

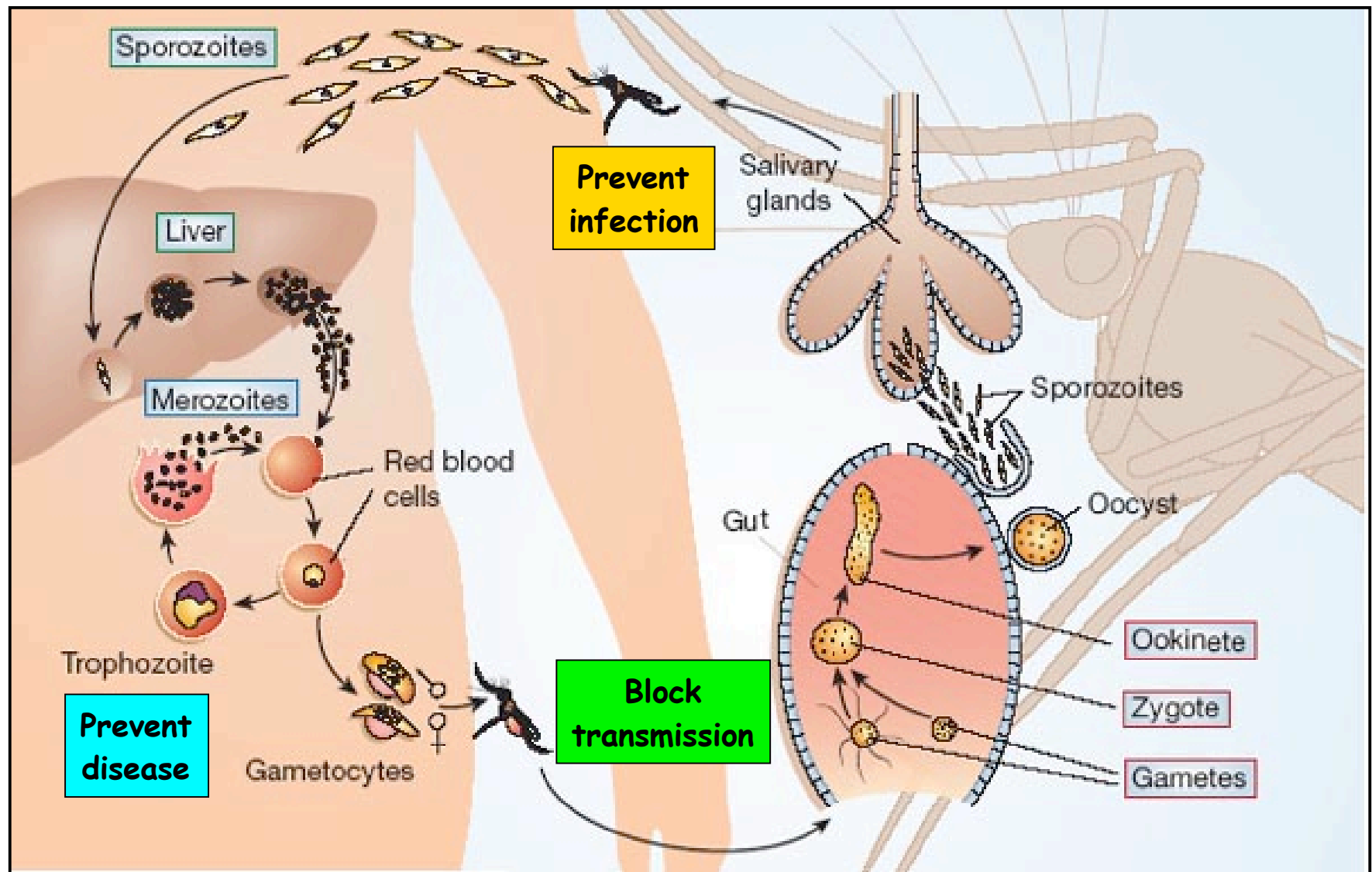


# Types of Vaccines

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- **Whole** pathogens **killed** prior to inoculation
  - Irradiated pathogens
- **Attenuated** live or **low virulence** vaccines
  - Genetically altered - now a possibility - proof-of-principle
- Protein **Subunit** vaccines
  - Purified proteins
  - Recombinant protein antigens - often lack post-translational modifications
  - Small peptide vaccines (can be chemically synthesized)
- **Nucleic acid** vaccines
  - Expression of parasite gene (protein produced) using promoter sequence of host
  - Relatively simple technology, low cost
  - Extremely stable - no refrigeration required

# Vaccine Development



# 3 Major Malaria Vaccine Types

## ■ Pre-erythrocytic vaccine - Prevents infection

- Sporozoites
- Aborts infection before or in the liver
- However, no resulting blood stage immunity



