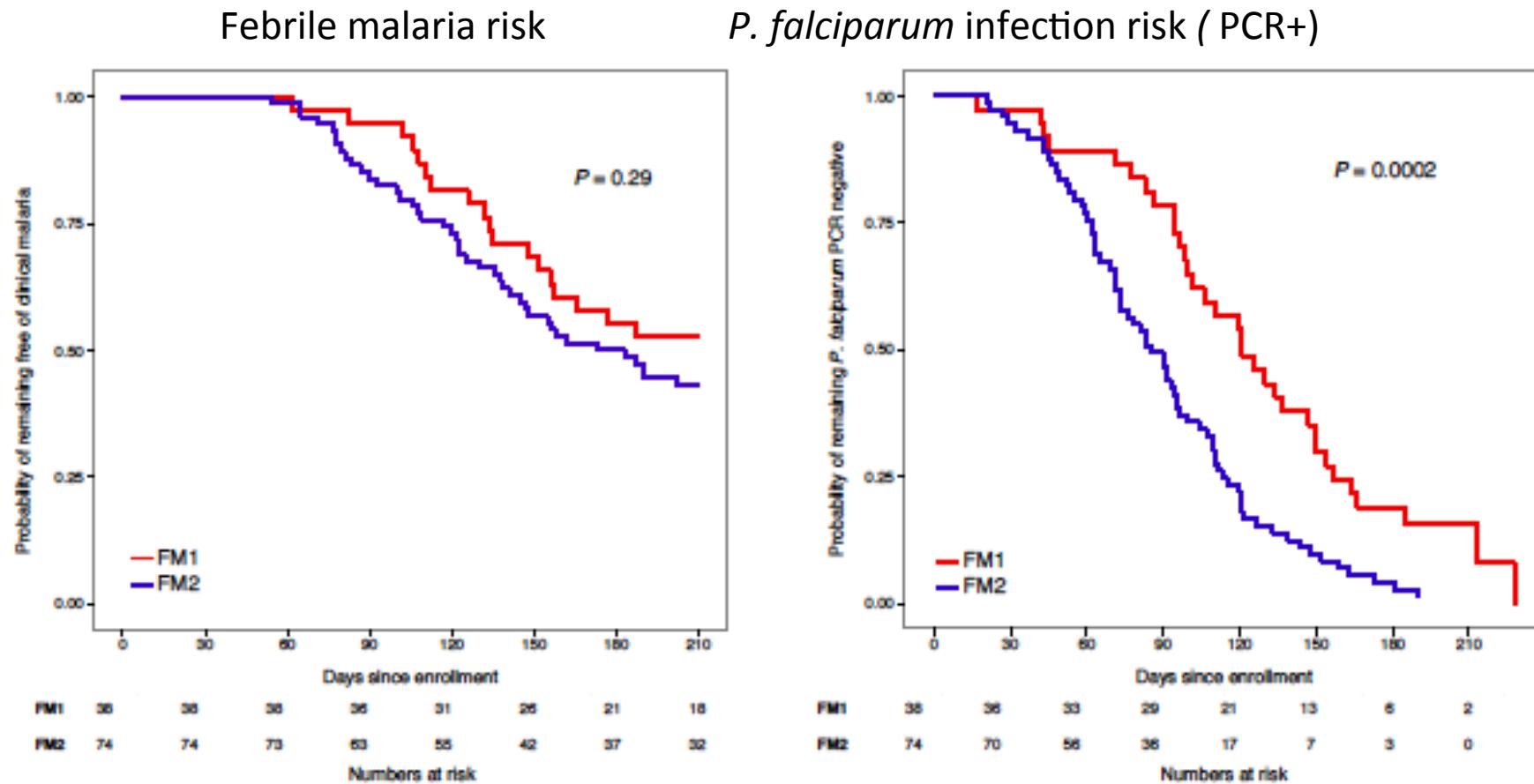


FONDATION MÉRIEUX

# Determining stool microbiome composition by 16s rRNA sequencing



# Microbiome composition is associated with protection from *P. falciparum* infection but not febrile malaria

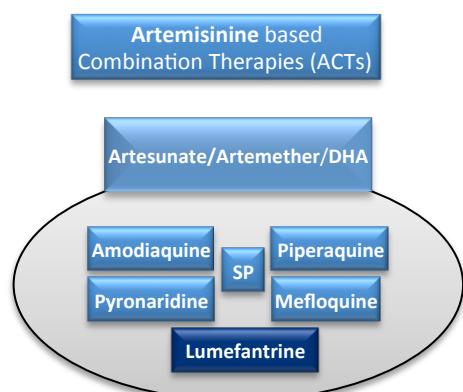


***Microbiomes of subjects at lower risk of *P. falciparum* infection had higher proportion of Enterobacteriaceae/Escherichia/Shigella compared to subjects at higher risk (3.2% versus 0.7%, respectively)***

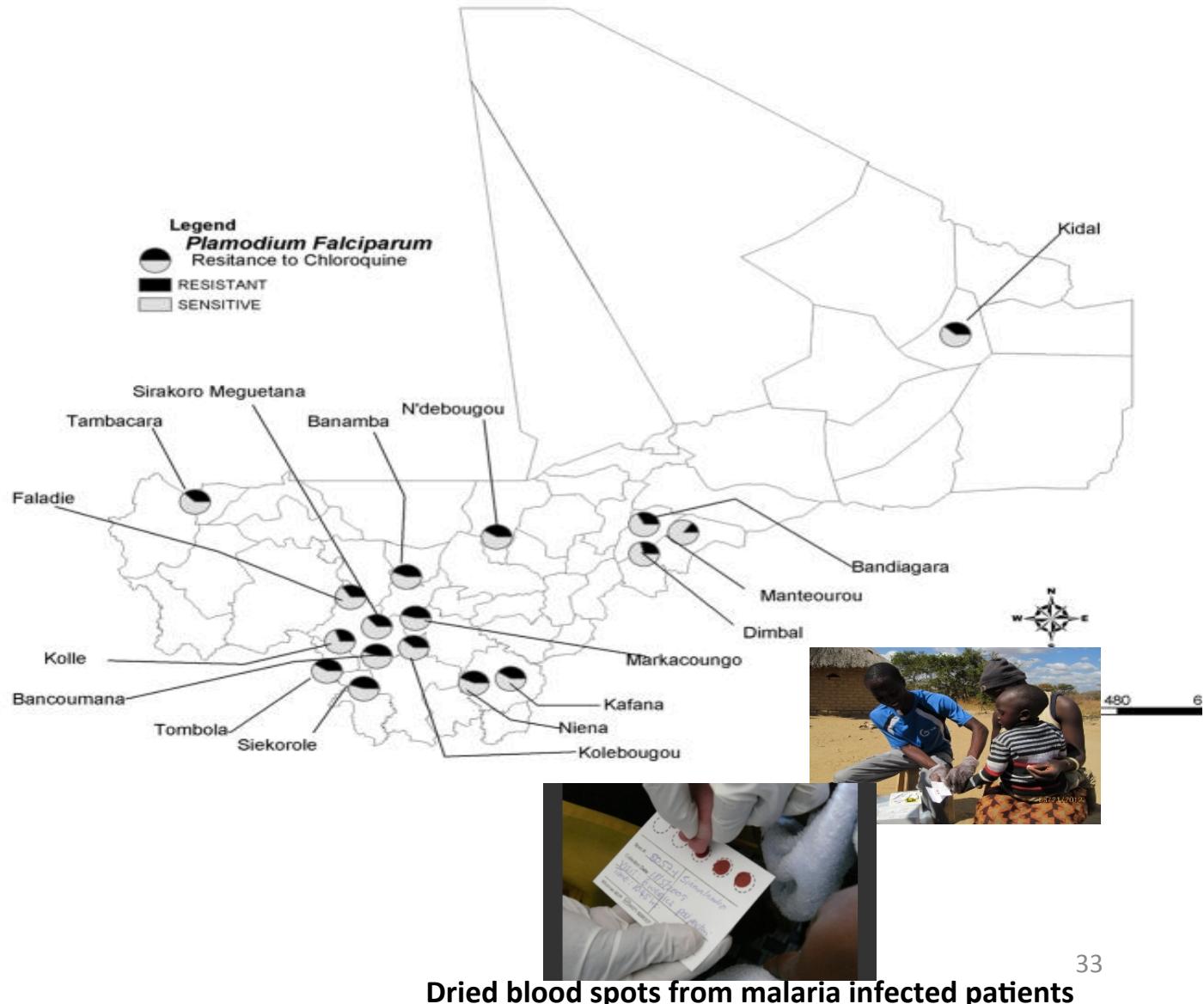
# Malaria chemotherapy and drug resistance molecular mapping in Mali

