Malaria parasite vaccine development Strategies & Targets

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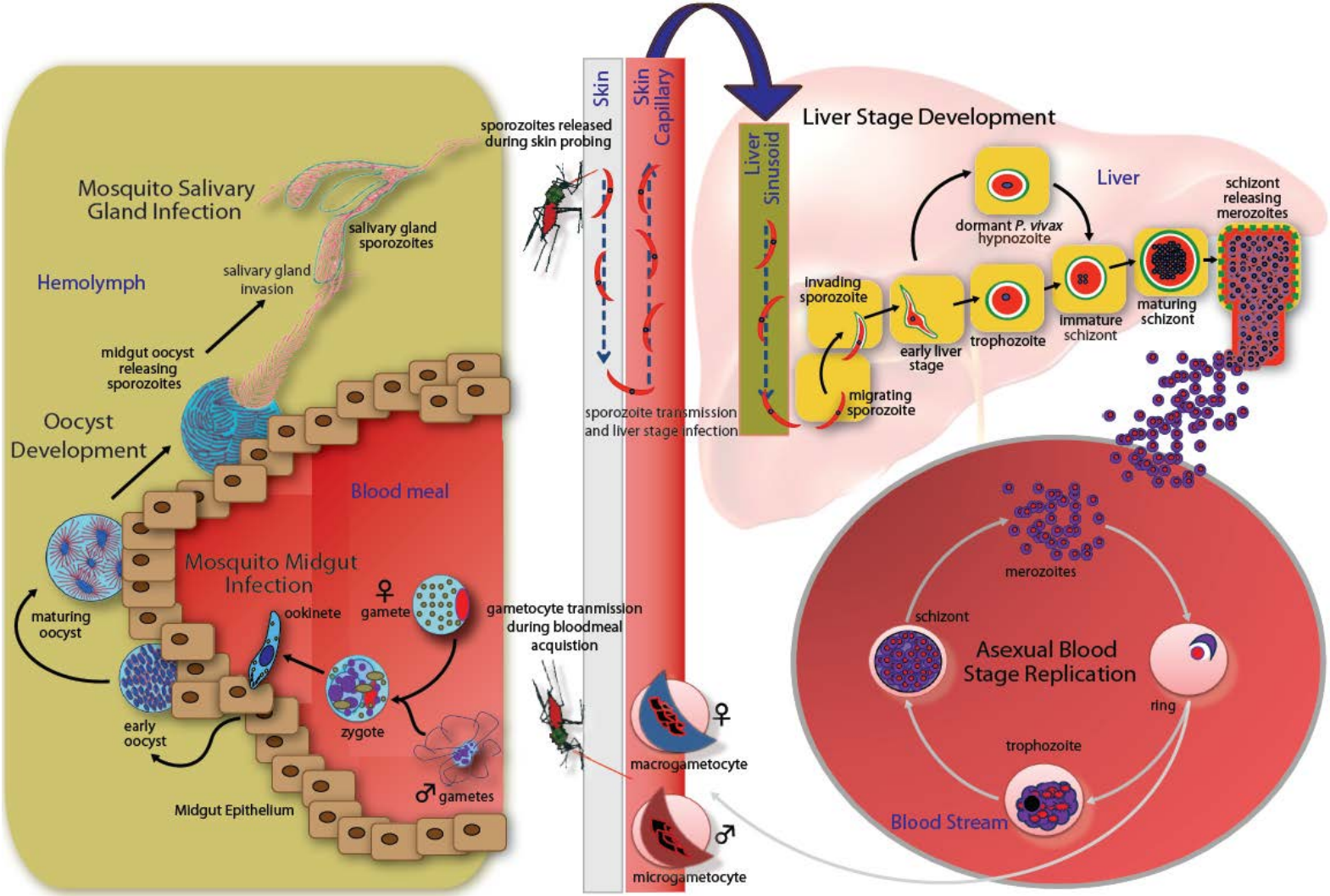
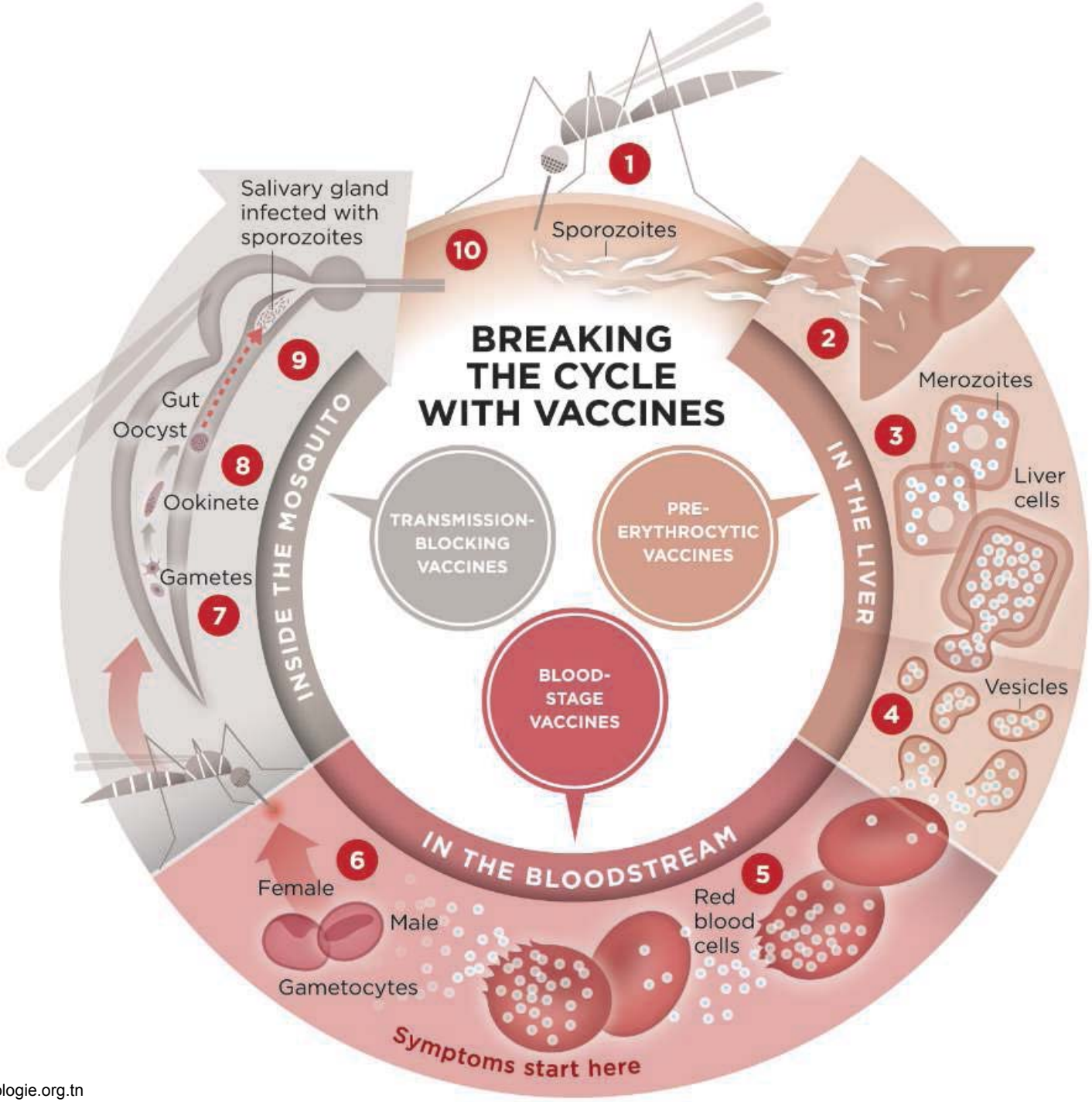
Most malaria disease deaths are among children and pregnant women