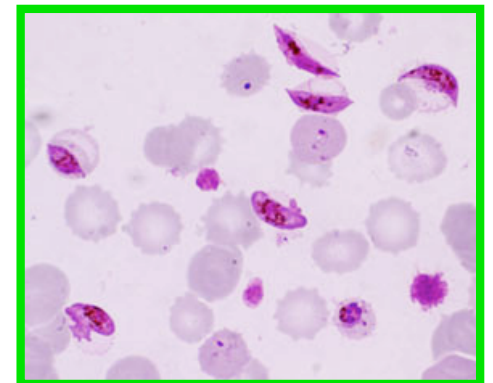
## ■ Blood stage vaccine - Prevent disease

- Merozoites
- Retards the infection (and disease)
- Enables development of immunity



## ■ Sexual stage vaccine - Transmission blocking

- Gametocytes
- Altruistic vaccine
- Host still has disease symptoms
- Contribute more to malaria control





# Pre-erythrocytic Vaccine

- Only a few (10-30) sporozoites are delivered to host during a blood meal, but give rise to large numbers of pathogenic merozoites
- Window of time to interrupt the pre-erythrocytic cycle is brief
- **Protection must be 100%**
  - 80-90% protection may provide decreased disease especially in children = anti-morbidity vaccine
- Lead candidate - RTS,S (circumsporozoite protein)
  - DNA vaccine - CS protein + Hepatitis B surface antigen
  - Gambia trials
  - Mozambique trials - protection in young children (1-4 yrs old)
    - New infection ↓45%, clinical episodes ↓29%, new episodes ↓57%
- Other candidates - TRAP - thrombospondin-related adhesive protein



# Blood Stage Vaccine

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- Rationale - anti-invasion, anti-complication
  - passive transfer of maternal antibodies
  - individual in endemic areas control parasitemia after repeated infection
  - Sera from chronically infected individuals can eliminate circulating parasites
- Merozoites are the target
  - MSP 1, 2, 3 - merozoite surface proteins - prevent invasion
    - Possible alternative route utilized for invasion!
    - Numerous other possible antigens
  - PfEMP1 - erythrocyte membrane protein 1
    - Cytoadherence
    - Antigenic variation - ~50 different genes



# Transmission Blocking Vaccine

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- Could break the cycle of parasite infection and transmission in local areas
  - Protect communities from infection
- Does not confer protection to the individual
  - Unattractive financially - altruism
- Widespread vaccine coverage that must be sustained
- Gametocytes are the target - sexual stages
  - Inhibit exflagellation and fertilization in mosquito



# Other possibilities

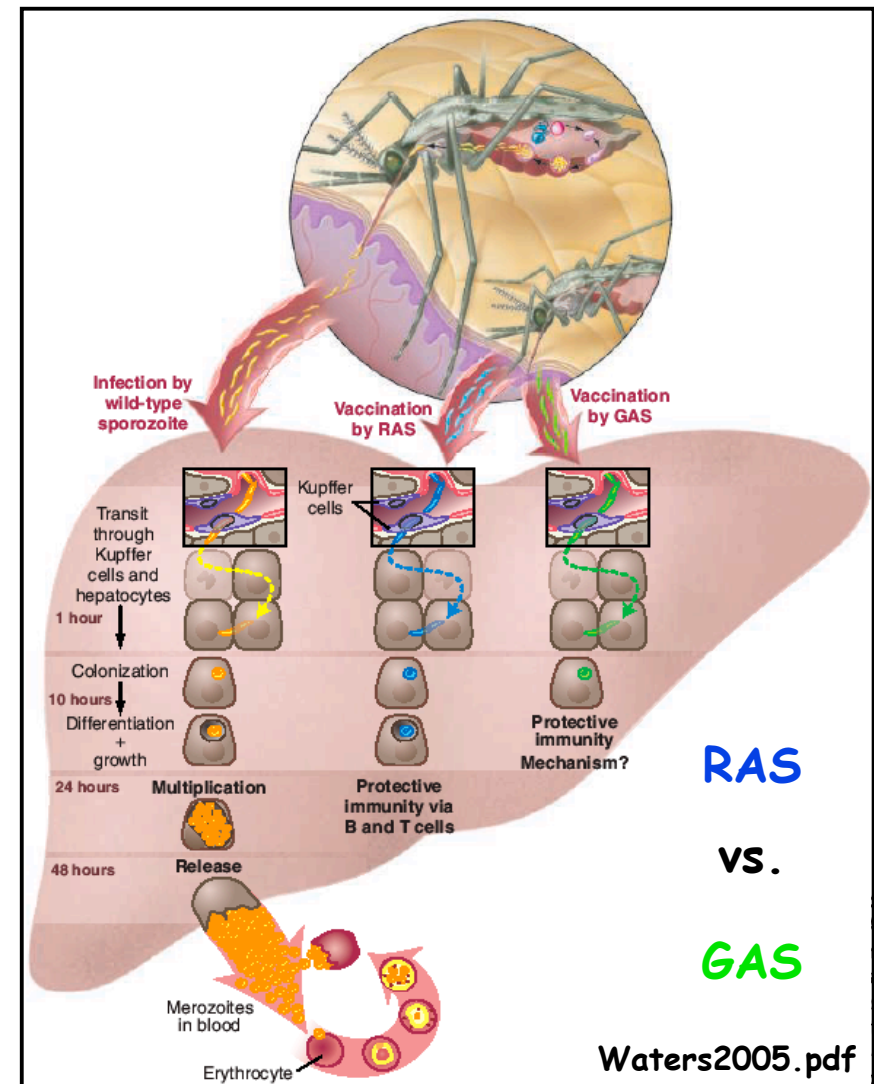
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## ■ **Combined vaccines**