Molecular surveillance of drug resistance

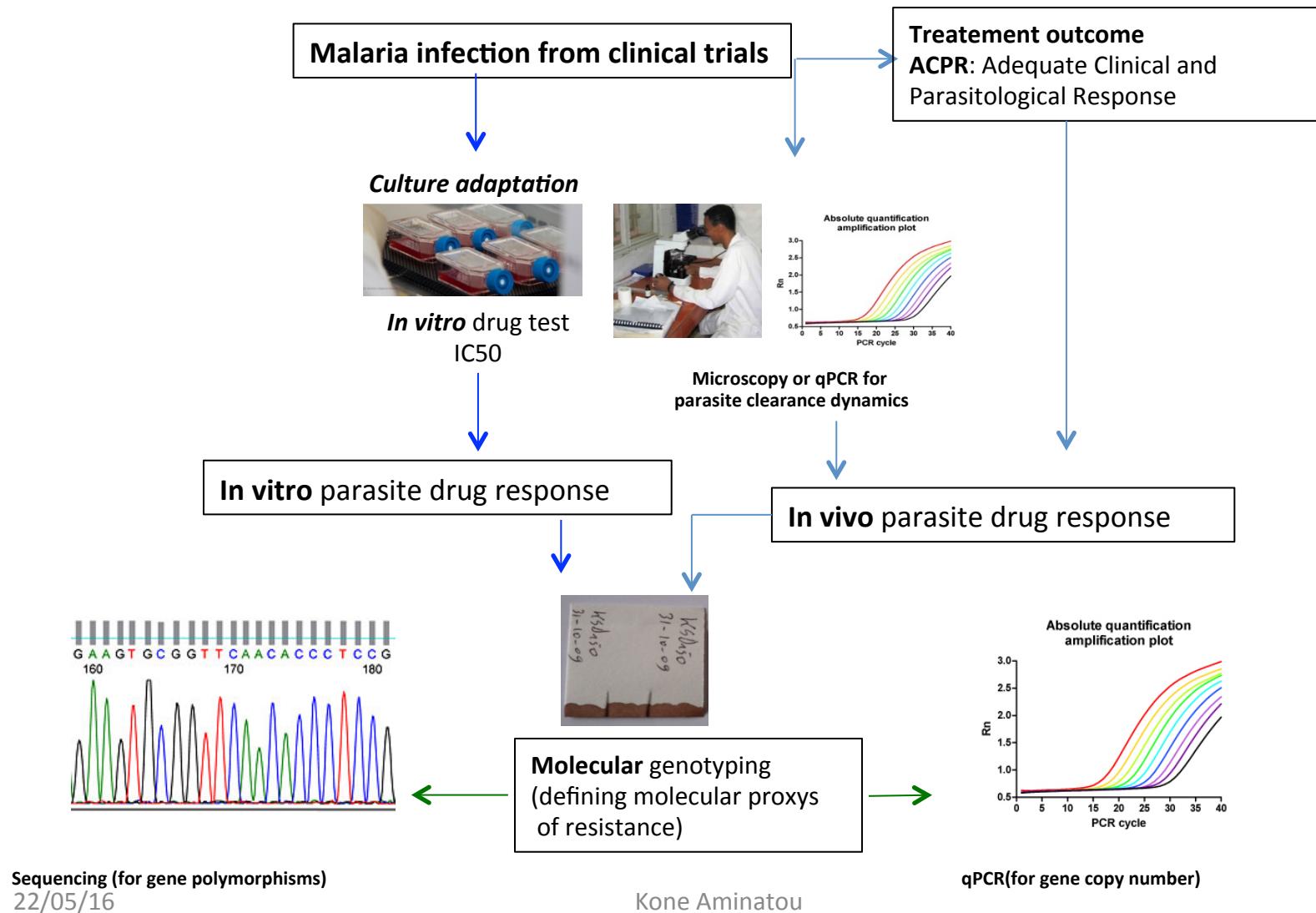
## Uncomplicated malaria



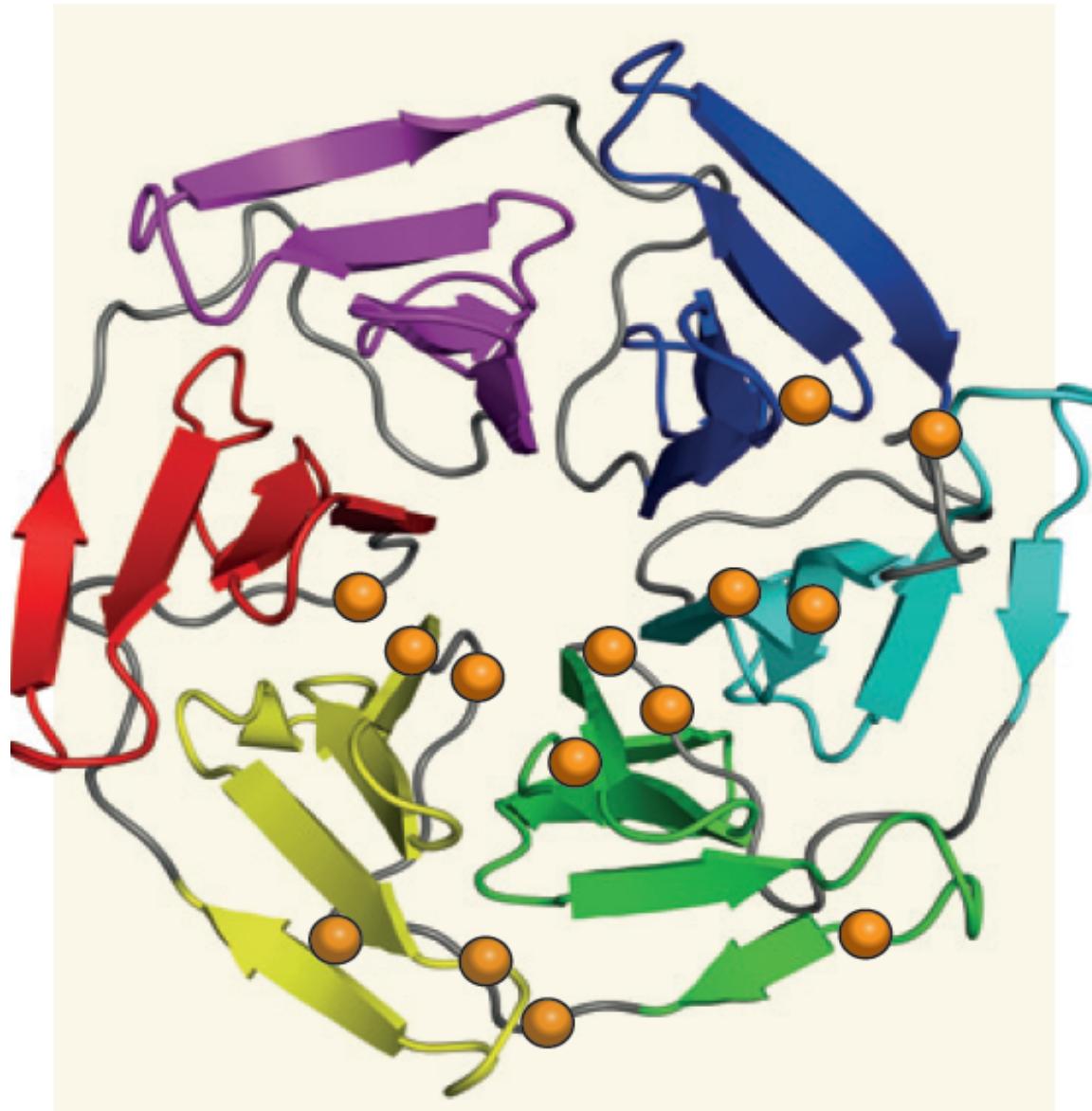
## Severe-complicated malaria



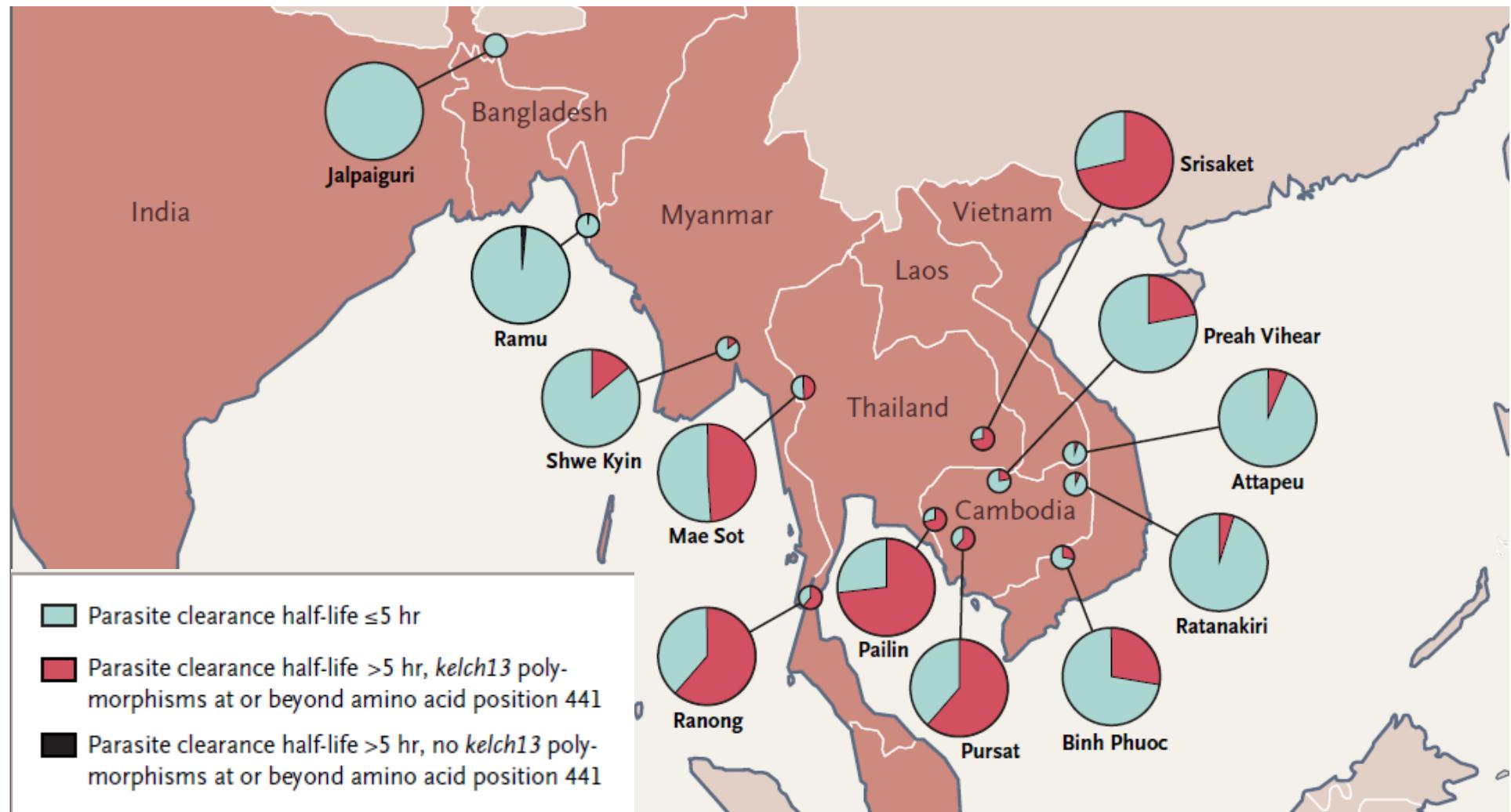
## Measurement of malaria drug resistance



# K13-propeller mutations and Artemisinine Resistance

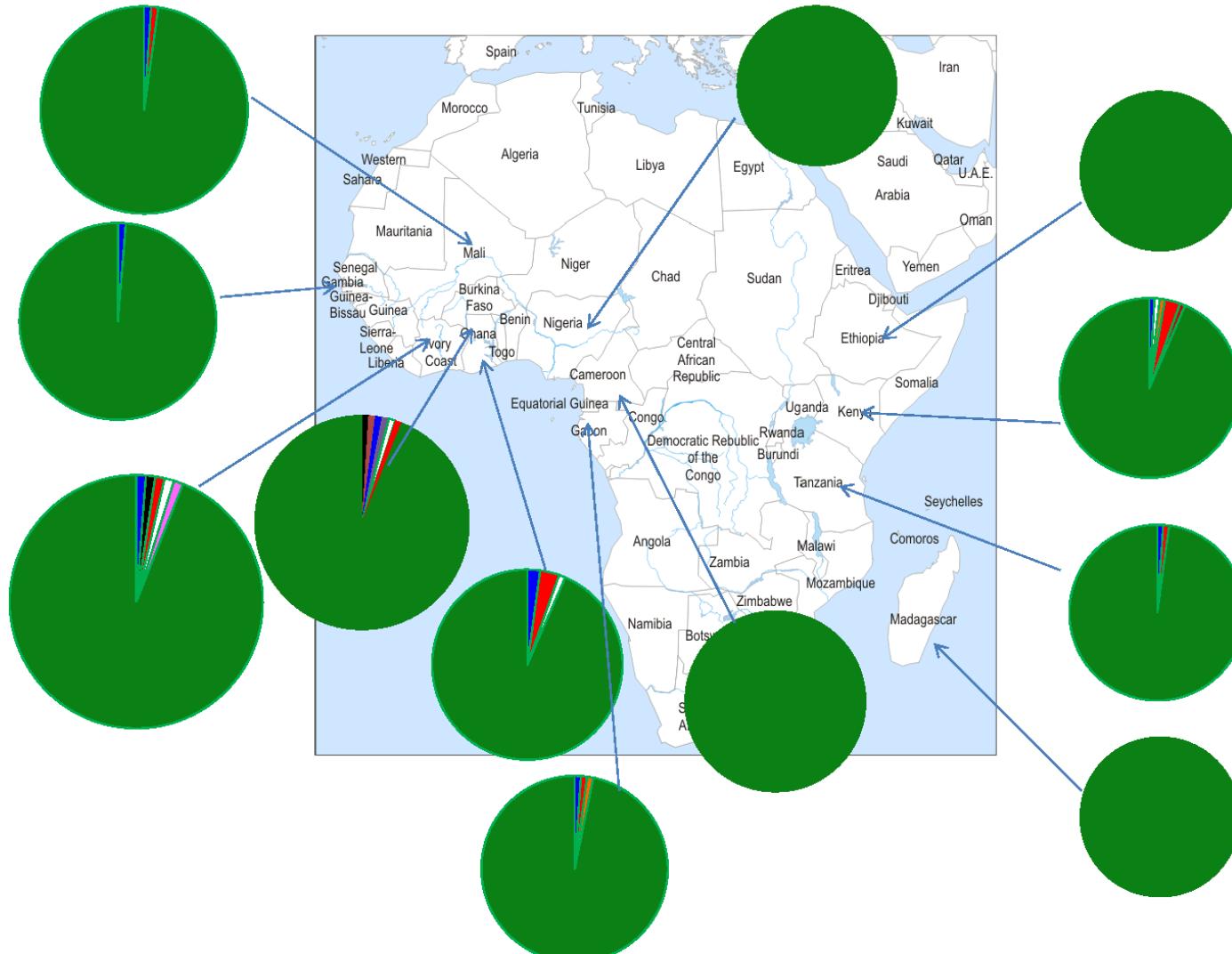


# Propagation of K13 mutants parasites in SEA: Molecular surveillance

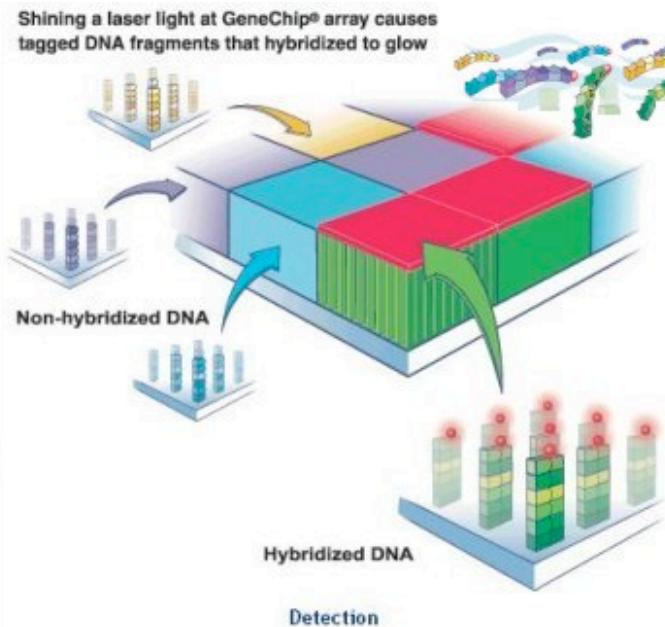
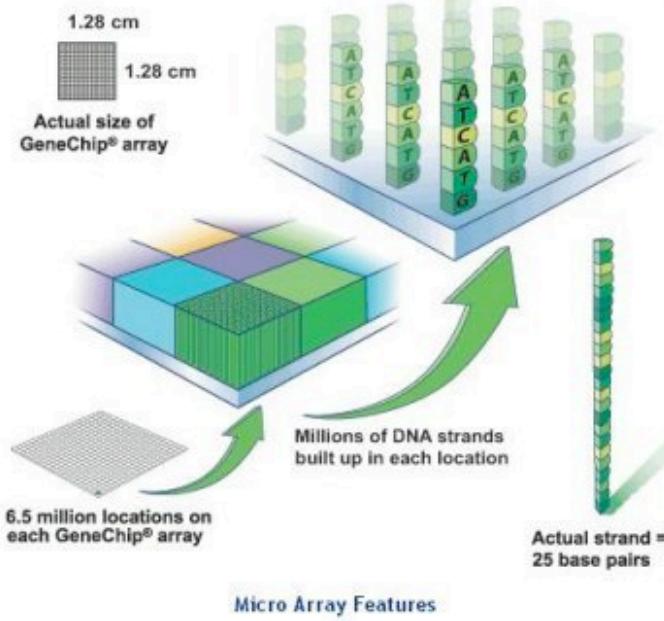