A child or a pregnant woman dies of malaria nearly every <u>33 seconds</u>

(WHO malaria report, 2010).

Malaria Parasite Life Cycle





Malaria vaccines

- (Anti-disease) **Blood stage vaccines**: Subunit, DNA and live whole vaccines that aim to prevent propagation of the pathogenic stages of *Plasmodium*. As of yet, none have shown any promising results.

- (Anti-Transmission) **Transmission blocking vaccines**: Subunit and DNA vaccines that aim to prevent *Plasmodium* transmission to the mosquito. Some candidates show promising results but have to be applied with anti-blood stage formulations, which is currently difficult.

- (Anti-infection) **Pre-erythrocytic vaccines**: Subunit, DNA and live whole organism vaccines that aim to prevent propagation of *Plasmodium* infection in the liver. Some candidates already show promising results.

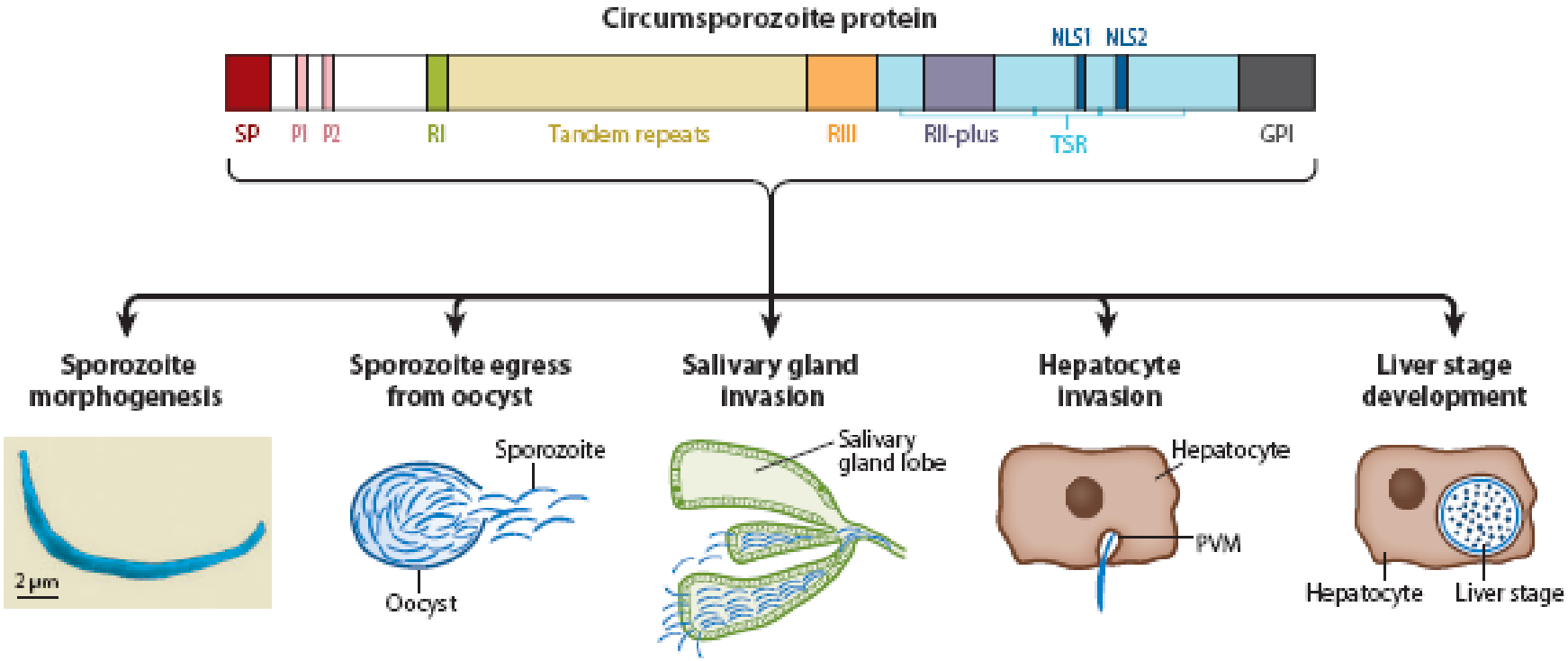
Subunit malaria vaccines

- Blood stages: AMA1, EMP1 (~ 60 *var* proteins) and MSP1, main surface antigens are used in vaccine formulations.

