

Malaria Research Program at CBER

Studies to improve Blood Safety
from the risk of transfusion-
transmitted malaria, and malaria
pathogenesis and control

Scientific Site Visit Review Summary

Sanjai Kumar, Ph.D.

Division of Emerging and Transfusion
Transmitted Diseases

CBER, FDA

CBER Mission-Oriented obligations and research projects

- **Blood safety from transfusion-transmitted malaria**
 - **Development of DNA and antibody based tests for blood donor screening**
- **Provide expertise for the review of malaria vaccine INDs**
 - **Studies on malaria biology, pathogenesis, vaccination and biomarkers of virulence and immunity**

Only malaria research program at CBER

Malaria Program Members:

Sanjai Kumar, Ph.D., Supervisory Biologist (PI)

Hong Zheng, M.S., Biologist

Victoria Majam, M.S., Biologist

Babita Mahajan, Ph.D., Post doctoral Fellow

Transfusion transmitted malaria in the US

1963-1998

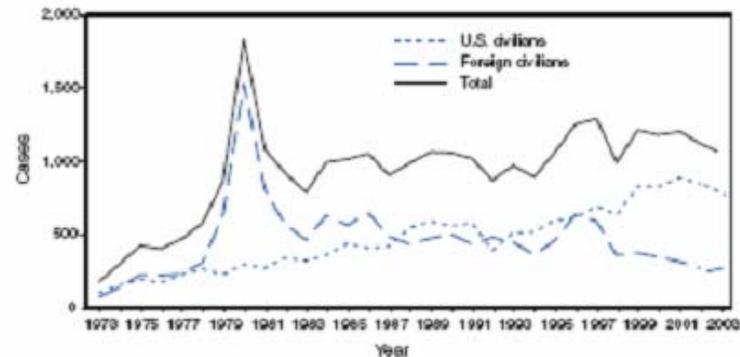
- 3 cases per year. All four species of *Plasmodium* implicated
 - Approximately 50% of cases were caused by malaria naïve travelers

1995 to 2005

- 0.5 case per year. *P. falciparum* is the predominant species
 - Caused by donors born in a malaria endemic country
- There is no laboratory test to screen blood donors for malaria infections
- Blood safety is maintained by risk based deferral policy
- Loss of approximately 150, 000 donors each year

US travelers: 28 million each year

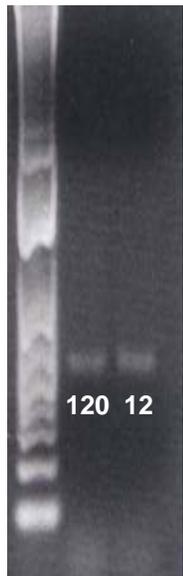
FIGURE 1. Number of malaria cases among U.S. and foreign civilians, by year — United States,* 1973–2003†



US immigrants: asymptomatic carriers

Laboratory tests to detect malaria parasite infections in blood donors

DNA Test



- 2.4 parasites/ml of blood
- Could leave up to 1200 parasites in a unit of blood
- For *P. vivax* infectious dose is 10 infected RBC or 0.02 parasites/ml in a unit of blood

Nested PCR - QIAmp kit

ELISA

Reactivity with recombinant *PfCSP*, *PfAMA-1*, and *PfMSP1₄₂*

Patient Sera	No. of Positives
<i>P. falciparum</i>	4/4
<i>P. vivax</i>	8/8
<i>P. malariae</i>	2/2
<i>P. ovale</i>	7/8
Normal Sera	0/2

- Three *P. falciparum* proteins can be used to detect antibodies to all four *Plasmodium* spp

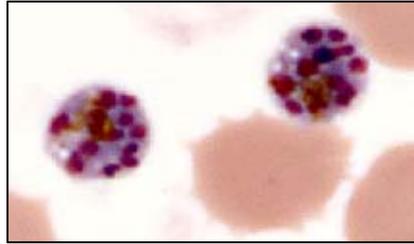
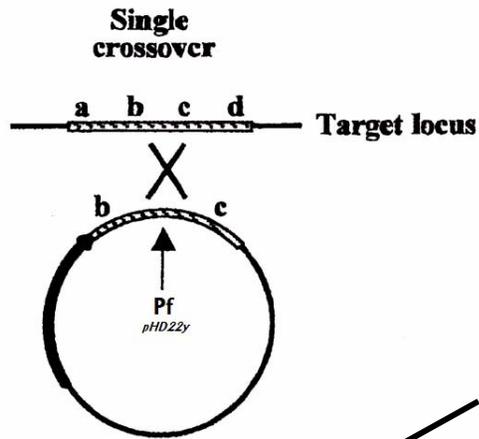
Live Attenuated Malaria Vaccines and Biomarkers of Virulence and Efficacy

- **R,TSS, the most successful recombinant malaria vaccine induced only 30% protection for a short duration**
- **Data from natural immunity, irradiated sporozoites and genetically attenuated sporozoites suggest that whole parasites are the most effective vaccines**
- **Current research trend suggests that several INDs based on live attenuated malaria vaccines will be submitted to FDA in the coming years**
- **A program to identify the molecules associated with parasite growth, and survival for targeted gene deletion to generate live attenuated vaccines**
- **To identify the biomarkers that could predict the virulence and efficacy of live attenuated vaccines**