Adapted from Ashley et. al, NEJM, 2014

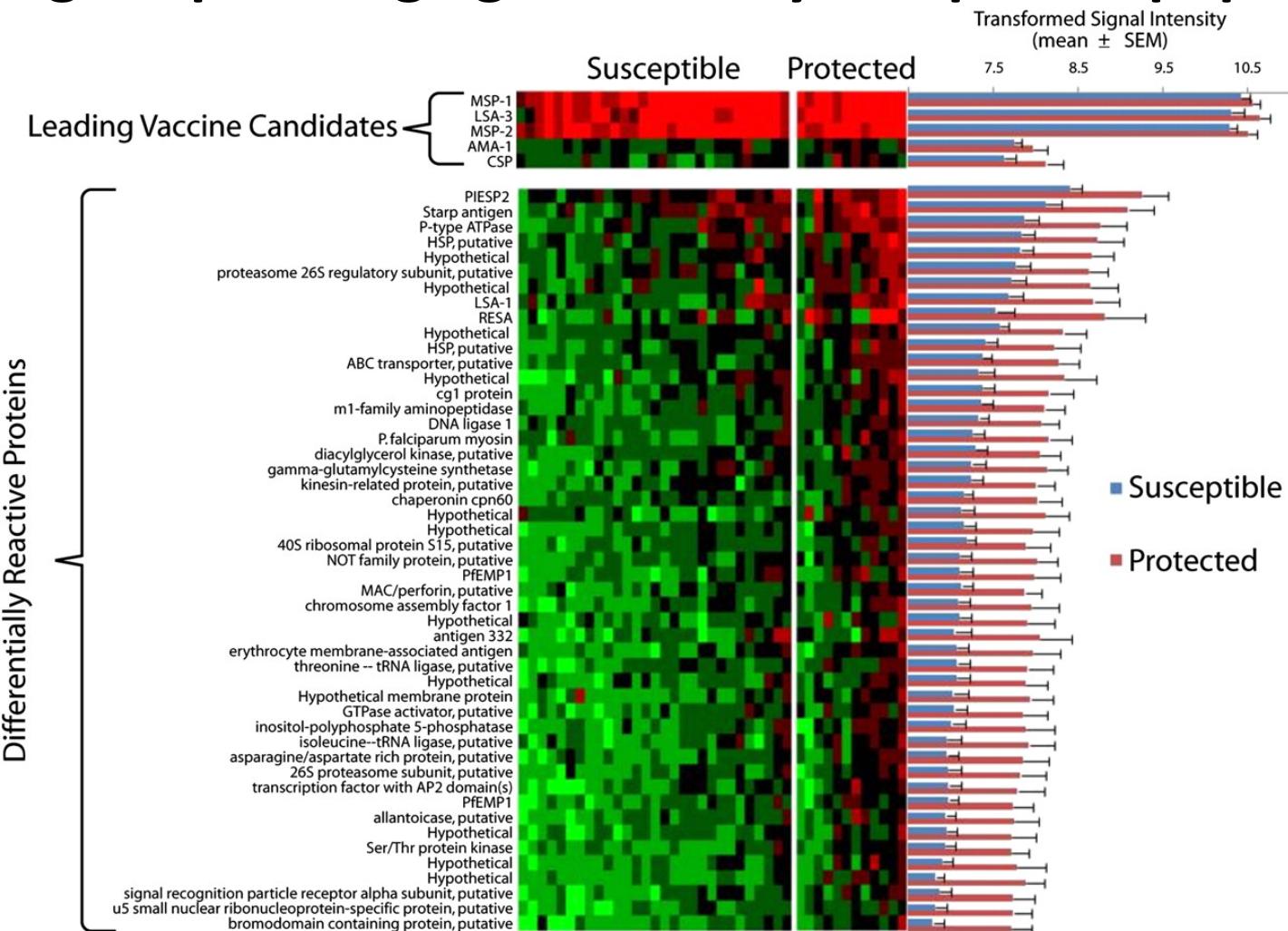
# K13 polymorphism in PDNA sites in Africa



# Affymetrix



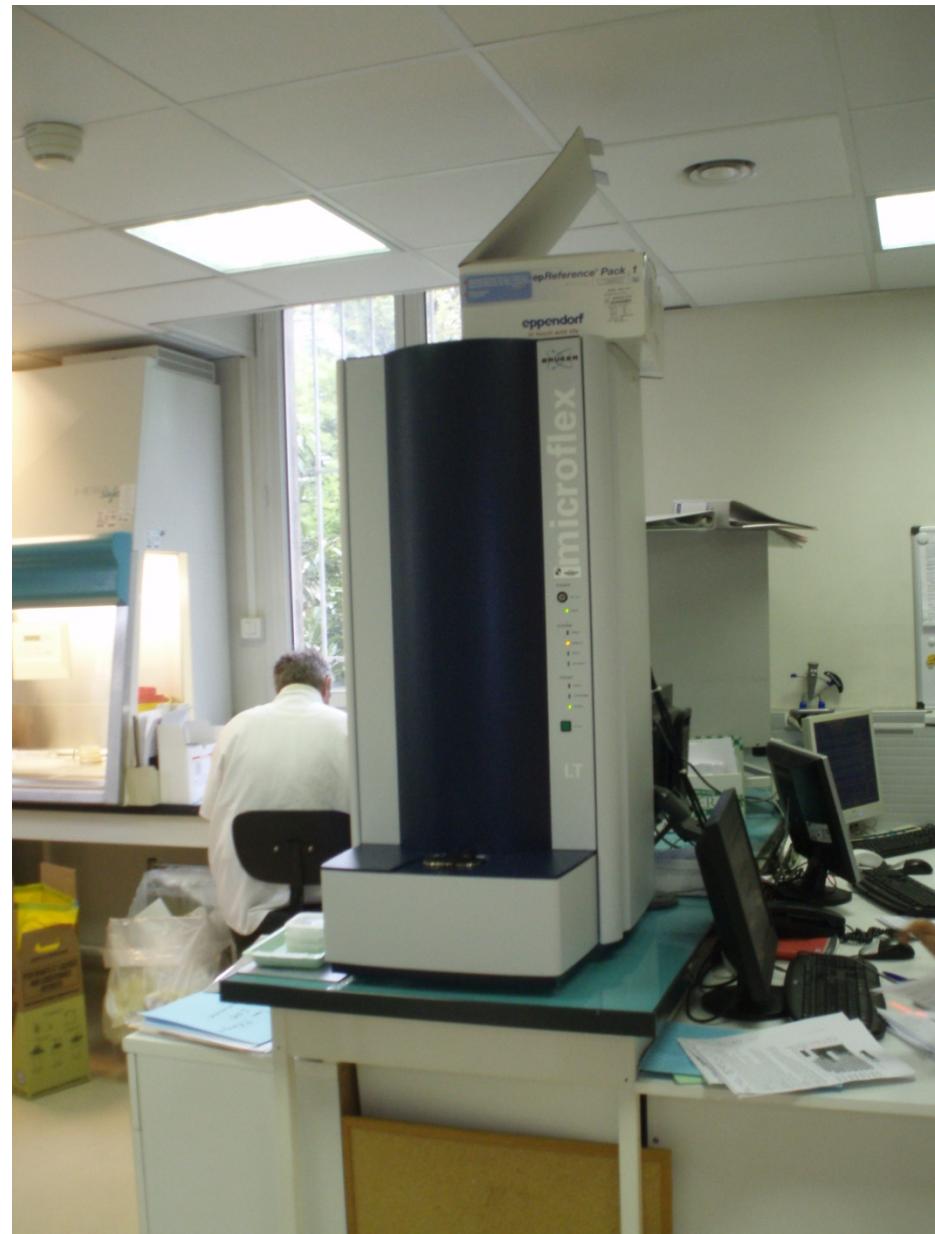
# Longitudinal cohort, tools in Infectious Diseases studies: Serological profiling against many falciparum peptides:

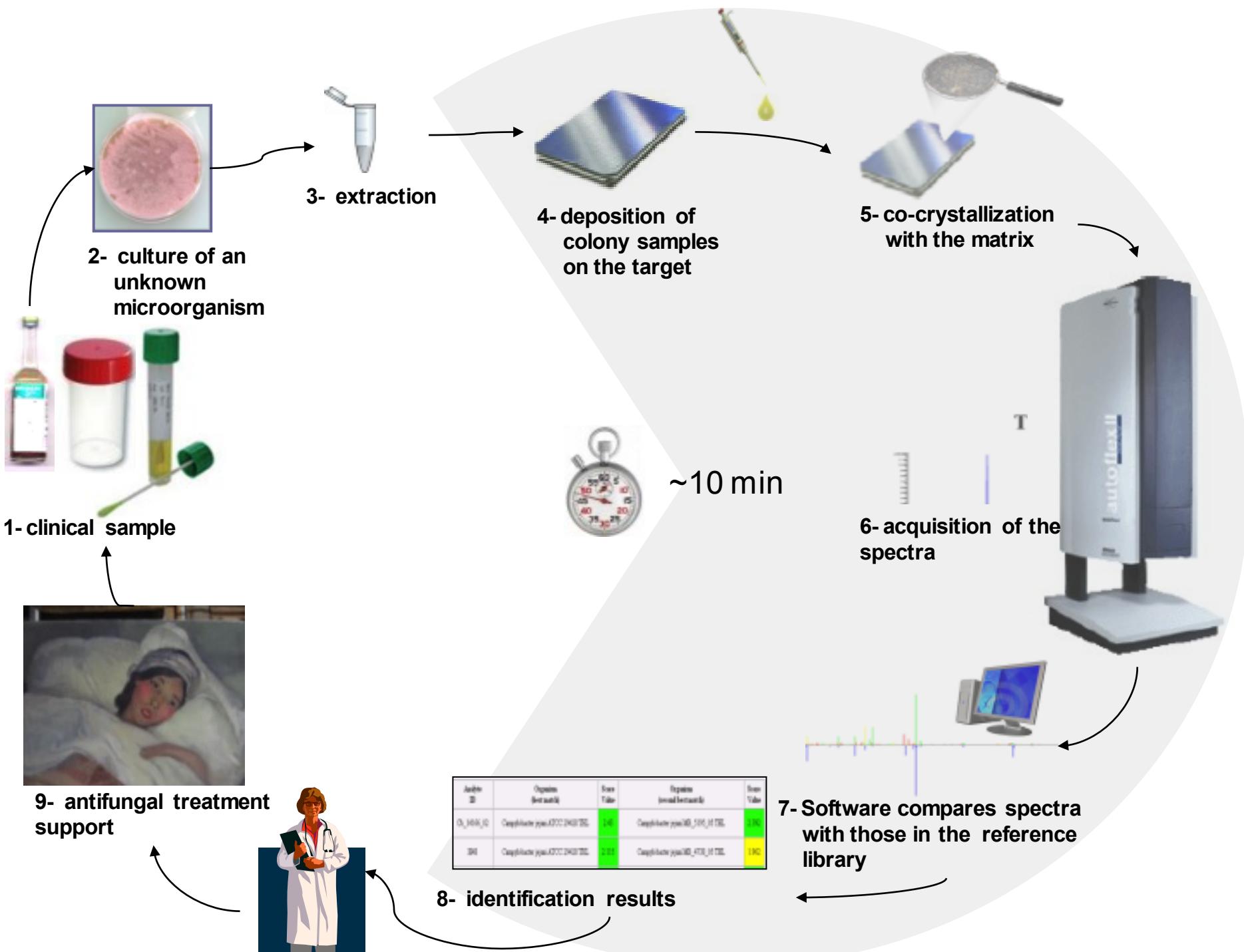


Crompton P D –Doumbo OK et al. PNAS 2010;107:6958-6963

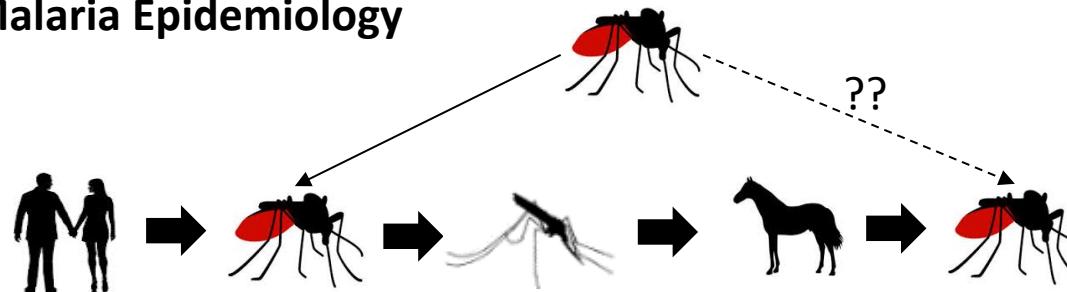
PNAS

# REVOLUTION OF MALDITOF TECHNOLOGY IN AFRICA





## MaldiTof and Malaria Epidemiology



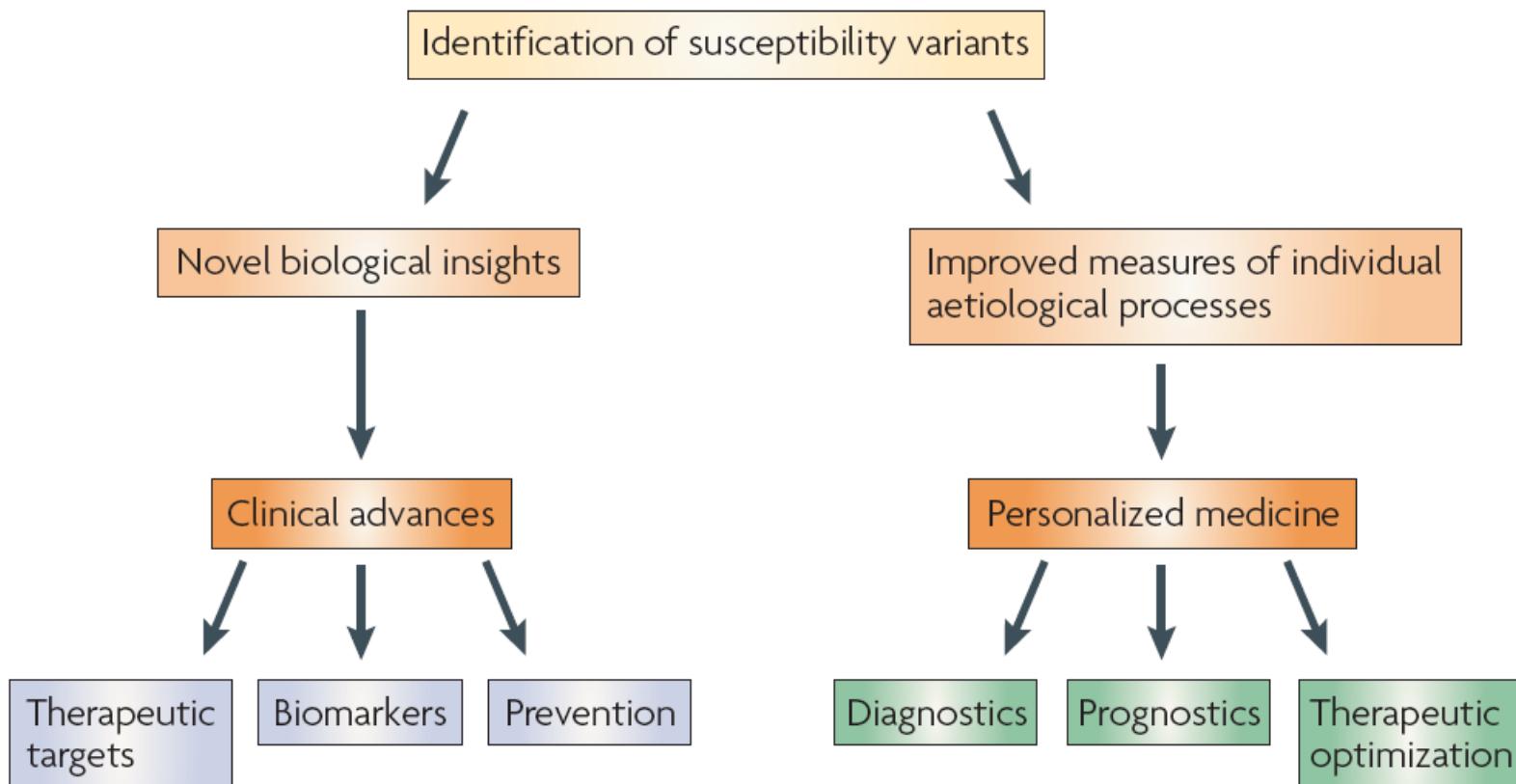
**Table 2** Mosquitoes used to determine the effect o the second blood blood meals and blind tests according to post-blood feeding period and blood meal source

Mosquito species <sup>(a)</sup>	First blood feeding source	Second blood feeding source	Number of specimens used blind tests against database <sup>(b)</sup>	High LSVs obtained from blind tests against database <sup>(c)</sup>	Vertebrate species identification of blood origin <sup>(d)</sup>
<i>An. gambiae</i> S	Human	/	10	[1.834-2.320] (10)	Human
<i>An. gambiae</i> S	Human	Goat	10	[2.173-2.670] (10)	Goat
<i>An. gambiae</i> S	Human	Dog	10	[2.139-2.764] (10)	Dog
<i>An. gambiae</i> S	Human	Cow	10	[1.810-2.396] (10)	Cow
<i>An. gambiae</i> S	Human	Sheep	10	[1.802-2.296] (10)	Sheep
<i>An. gambiae</i> S	Human	Rabbit	10	[1.826-2.101] (10)	Rabbit
Total	10		60		

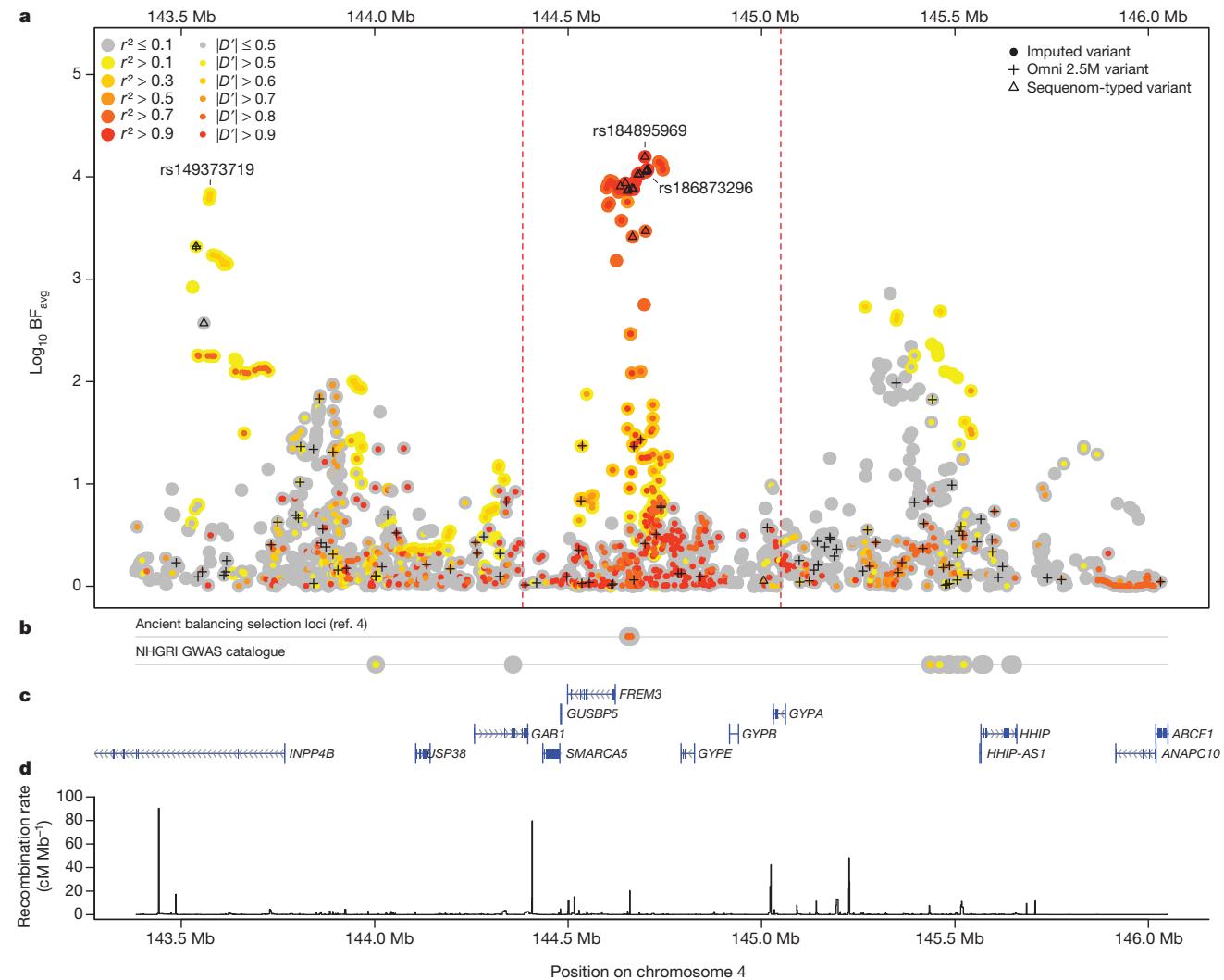
<sup>(a)</sup> Mosquitoes were collected from 12 hours following blood meals and their abdomen protein extracts were submitted to MALDI-TOF MS. <sup>(b)</sup> Number of specimens used to blind test against the Database. <sup>(c)</sup> Into brackets are indicated the number of specimens with LSVs upper and lower than 1.8. <sup>(d)</sup> Vertebrate species blood origin are indicated only for specimens with LSVs greater than 1.8. LSVs, log score values.

# Potential of GWAS

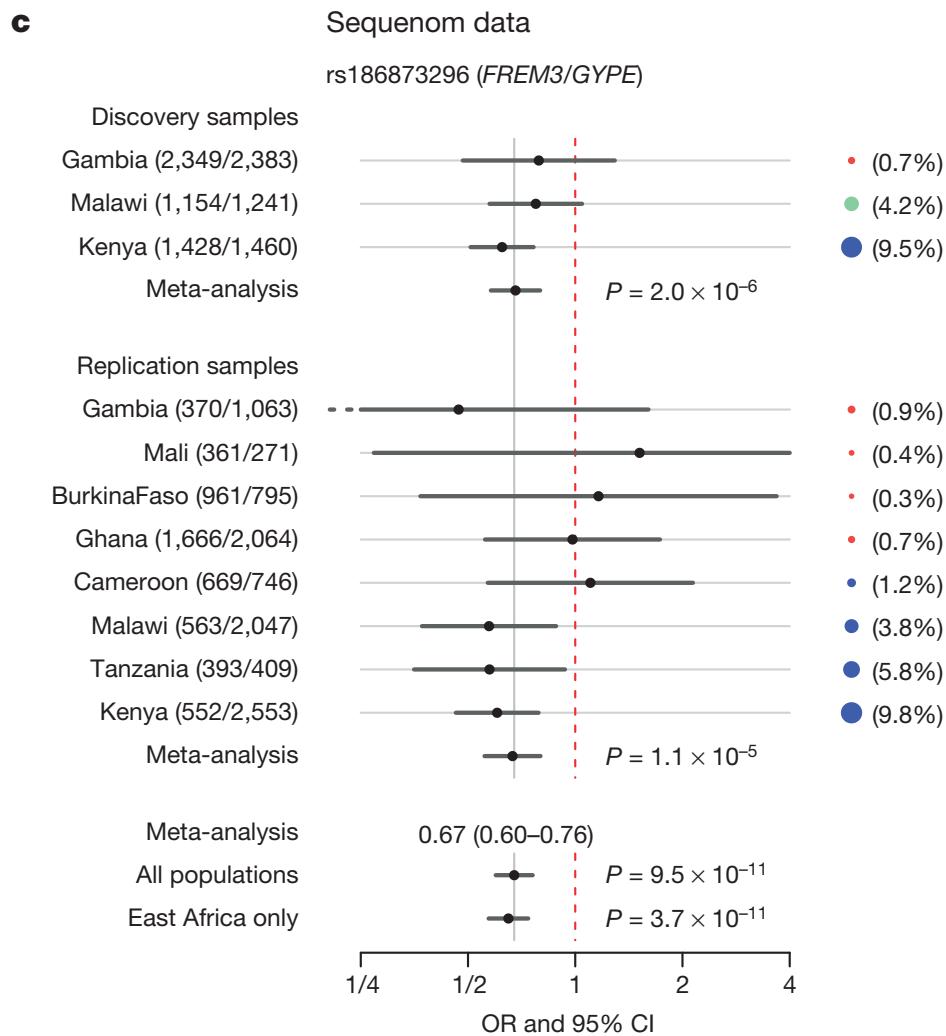
(*MalariaGEN Nature 2015*)