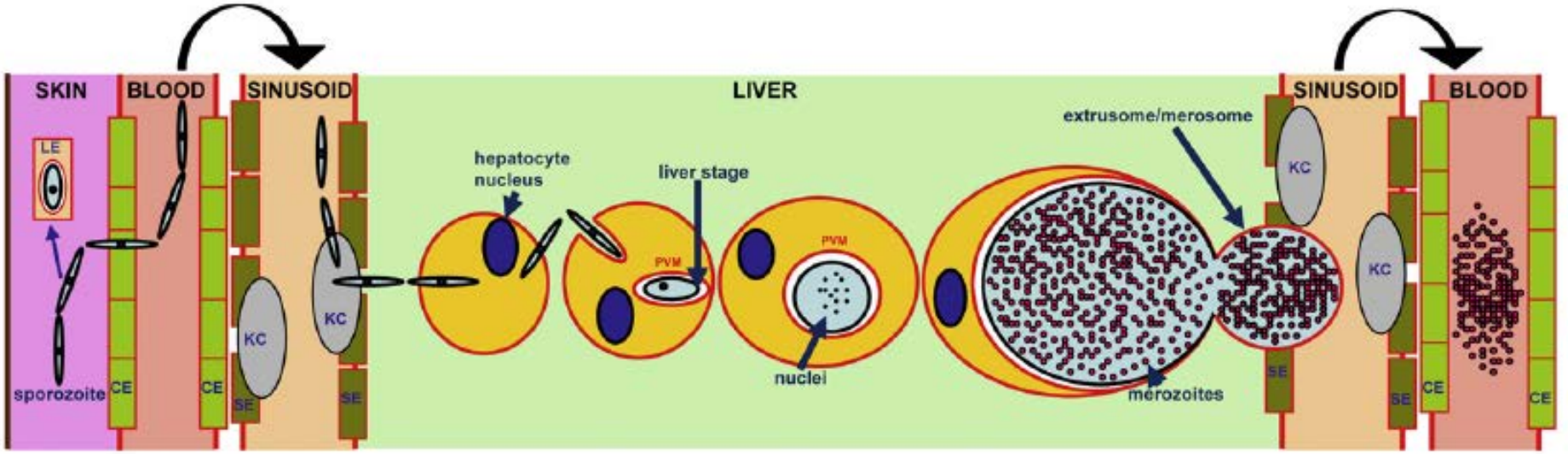
- Transmission blocking: P48/45 (male gamete specific), P25, P28, P230, HAP2 and Peg3 are all gamete surface proteins used in vaccine formulations. Antibodies against antigens act inside the mosquito midgut after gametogenesis to prevent fertilization.

- Pre-erythrocytic: TRAP (ME-TRAP), LSA1 and CSP (most recent trials with **RTS,S**) antigens are used in vaccine formulations.

The Circumsporozoite Protein Functions



Route to the liver



RTS,S

- Using recombinant DNA technology to express in yeast a fusion gene encoding the NANP Repeat region and the C-terminus (T-cell epitope) of *P. falciparum* CSP and Hepatitis B surface antigen S (HBsAg), formulated with AS0X adjuvants of GlaxoSmithKline to generate the RTS,S subunit malaria vaccine in virus like particles.

Clinical Trials on RTS,S subunit vaccine

- Phase III: RTS,S/AS01

Study Site

Burkina Faso, Gabon, Ghana (2 sites),Kenya (3 sites), Malawi, Mozambique and Tanzania.

Population

15,460 children enrolled in total.

6537 infants in 6-12 week age group.

8923 children in the 5-17 month age group.

Agnandji ST, et al. 2011. First results of phase 3 trial of RTS,S/AS01 in African children. N Engl J Med. 2011 Nov 17;365(20):1863-75.