- Multivalent - several antigens from the same stage
  - Heterogeneity of host response to malarial infection
- Target multiple stages
  - Pre-erythrocytic and blood stage vaccine
    - Would not need 100% sterile protection of sporozoite vaccine
    - Multi-gene, multi-stage vaccine - appear well tolerated, no challenge yet
      - MuSTDO - 9 plasmid DNA vaccine
        - 5 plasmids - liver and sporozoite stage antigens
        - 4 plasmids - blood stage
- Reduce the rate at which the parasite might develop resistance

# Vaccine Development

- Effective subunit vaccine has remained elusive
- Renewed interest in whole organisms vaccine approaches
- Genome completed - expression studies for the various stages
- Pre-erythrocytic genes called *UIS* are upregulated in sporozoites when they gain infectivity
- Hypothesis - disruption of one of these genes may lead to attenuation of liver stage parasites (sporozoites)







# New Approach to Malaria Vaccines

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## **Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine**

**Ann-Kristin Mueller<sup>1</sup>, Mehdi Labaied<sup>2</sup>, Stefan H. I. Kappe<sup>2,3\*</sup>  
& Kai Matuschewski<sup>1\*</sup>**

<sup>1</sup>*Department of Parasitology, Heidelberg University School of Medicine,  
Heidelberg 69120, Germany*

<sup>2</sup>*Seattle Biomedical Research Institute, Seattle, Washington 98109, USA*

<sup>3</sup>*Department of Pathobiology, University of Washington, Seattle, Washington  
98195, USA*

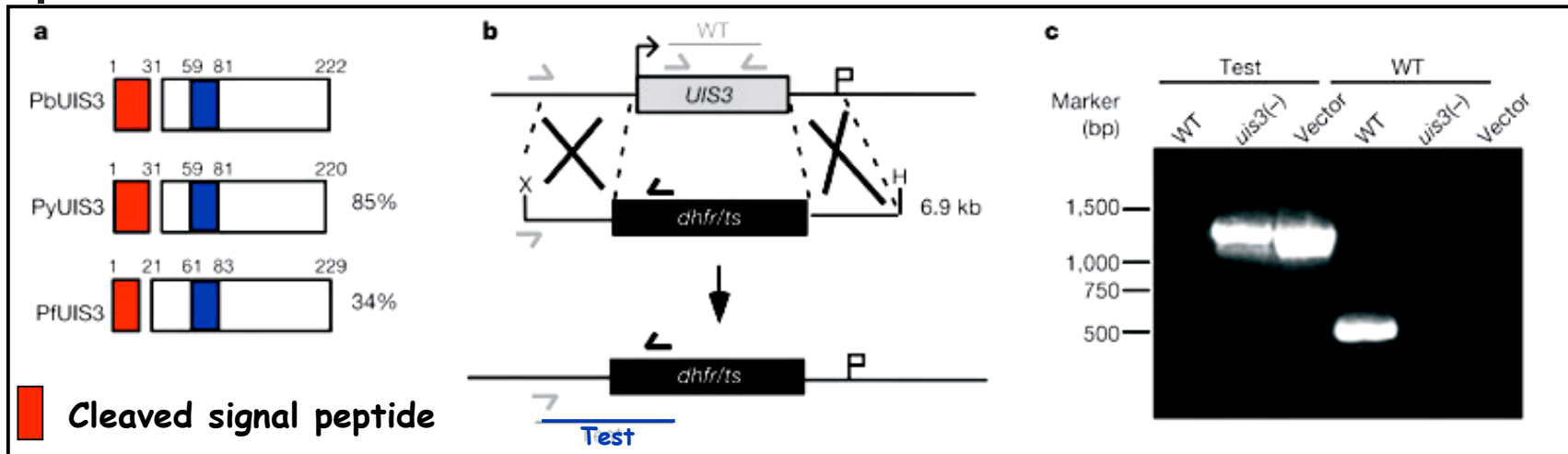
\* These authors contributed equally to this work