Genetically altered live attenuated malaria vaccines

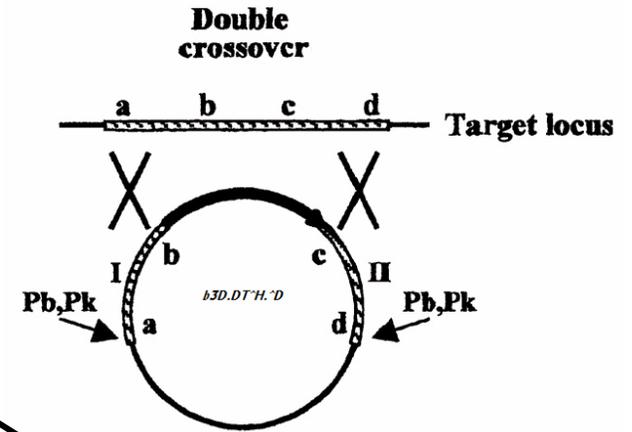
Gene KO strategy for Centrin

P. falciparum



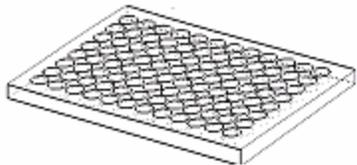
Blood Stage Schizonts

P. berghei



Electroporation & Transformation

In vitro drug selection



Genetic analysis and microarray

In vivo selection of resistant parasites

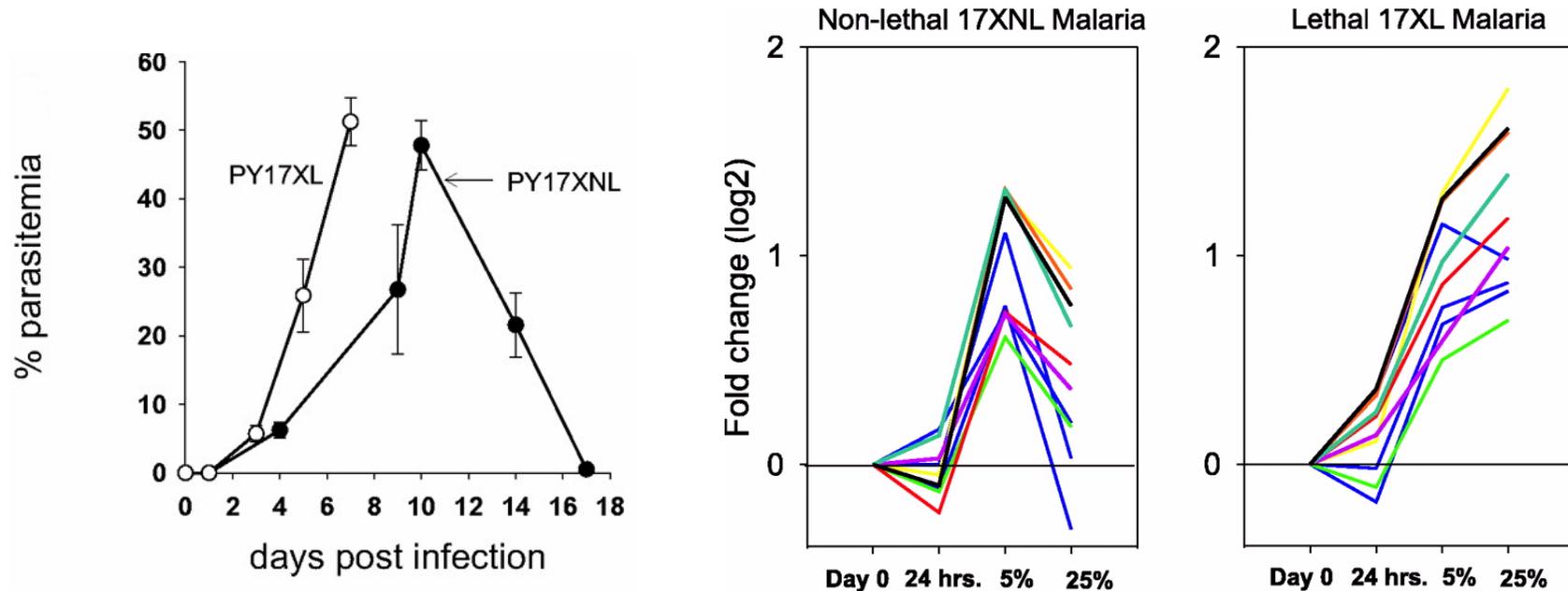


Genetic analysis and microarray

Attenuated Sporozoite



Genome-Wide Expression Profiling in Malaria *infection* Reveals Transcriptional Changes Associated with Lethal and Nonlethal Outcomes



- Identify a set of host biomarkers that distinguishes between lethal and non-lethal malaria infections
- Metabolic acidosis resulting from the accumulation of lactate is an important prognostic indicator for the severity of malaria. Increased activity in the glycolysis pathway is associated with pyruvate conversion to lactate
- In 17XL, all genes in the glycolysis pathway are upregulated during the entire course of infection

Summary

- A nested PCR that detects 2 *P. falciparum* parasites/ ml of blood and a pan-*Plasmodium* ELISA that recognizes all four species
- Identified *P. falciparum* parasite molecular factors and associated biological pathways induced in response to febrile temperatures
- Identified both host and parasite biomarkers that distinguish between virulent and non-virulent strains of malarial

Future Directions

- DNA and antibody based tests for the detection of malaria parasites in blood donors
- Suitable molecules for targeted gene deletion to create growth deficient malaria parasites
- Genetically attenuated live malaria vaccines and their safety, efficacy and virulence profiling
- Biomarkers that could predict the safety and efficacy of attenuated malaria vaccines

Collaborations

- **FDA**

Hira Nakhasi, Anangmuthu Selvapandian

- **NIH**

Tom McCutchan

- **WRAIR**

Chris Ockenhouse

- **Virginia Tech**

Dharmendar Rathore

- **NCBI**

Aravind Iyer