# Signal of association with severe malaria across the FREM3/GYPE region



# Forest plot on Sequenom data



# Summary

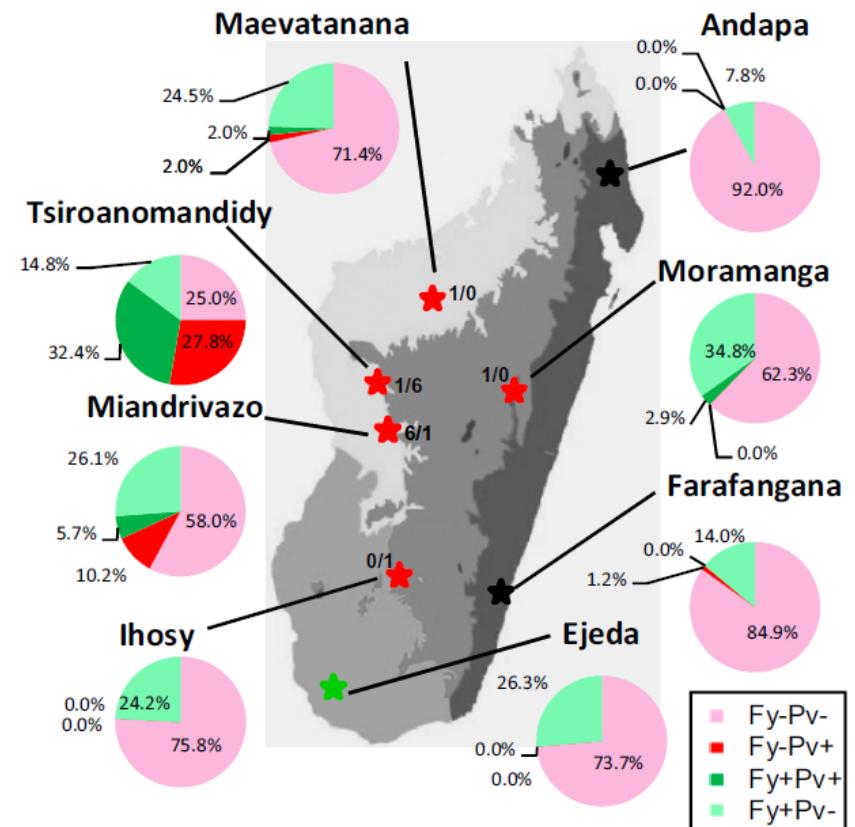
- Identification a novel malaria resistance locus close to a cluster of genes encoding glycophorins
- Identification of a haplotype at this locus that provides 33% protection against severe malaria

# Plasmodium vivax infections in Duffy-negative patients (2)

- Brazil<sup>1</sup>: 2 cases of *P. vivax* identified in Duffy (-) patients by PCR.
- Madagascar<sup>2</sup>:
  - *P. vivax* prevalence: 8.8% in Duffy (-) patients
  - High parasitemia observed
  - Duffy (-) genotype match phenotype
  - *P. vivax* gametocytes observed in Duffy (-) patients
  - More than 50% of *P. vivax* infections in Duffy (-) in some areas
  - *P. vivax* break dependency to Duffy antigen to invade RBCs

<sup>1</sup> Cavasini CE. 2007

<sup>2</sup> Ménard D. 2010



**Figure 15.** Distribution of *P. vivax* in Madagascar<sup>2</sup>

# *Plasmodium vivax* infections in Duffy-negative and in Mali

- *P. vivax* is absent or rare in West and Central Africa
- East Africa: low prevalence
- *P. vivax* is endemic in some populations of Sudan, Somalia, Ethiopia, Djibouti (predominantly Duffy positive)<sup>1</sup>.
- *P. vivax* identified in Equatorial Guinea (8 cases) and Angola (7 cases) by PCR and Duffy antigen genotyping<sup>2</sup>.
- Mali<sup>3</sup>:
  - Prevalence in north Mali: 30%
  - Duffy antigen not confirmed by molecular techniques



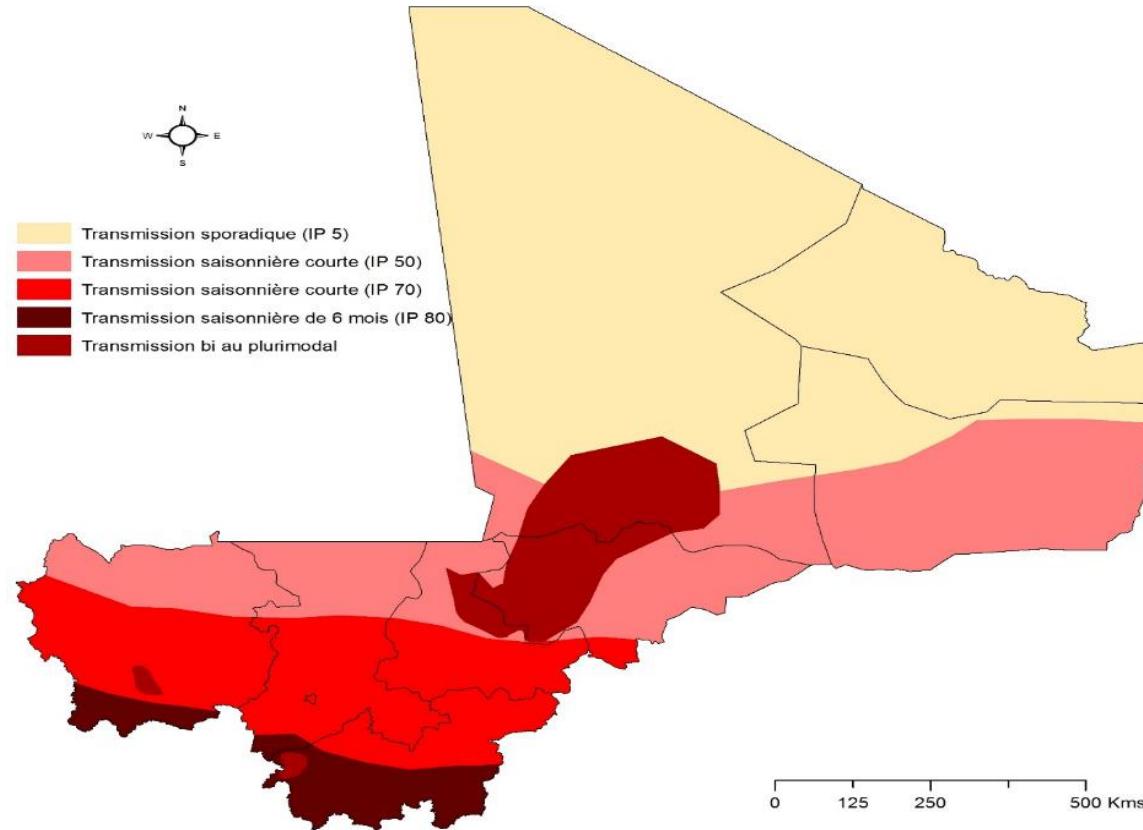
Figure 16. *P. vivax* in Mali<sup>3</sup>

<sup>1</sup>Mathews HM. 1981

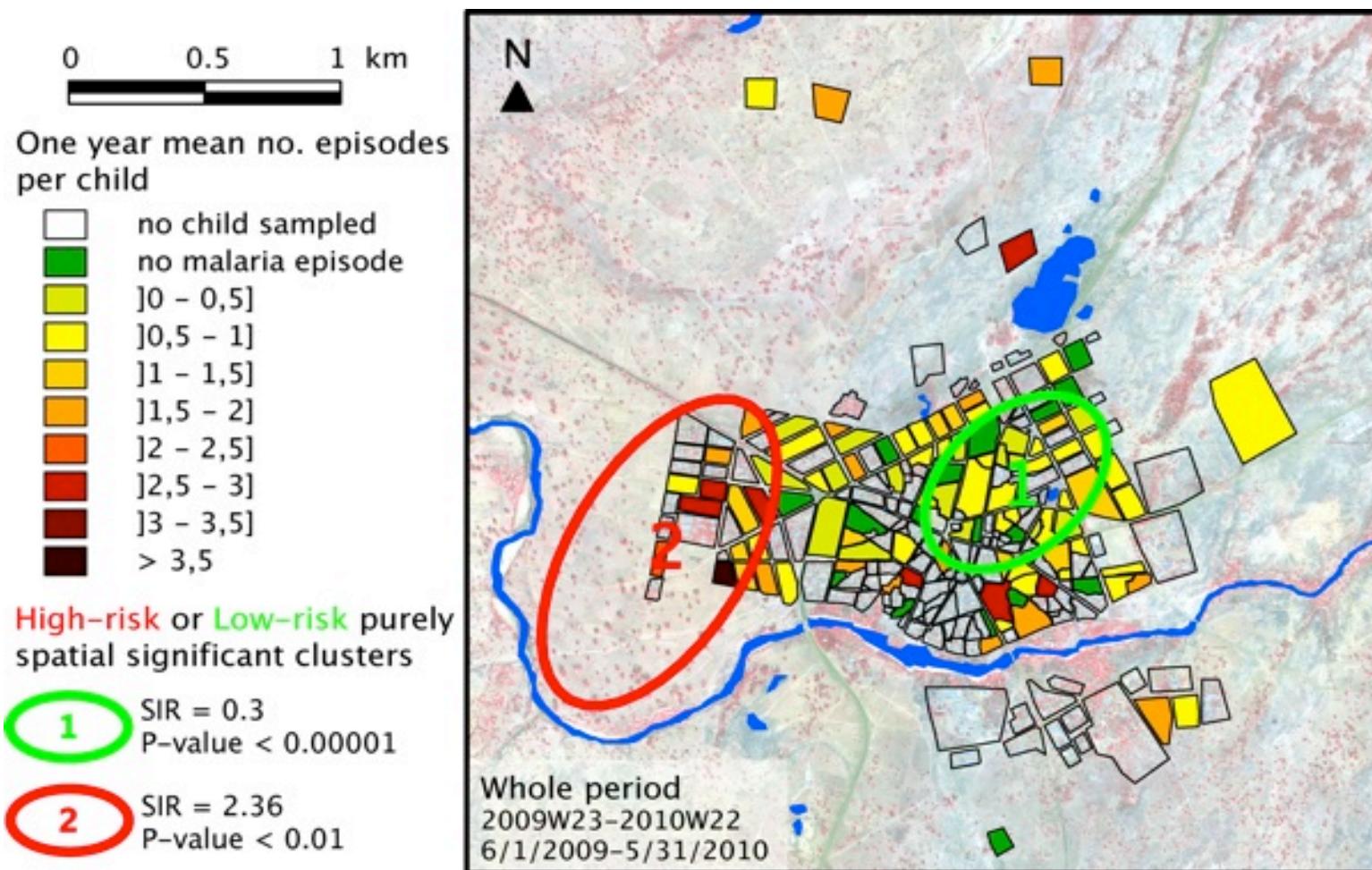
<sup>2</sup>Mendes C. 2011

<sup>3</sup>Bernabeu M. 2012

# MALARIA RISK MAP OF MALI (*D Coulibaly et al., 2015*)

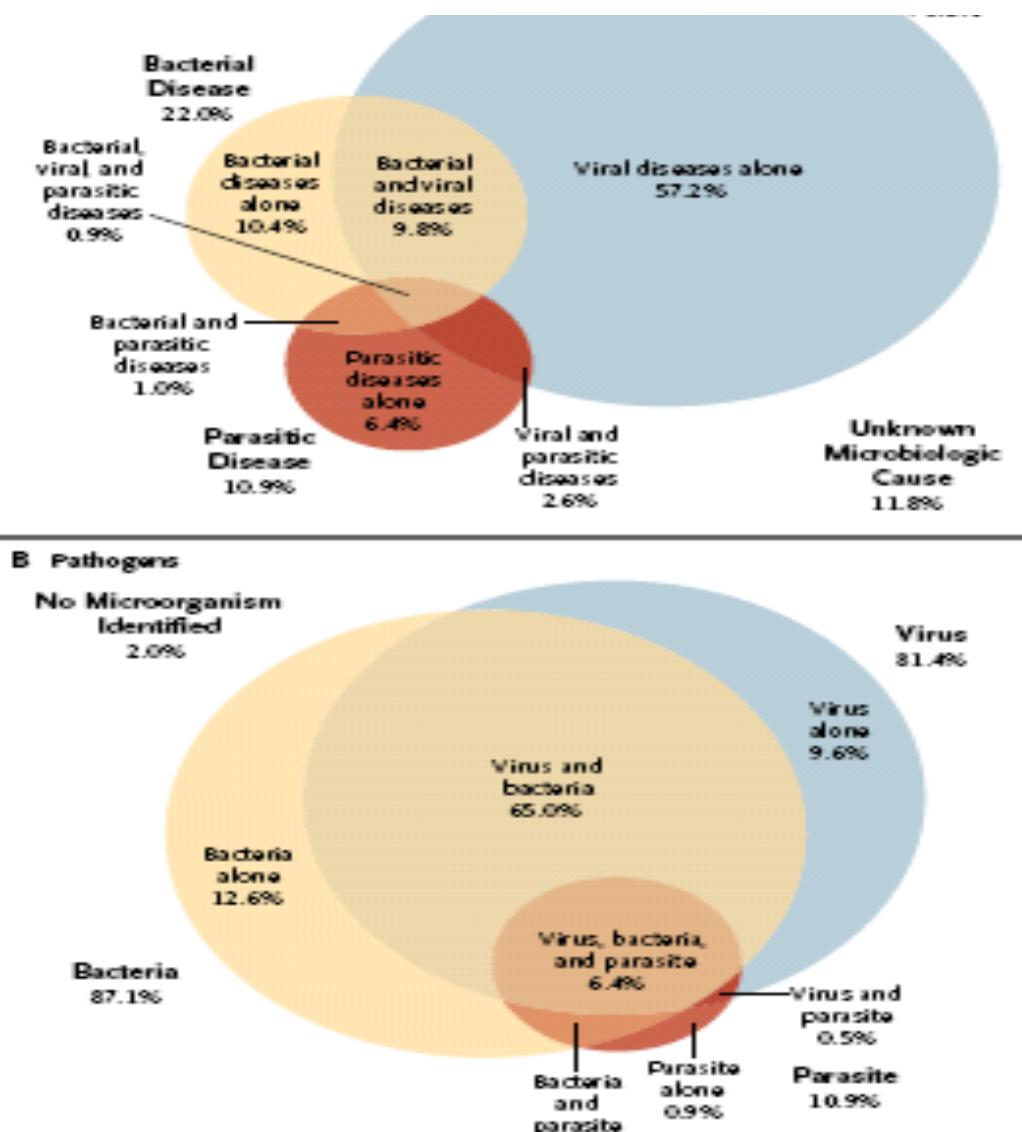


**Carte des zones à risque de paludisme au Mali [MRTC, PNLP 2015]**  
**Combinaison des zones climatiques, la prévalence de l'infection et la durée de la saison de transmission**