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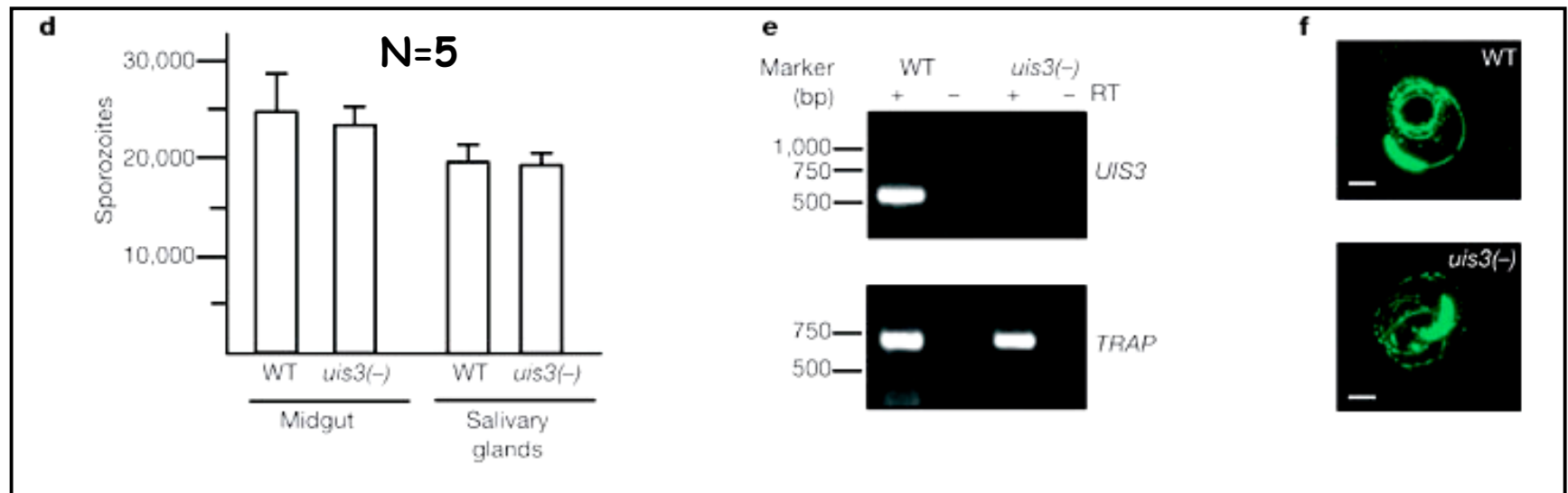
**Malvacc2005.pdf | MalariaKO.pdf | Waters2005.pdf - reading that might be helpful**