Clinical Trials on RTS,S subunit vaccine

- Phase III: RTS,S/AS01

Design

Double blind randomized clinical trial.

Vaccines were given at time-points 0, 1, 2 months.

Control group for infants received meningococcal vaccine.

Control group for children received rabies vaccine.

Statistical Analysis

Study is still ongoing.

Data collected up to May 31, 2011 was utilized. Participants had to have at least 12 months of follow-up.

Clinical Trials on RTS,S subunit vaccine

- Phase III: RTS,S/AS01

Outcomes of Interest

Safety

Immunogenicity

Efficacy

Clinical Trials on RTS,S subunit vaccine

- Phase III: RTS,S/AS01

First Results

Safety: Serious Adverse events occurred in a similar frequency amongst both vaccination and control groups for both age groups.

Immunogenicity: Vaccine was found to be immunogenic (99.9% of participants had a positive CSP titer after 3 doses with a geometric mean titer of 621 EU/ml)

Efficacy: About 50% protective efficacy against clinical malaria cases.

Whole organism live attenuated malaria vaccines

- Attenuation of *Plasmodium* parasites used as live vaccines can occur by three different methods in blood and pre-erythrocytic stages:

1- Irradiation: (RAP: Radiation Attenuated Parasites).

Gamma radiation of pre-erythrocytic stages.

2- Genetic manipulation: (GAP: Genetically Attenuated Parasites).

Deletion of essential genes in blood and pre-erythrocytic stages.

3- Infection-Treatment: (ITV)

Infection followed by clearance of the parasite using drugs and antibiotics against blood and pre-erythrocytic stages.

Infection-Treatment-Vaccination

- **Historically, human *Plasmodium* infections that induced fever, with only symptomatic non-curative anti-malarial drug treatment, were used to cure infections with neurosyphilis bacterium before the wide-spread use of Penicillin (Collins and Jeffrey, 1999).**

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- **Experimental repeated immunization with *P. falciparum* in humans (Pompo et al, 2002), monkeys (Jones et al, 2000) and *P. chabaudi* in mice (Elliot et al, 2005), with simultaneous curative anti-malarial treatment, induced sterile protective immunity only against the same strain of the malaria parasite.**

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- **Recently, Infection-Treatment-Vaccination have been successfully tested against pre-erythrocytic stages in the mouse model after treatment with different antibiotics (Friesen et al, 2010).**

BS Genetically Attenuated Parasites

- Example: Targeted deletion of nucleoside transporter 1 (NT1) in the mouse malaria model, and using *nt1(-)* parasites as live experimental vaccines.

- Parasites can make their own pyrimidines from scratch. However, they have to salvage purines from the host.

- Deletion of NT1 deprives intracellular blood stage parasites from the tunnel through which they get purines so they grow and replicate extremely slowly.

- Thus, *nt1(-)* parasites do not cause patent disease in mice and are safe as experimental vaccines.