Malaria Transmission hotspot in Bandiagara, Mali, D Couloubaly et al., 2015

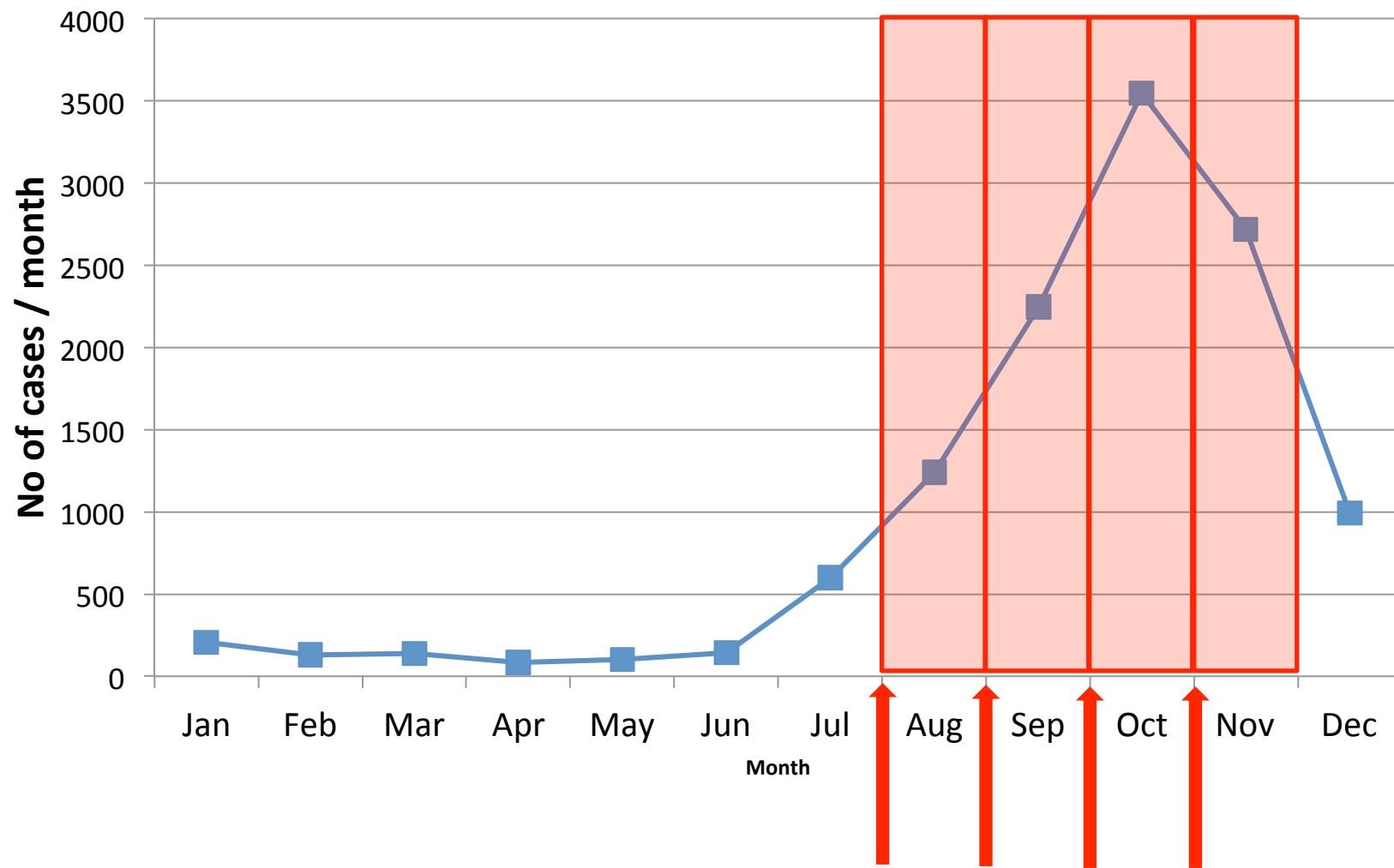
tribution des maladies en  
e la nature des pathogènes  
nes, virales ou parasitaires)  
evauchement entre tous  
ènes identifiés,  
mment des critères  
t biologiques utilisés pour  
nation du diagnostic



**Figure 4.** Overlap among Disease Types and Pathogen Types in the 1005 Febrile Children.

Panel A shows all diseases diagnosed in the present study; disease definitions are given in Table S1 in the Supplementary Appendix. Panel B shows types of pathogens identified, regardless of the clinical and laboratory criteria used to establish final diagnoses.

can be generalized to the nation as a whole and,



# SMC and the Nouakchott initiative 2013



RESEARCH ARTICLE

## Sub-National Targeting of Seasonal Malaria Chemoprevention in the Sahelian Countries of the Nouakchott Initiative

Abdisalan Mohamed Noor<sup>1,2</sup>\*, Eliud Kibuchi<sup>1</sup>, Bernard Mitto<sup>1</sup>, Drissa Coulibaly<sup>3</sup>, Ogobara K. Doumbo<sup>3</sup>, Robert W. Snow<sup>1,2</sup>

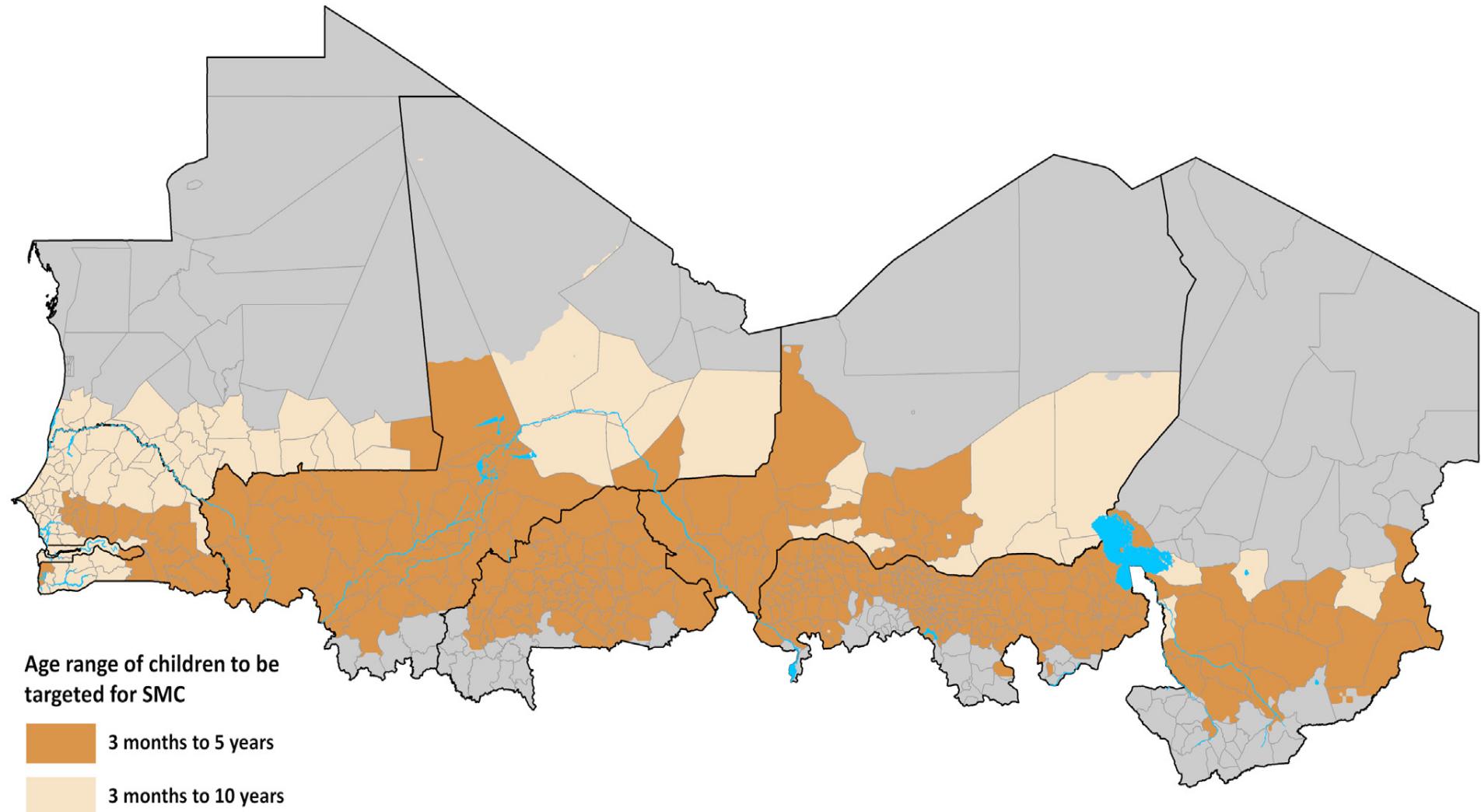
1 INFORM (Information for Malaria – [www.inform-malaria.org](http://www.inform-malaria.org)), Spatial Health Metrics Group, Kenya Medical Research Institute/Wellcome Trust Research Programme, Nairobi, Kenya, 2 Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom, 3 Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali

\* These authors contributed equally to this work.

\* [anoor@kemri-wellcome.org](mailto:anoor@kemri-wellcome.org)



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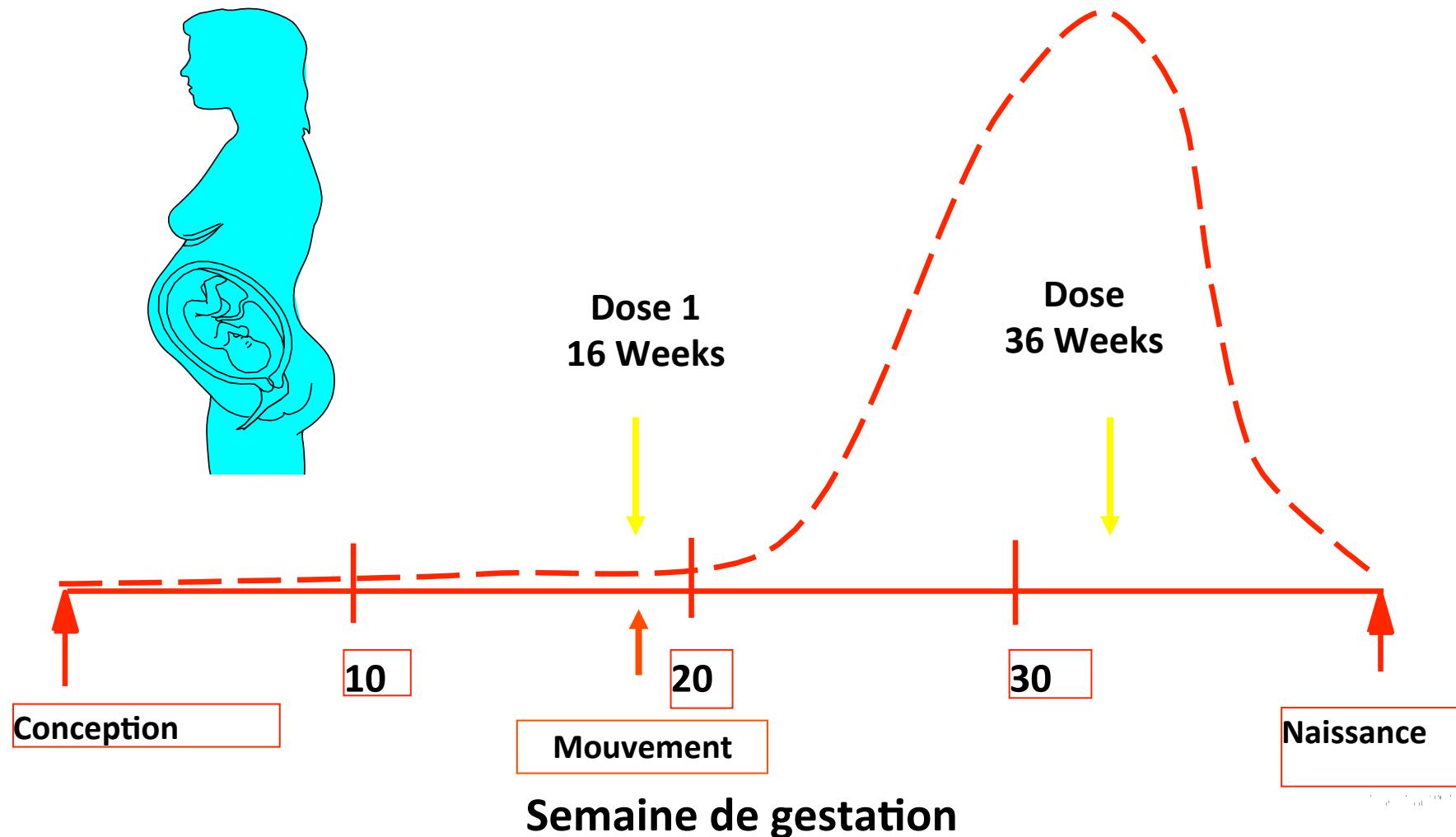
**SMC target areas in West Africa: D. Coulibaly et al., 2015)**