# Targeted disruption of *UIS3*



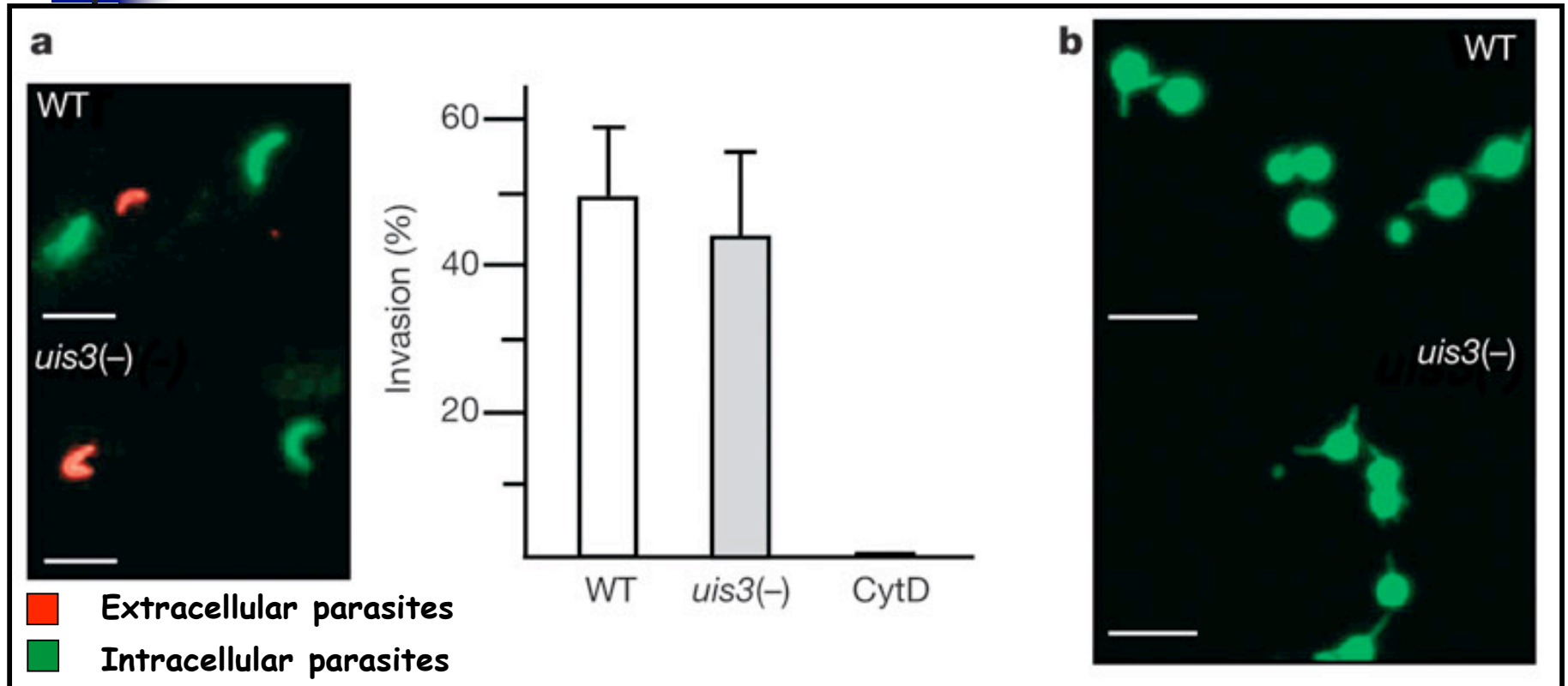
- *UIS3* - encodes a small transmembrane protein
  - Conserved among *Plasmodium* species
  - Only 34% identity in *P. falciparum*
  - **Expressed in infectious sporozoites and after infection in hepatocytes**
- *P. berghei* is good model system to study liver stage immunity
- Gene replacement with *T. gondii* *dhfr/ts* selectable marker
- PCR confirmation of replacement

# Targeted Disruption of *UIS3*



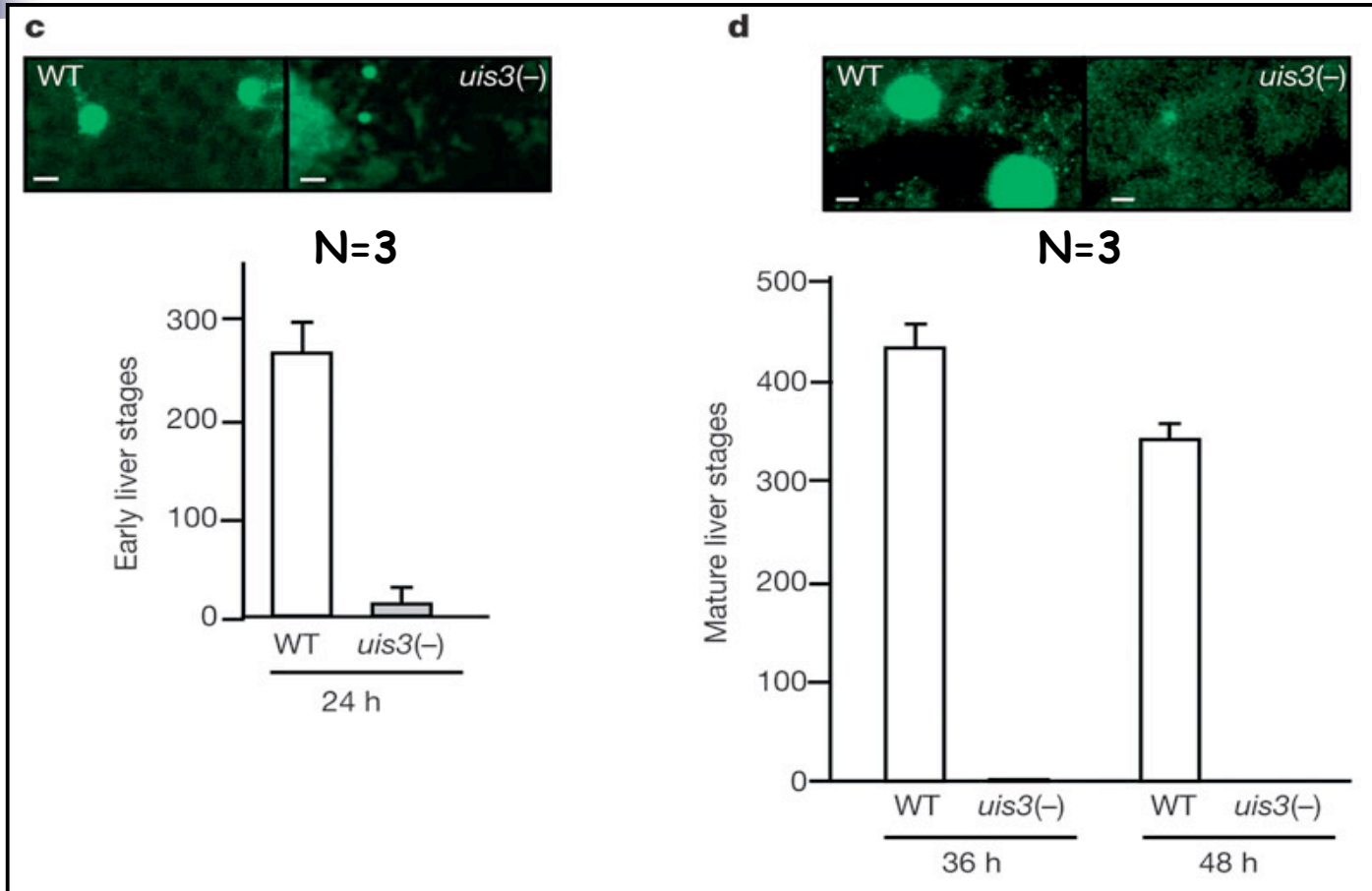
- *UIS3* is not essential for sporozoite development in mosquito midgut
- *UIS3* is not essential for sporozoite invasion of salivary glands
- *UIS3* is not essential for gliding activity
  - Gliding (substrate-dependent motility) is essential for transmission and infectivity