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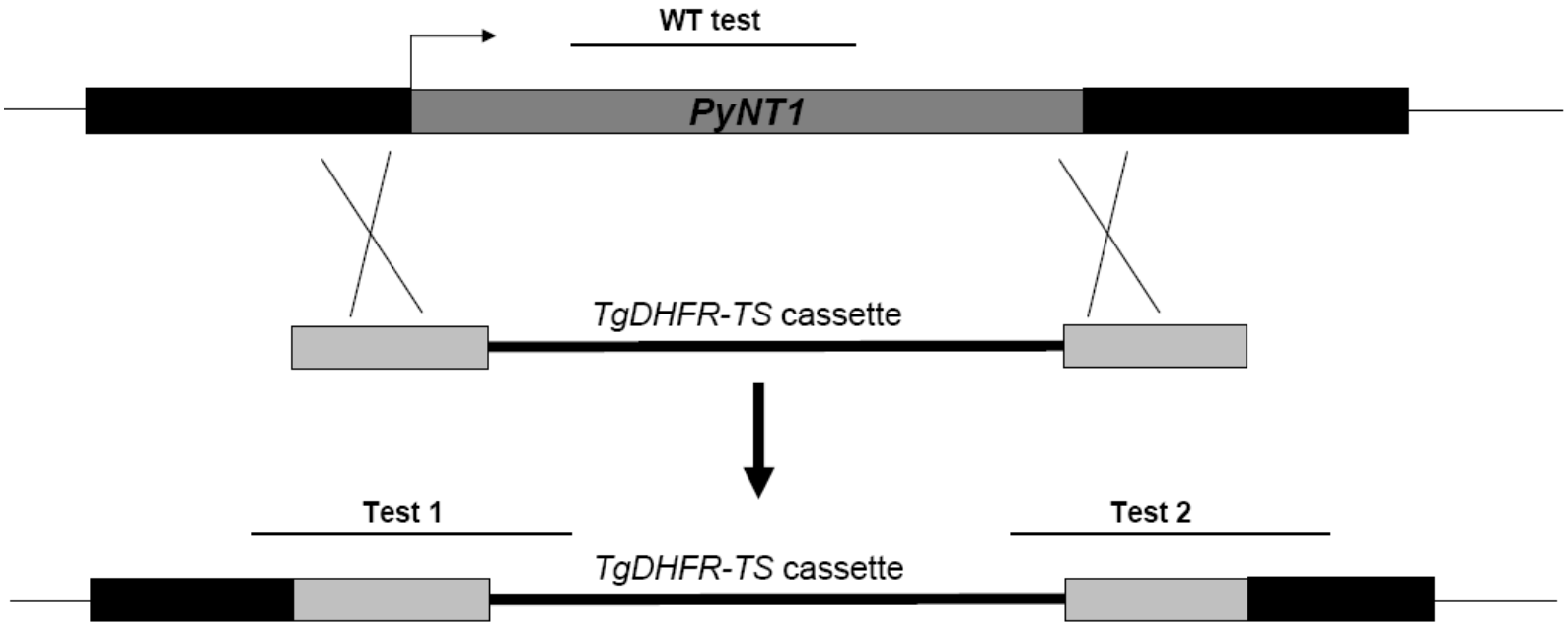