# IPTp-SP-standard 2 doses vs 3 doses

*Kayentao K....Doumbo OK et al., JID 2005*

*Diakite O. Maiga ... Doumbo OK et al., CID 2011*

*Kayentao K....Doumbo OK & Feiko et al., JAMA 2013*



# Intermittent Preventive Therapy for Malaria During Pregnancy Using 2 vs 3 or More Doses of Sulfadoxine-Pyrimethamine and Risk of Low Birth Weight in Africa

## Systematic Review and Meta-analysis

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Kassoum Kayentao, MD

594 JAMA, February 13, 2013—Vol 309, No. 6

**Authors:** *Kayentao K, Garner P, Anne van Eijk A, Naidoo I, Roper C, Mulokozi A, MacArthur J., Luntamo M, Ashorn P, Doumbo O, ter Kuile F*



WHO Global Malaria Programme

WHO Department of Reproductive Health and Research

WHO Department of Maternal, Newborn, Child and Adolescent Health

**WHO Policy Brief for the Implementation of  
Intermittent Preventive Treatment of Malaria in Pregnancy  
using Sulfadoxine-Pyrimethamine (IPTp-SP)**

11 April 2013



WHO Global Malaria Programme

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WHO Policy Brief for the Implementation of

Intermittent Preventive Treatment of Malaria in Pregnancy  
using Sulfadoxine-Pyrimethamine (IPTp-SP)

11 April 2013

# Mise à jour de la Politique de l'OMS sur IPTp-SP , Avril 2013

- Tot au deuxième trimestre
- **A chaque visite de CPN programmée jusqu'à l'accouchement**, au moins un mois d'intervalle
- Dernière dose jusqu'à l'accouchement sans dangers
  - DOT
  - Peut être administré sans manger.
  - Pas administré chez les femmes sous co-trimoxazole
  - Eviter l'acide folique à > 5mg par jour

## **Chimioprévention du Paludisme dans la population cible en zone d'endémie.**

- **FEMMES ENCEINTES**
  - MILDA+ TIPp-SP  $\geq$  3 DOSES A PARTIR DU SECOND TRIMESTRE
  - 1ER TRIMESTRE: MILDA + TDR ET SELS QUININES (EN CAS DE SIGNES)
  - CTAs a partir du second trimestre
- **ENFANTS DE MOINS DE 5 ANS**
  - MILDA + CPS AVEC AQ+SP **POUR PREVENTION DU PALUDISME SAISONNIER**
  - TDR + CTA, EN CAS DE SIGNES

# **Chimioprévention du Paludisme dans la population cible en zone d'endémie.**

- **POPULATIONS DU NORD**
  - NON PREMUNIE DONC POSSIBILITE D'EPIDEMIES (1988, 1999, 2015)
  - CIRCULATION DE *Plasmodium vivax* (*avec possibilité de reviviscence a partir des hypnoozoites dans le foie des mois, voir des années après le retour du nord des soldats et leurs familles*).
  - MILDA ET CPS –AQ+SP EN CAS D'EPIDEMIE POUR TOUS LES AGES (exemple 2015).
- **MILITAIRES ET LEURS FAMILLES**
  - GRAND RISQUE DES MILITAIRES DU NORD SERVANT AU SUD ==→ MILDA et CPS-AQ+SP ET DES MILITAIRES DU SUD AYANT SEJOURNER PLUS DE 2 ANS AU NORD AVEC LEURS FAMILLES.
  - RESTE IDEM POPULATIONS ENDEMIQUES (MILDA, CPS, TPI, TDR/ACT)

## **QUESTION3:**

**PEUT UTILISER LES CTAs POUR PRENDRE EN CHARGE UN PALUDISME *PER OS* CHEZ UNE FEMME ENCEINTE ?**

- 1] OUI MAIS  $\geq$  Second trimestre de grossesse**
- 2] AL ET DHA-PQ sont mieux tolérées a efficacité comparable vs ASAQ, MF-AS**
- 3] Importance des données de pharmacovigilance chez la femme enceinte**

# Umberto D et al., NEJM 2016

## Flow chart de répartition des femmes

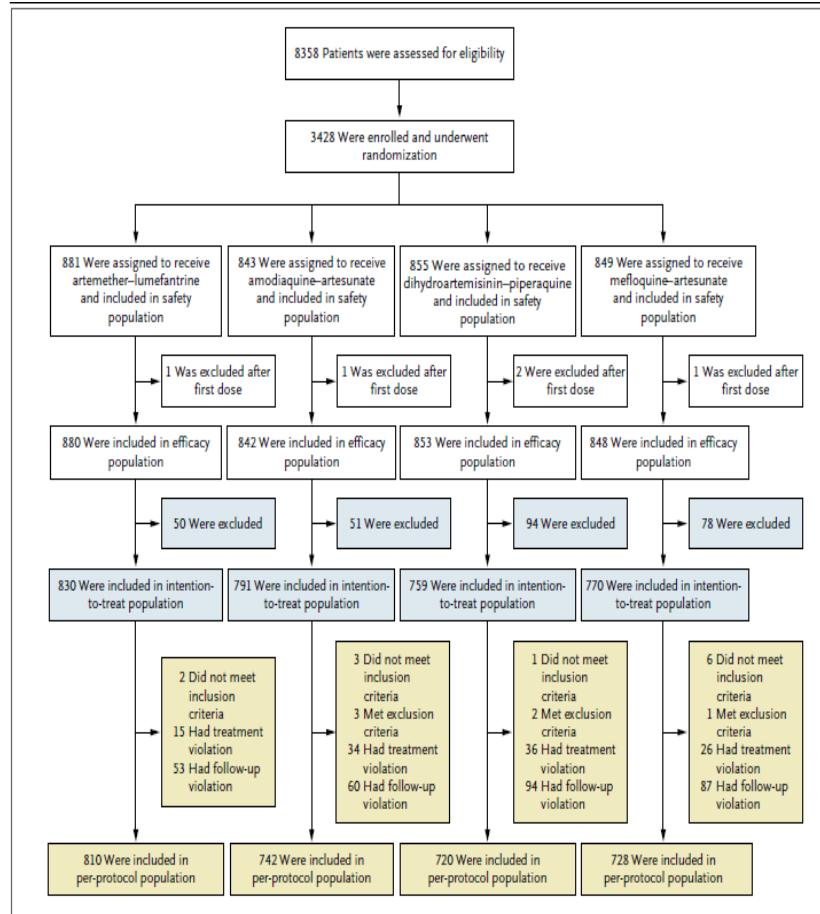


Fig.1: Randomisation des femmes et analyse de la population

# Résultats (*Umberto D et al., NEJM 2016*)

- N= 3428 de femmes enceintes incluses
- Tableau I: répartition des femmes par bras de traitement

	Nbre inclus
AL	881
ASAQ	843
DHA-PQ	855
MF-AQ	849
Total	3428

# Efficacité thérapeutique CTAs

*Umberto D et al., NEJM 2016)*

- Tableau II: Efficacité thérapeutique des CTAs dans le Paludisme Associe a la Grossesse

	PCR corrigée	PCR non corrigée
AL	96,1 p<0,001	52, 5 p<0,001
ASAQ	98,5	82,5
DHA-PQ	99,2	86,9
MF-AS	96,8	73,8

*Umberto D et al., NEJM 3016*

## Niveau de clairance parasitaire sous les 4 CTAs au cours du suivi

- D1: clearance parasitaire était lente dans le bras AL  
 $p<0,001$

AL: **24,8% positif à D1 (217/875)**

ASAQ: 6,9% (57/828)

DHA-PQ: 8% (67/837)

MF-AS: 13,5% (113/837)

- D2 : GE négative chez 99,5% des femmes (4 CTAs)

- Infection placentaire :  $p=0,47$  (4 CTAs=comparables)

- Poids de naissance: :  $p=0,40$  (4 CTAs = comparables)

# TOLERANCE DES CTAs au cours du PAG

- *(Umberto D et al., NEJM 2016)*
  - SAE: 72 femmes à D63
    - 1 décès par méningite : MF-AS
    - 10 SAE → ASAQ: 5, DHA-PQ: 1, MEF-AS: 4

**Evénements indésirables +++ avec ASAQ et MEF-AS  
p<0,001**

Asthénie, perte de poids , douleurs, nausées,  
vomissements

Hallucinations: ASAQ

Insomnie

Faible pouls

Hypotension

# **IMPACTS DES CTAs SUR L'ISSU DE LA GROSSESSE<sup>1</sup> (*Umberto D et al., NEJM 2016*)**

Avortements: 13

AL: 1 et 3 dans chacun des autres bras

Mort-nés: 78

AL: 1,9% (16/856)

ASAQ: 2,1% (17/815)

DHA-PQ: 2,77% (22/818)

MF-AS: 2,8% (\_23/821)



Dicko AA, Sagara I, Doumbo OK et al.,  
Un essai de chimio prévention du  
paludisme saisonnier plus  
l'azithromycine chez les enfants africains

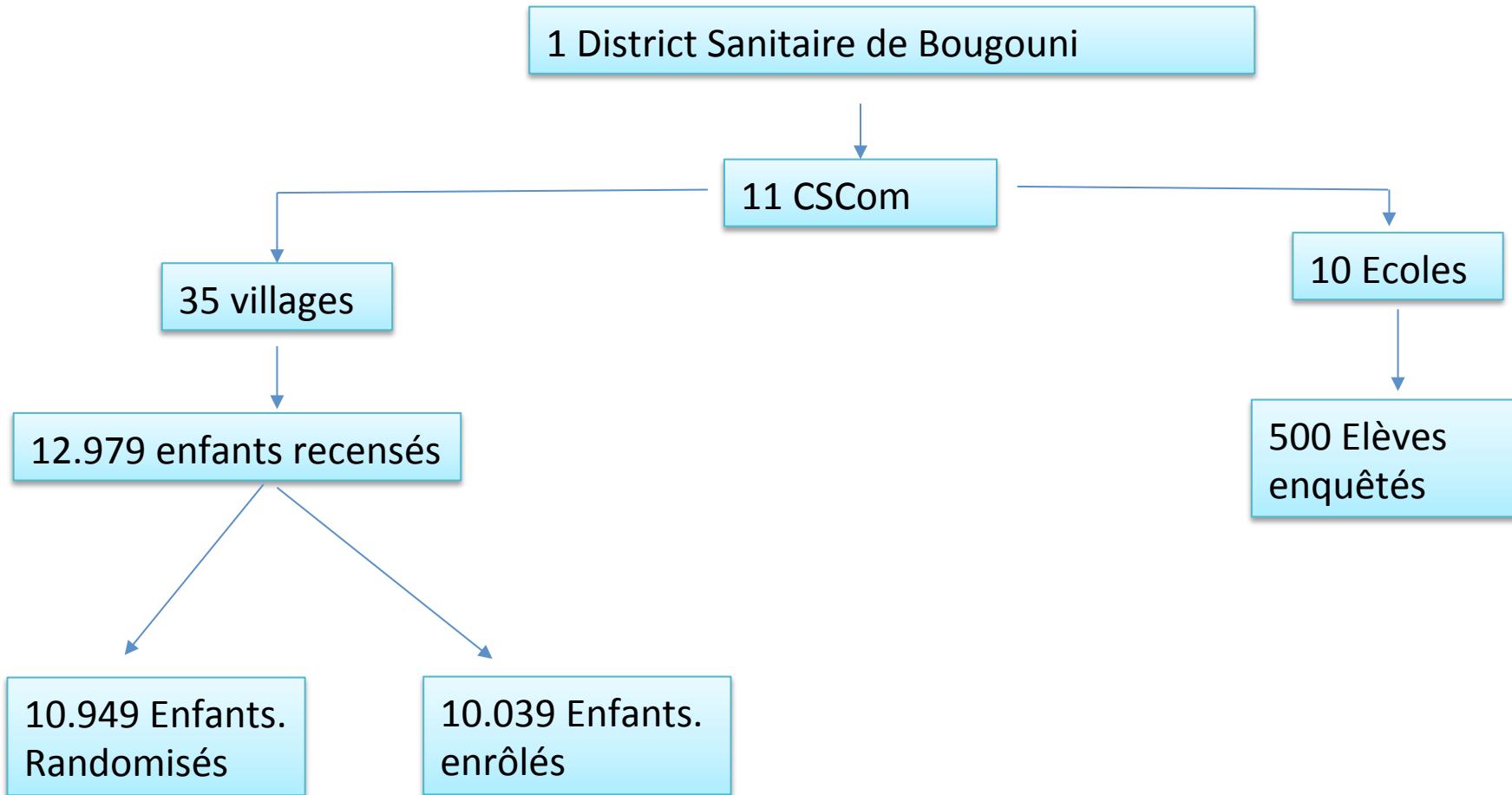
# Site d'étude : Bougouni

- 150 km sud de Bamako
- Bonne route goudronnée
  - 35 villages couverts
  - 11 CSCom
  - 1 CSRef