# Target disruption of UIS3



- UIS3 is not required for liver cell invasion (cultured cells)
- UIS3 is not required for the initial sporozoite/trophozoite transformation - *in vitro* test (cultured cells)

# USI3 is required for liver stage development (*in vitro*)

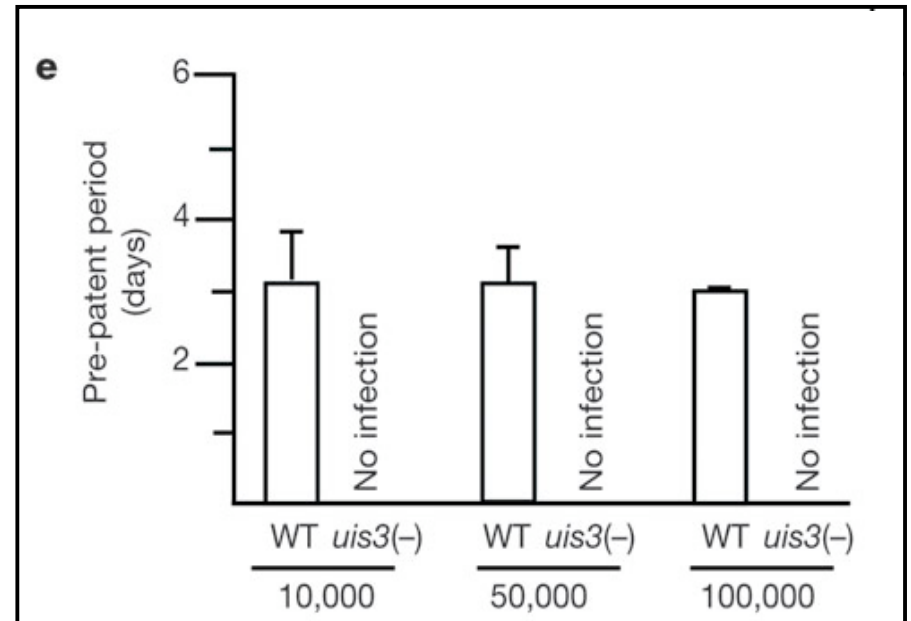


Few develop completely  
into trophozoites

No schizonts formed

# UIS3(-) Parasites in Host

- Infect young Sprague-Dawley (highly susceptible) rats with sporozoites
- Occurrence of blood stages were monitored by Giemsa-stained blood smears
- **UIS3(-) parasites DO NOT progress to blood stage infections**



Experiments were in duplicate

Each experiment used 4 rats for WT and 4 rats for *uis3*(-) sporozoites

# New Whole Organism vaccine?

UIS3(-) sporozoites lost capacity to develop to blood-stage parasites

UIS3(-) sporozoites might be a good whole organism vaccine

Mouse/sporozoite challenge model - well characterized

Variation in the prime/boost regimen

Looking for sterile protection - WHY?