Aly, et al, 2010_Cellular Microbiology

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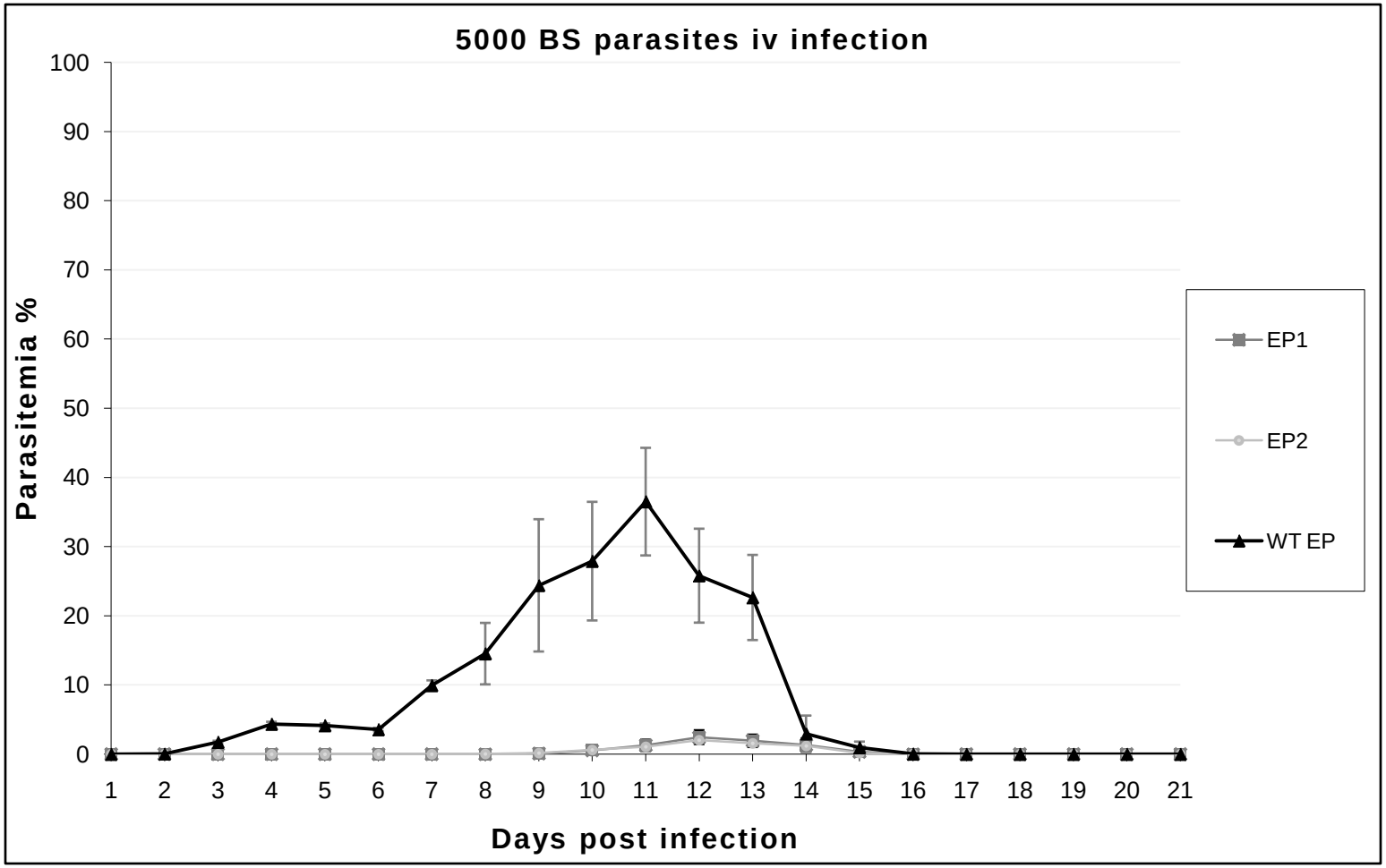