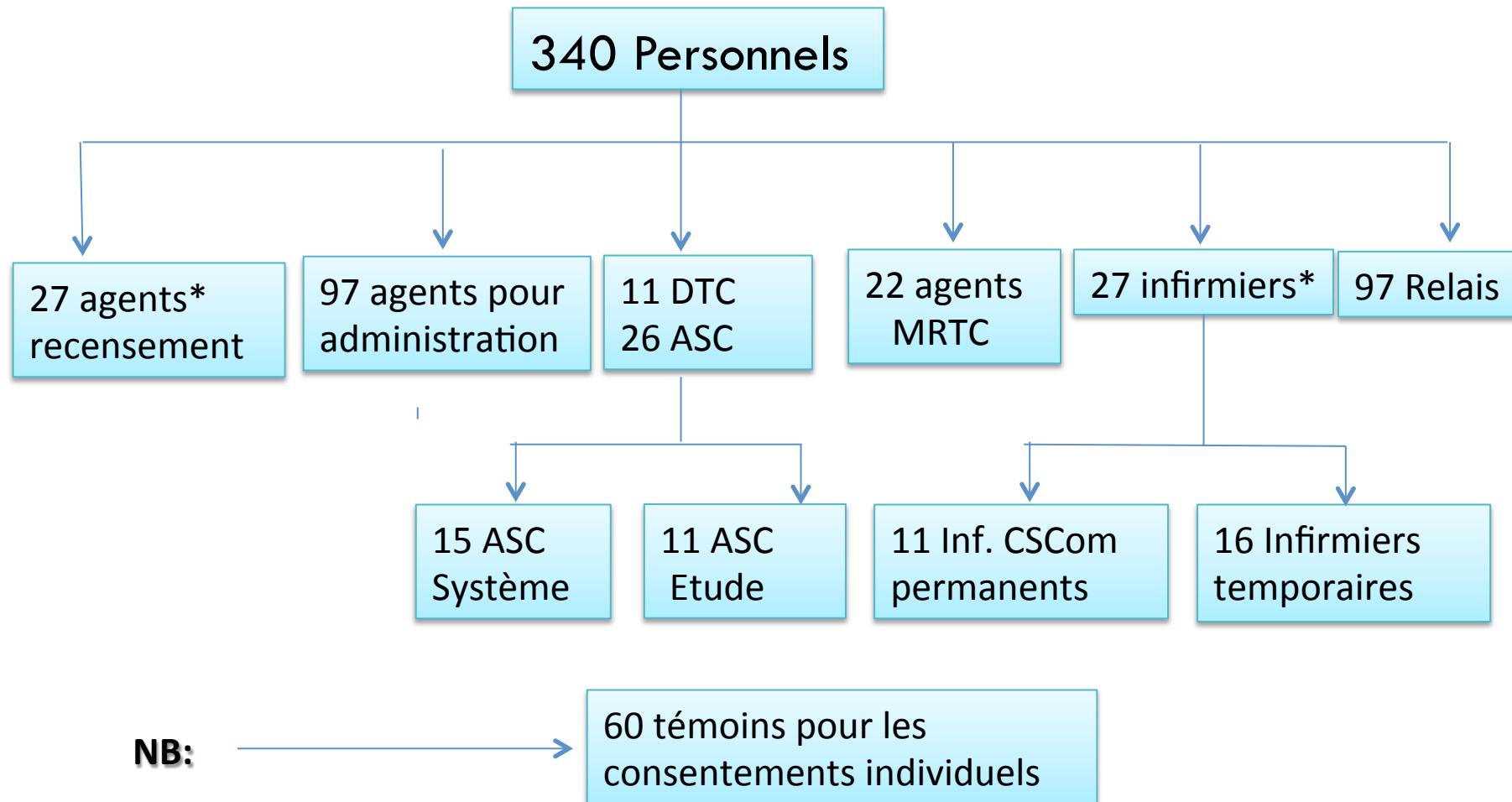
Bougouni, Mali



# Organisation et mise en œuvre



# Personnels impliqués dans la mise en œuvre

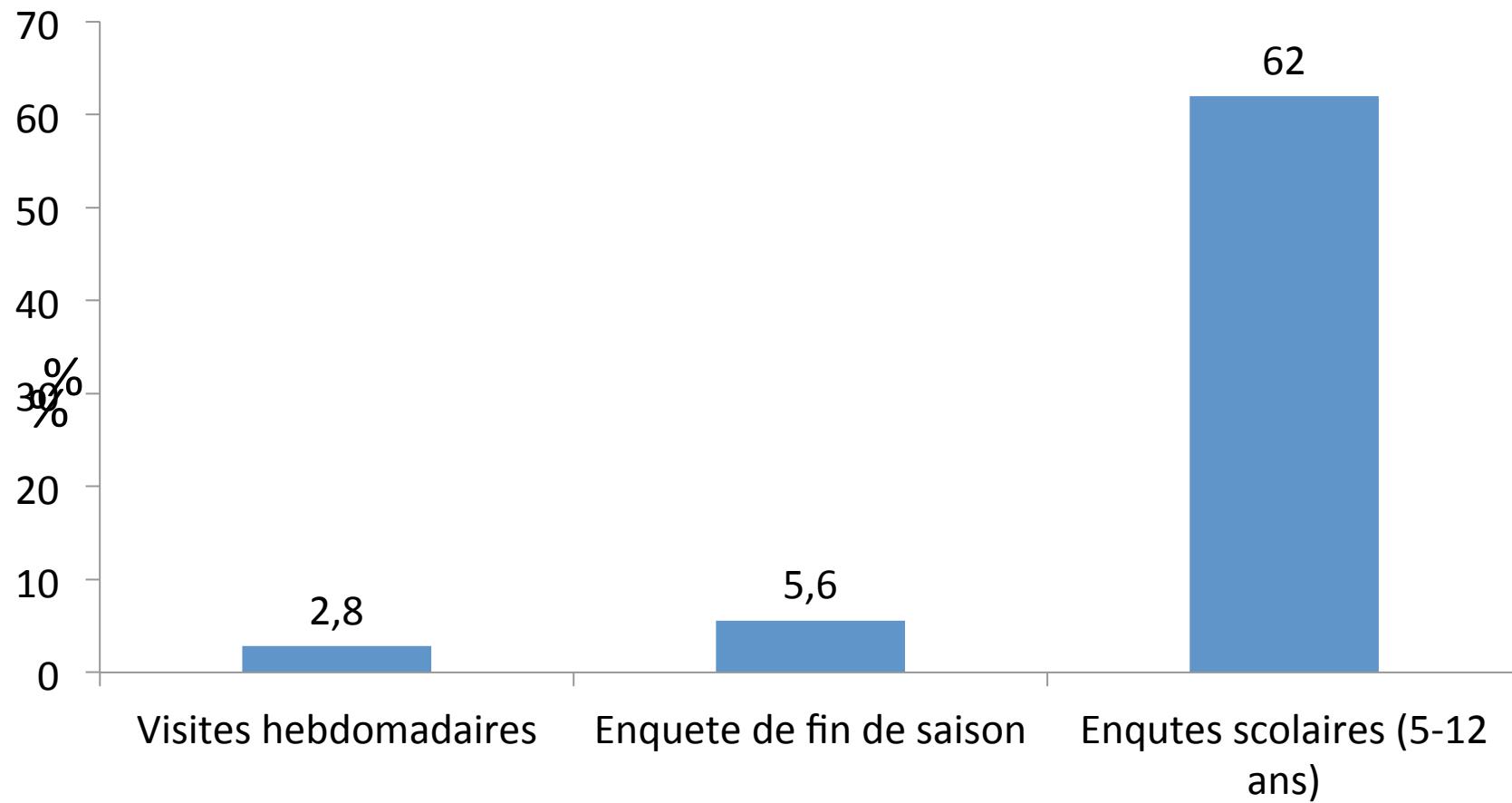


\* Les 27 infirmiers sont aussi les agents qui ont effectué le recensement

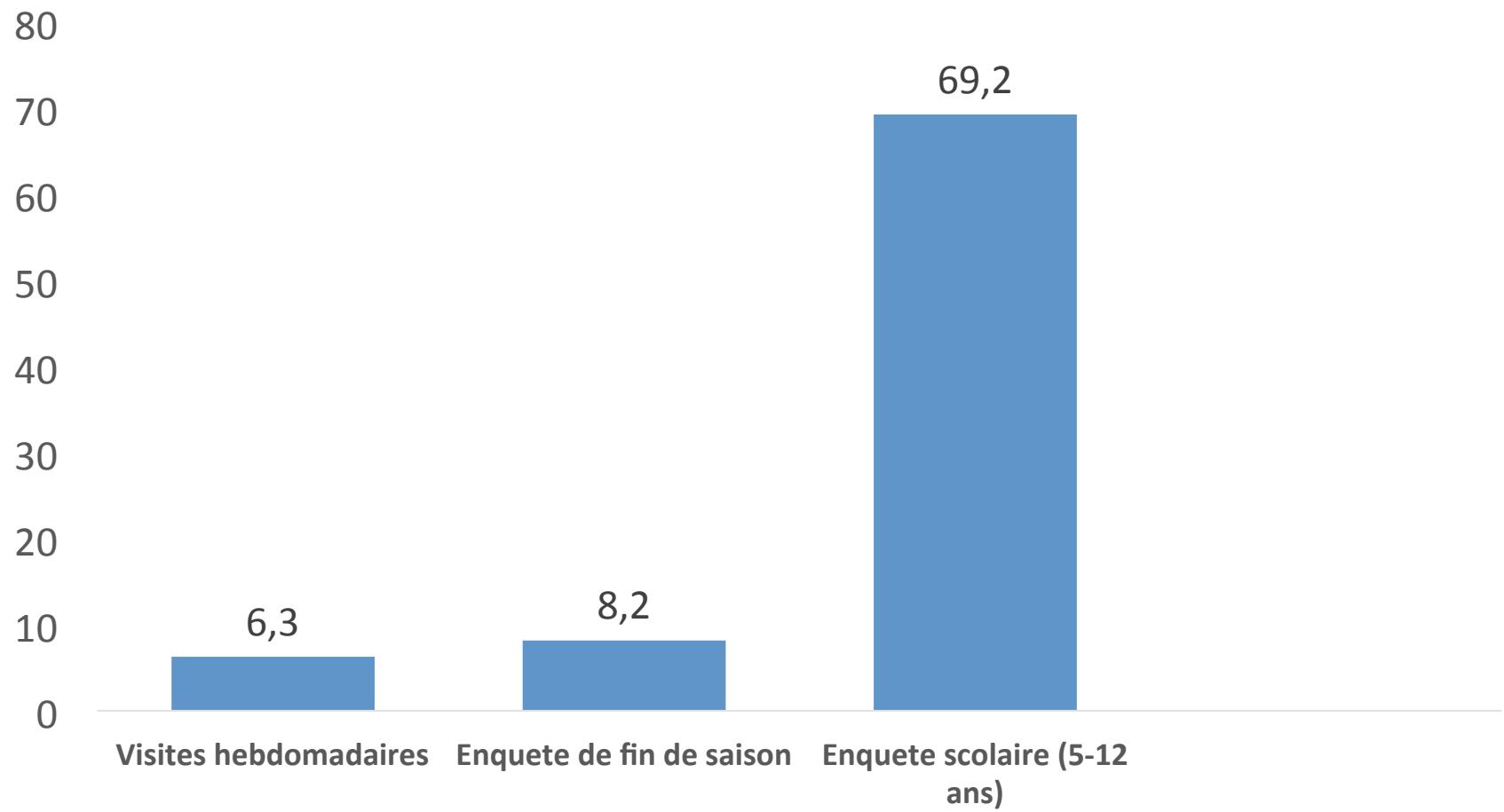
# Quelques chiffres (1)

- 10.949 Randomisés (2015)
  
- 9405 ont reçu les médicaments de l'étude (2014)

# Infection Palustre (2014)



# Infection Palustre (2015)





# MALI TEAMS