Table 1 Protection of C57Bl/6 immunized mice against challenge with wild-type *P. berghei* sporozoites

Experiment	Immunization (uis3(-) sporozoites)	Boosts*	Challenge dose (time point)†	Number protected/number challenged (pre-patency)‡
1	50,000	25,000 (d 14)/25,000 (d 21)	10,000 sporozoites (d 7)	10/10 (no infection)§
1	10,000	10,000 (d 14)/10,000 (d 21)	10,000 sporozoites (d 7)	10/10 (no infection)§
1	–	–	10,000 sporozoites	0/9 (d 3)
2	50,000	25,000 (d 34)/25,000 (d 45)	10,000 sporozoites (d 30)	5/5 (no infection)
2	10,000	10,000 (d 34)/10,000 (d 45)	10,000 sporozoites (d 30)	5/5 (no infection)
2	–	–	10,000 sporozoites	0/6 (d 4.5)
3	50,000	50,000 (d 14)/10,000 (d 21)	Ten infectious mosquitoes (d 38)	5/5 (no infection)
3	10,000	10,000 (d 14)/10,000 (d 21)	Ten infectious mosquitoes (d 38)	5/5 (no infection)
3	–	–	Ten infectious mosquitoes	0/5 (d 3)
4	10,000	10,000 (d 14)/–	10,000 sporozoites (d 7)	7/10 (d 8)
4	–	–	10,000 sporozoites	0/5 (d 3)
5	50,000	25,000 (d 14)/25,000 (d 21)	10,000 blood stage (d 30)	0/5 (d 2)
5	10,000	10,000 (d 14)/10,000 (d 21)	10,000 blood stage (d 30)	0/5 (d 2)
5	–	–	10,000 blood stage	0/3 (d 2)

Mice were immunized with *P. berghei* uis3(-) sporozoites.

\*Data are presented as numbers of sporozoites for first boost/second boost. Day of boost is indicated in parentheses.

†Mice were challenged with infectious *P. berghei* wild-type sporozoites or blood stages. Mice were from the same age group (50–80 days old) and sporozoites were from the same mosquito batch. Time points indicate the day of challenge after the final boost.

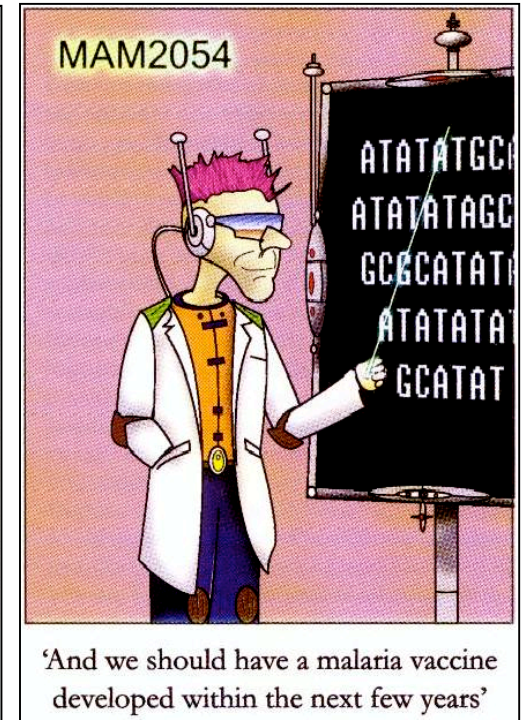
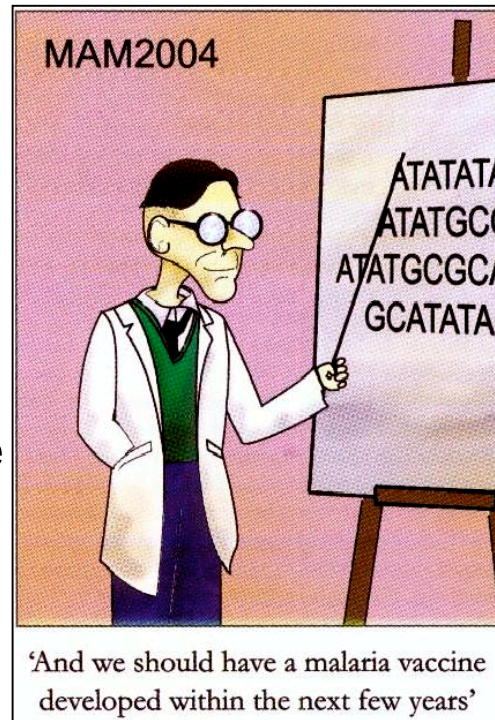
‡The pre-patent period is defined as the time until the first appearance of a single erythrocytic stage in Giemsa-stained blood smears.