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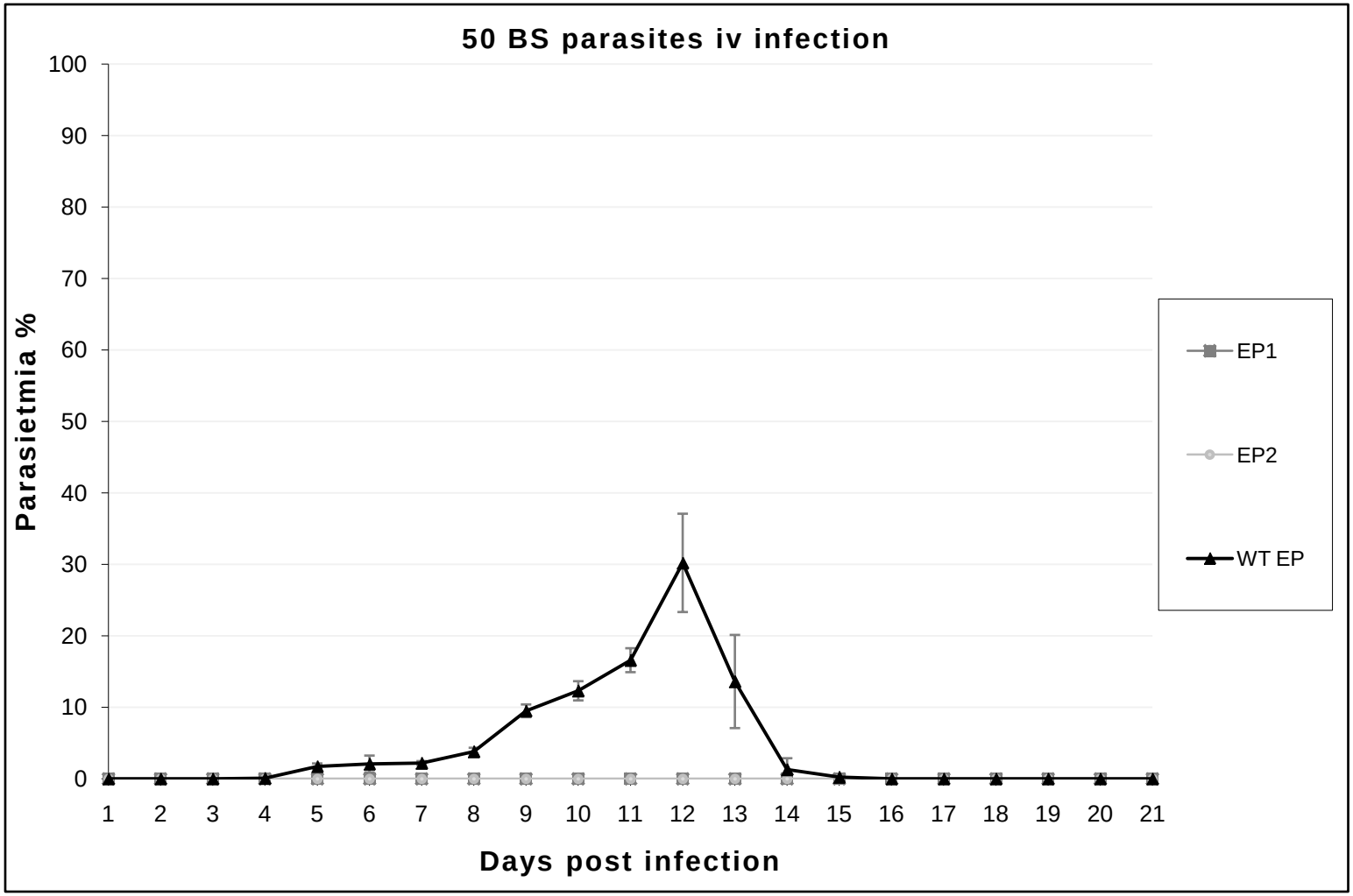