§Five mice of the group were re-challenged with one dose of 50,000 wild-type sporozoites 2 months after the first challenge and remained protected.



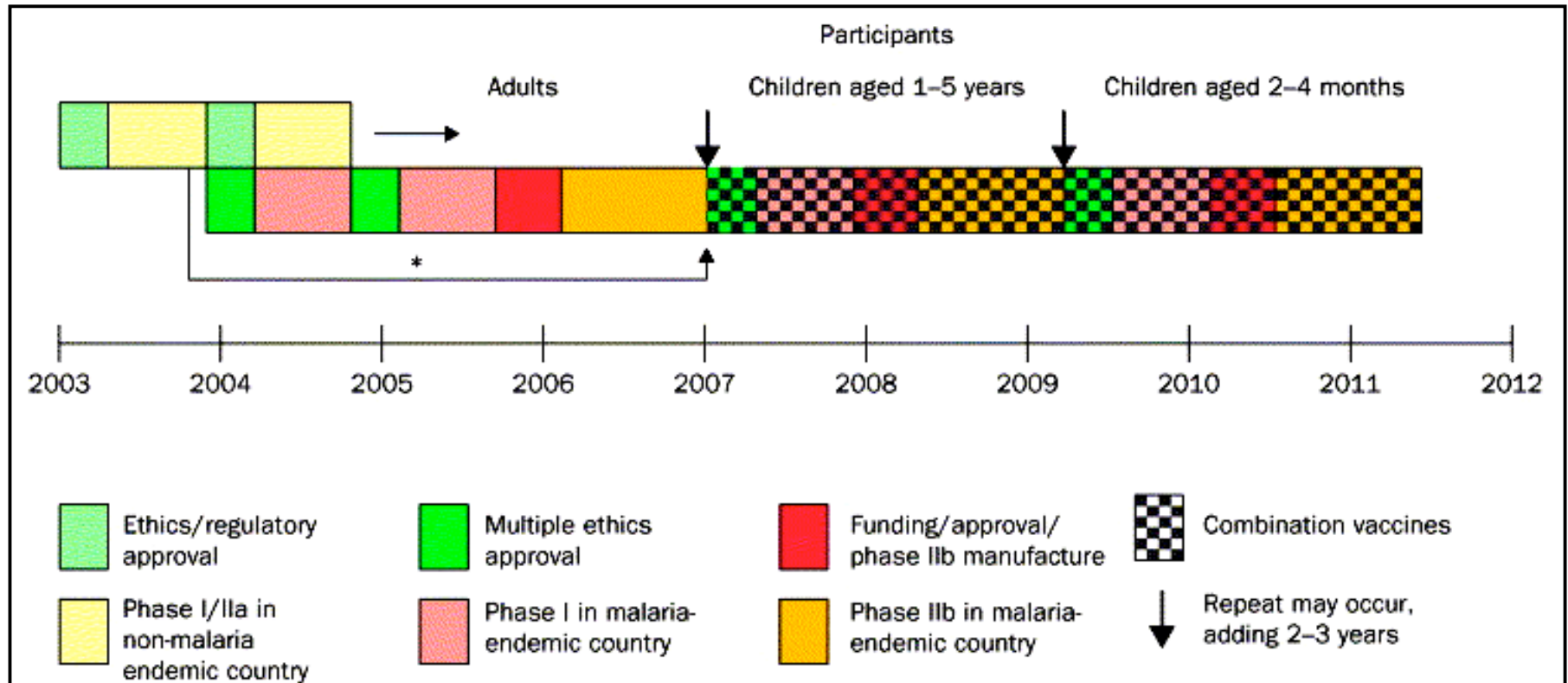
# Good Experimental Evidence!!

- Possible to develop genetically altered parasites that are attenuated at the liver stage
- **PROOF OF PRINCIPLE!!!**
- Sterile protection is absolutely required for a pre-erythrocytic stage vaccine
- No protection against blood stage parasites
- Technical Hurdles ahead
- Breakthrough that the Malaria Vaccine field has been looking for?

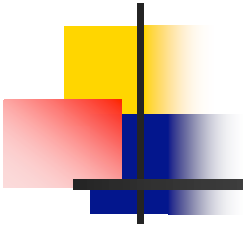




# Time to Market Considerations



**Proposed timeline when first large scale meetings on Malaria vaccines were organized.**



**Please refer to the Hill 2011 review for some additional background and history related to Malaria vaccine development.**

**I also posted an audio file - this is a bit more detailed - the earlier parts of the audio contain historical aspects.**