BS Genetically Attenuated Parasites

Parasite	Primary Dose[1]	Infection[2]	Mouse strain	Challenge Dose[1] / Days After infection	Protection[3]
Pynt1(-) Cl. 1	100	0/4	BALB/c	1000 / 3 months	4/4
Pynt1(-) Cl. 1	100	0/5	C57BL/6	25,000 / 3 months	5/5
Pynt1(-) Cl. 1	100	0/4	SW	25,000 / 3 months	4/4

[1] infection by iv injection.

[2] number of mice that showed BS patency from the total number of mice injected.

[3] determined by complete absence of blood stage parasites up to 14 days post challenge.

BS Genetically Attenuated Parasites

Parasite	Primary Dose[1]	Infection[2]	Mouse strain	Challenge Dose[1] / Days After infection	Protection[3]
Pynt1(-) Cl. 1	10,000	0/5	BALB/c	1000 / 1 month	5/5

[1] infection by subcutaneous injection.

[2] number of mice that showed BS patency from the total number of mice injected.

[3] determined by complete absence of blood stage parasites up to 14 days post challenge.

Aly, et al, 2010_Cellular Microbiology

Spz. Genetically Attenuated Parasites

- Example: Targeted deletion of Sporozoite Asparagine-rich Protein1 (SAP1) in the mouse malaria model, and using *sap1(-)* sporozoites as live experimental vaccines.

- SAP1 is a ***master regulator*** of other genes that encode essential proteins during liver stages development.

- Deletion of SAP1 deprived sporozoites of those essential proteins needed after hepatocyte invasion.

- Thus, *sap1(-)* parasites do not cause patent infection in mice and are safe as an experimental vaccine candidate.

Spz. Genetically Attenuated Parasites

No. of injected sporozoites	*Pysap1(-)*		*PyWT*	
	Infected	pre-patent period*	Infected	pre-patent period*
20	ND	ND	2/2	4 days
100	ND	ND	6/6	4 days
100,000	0/15	-	3/3	2.5 days
>2,000,000	0/4	-	ND	ND

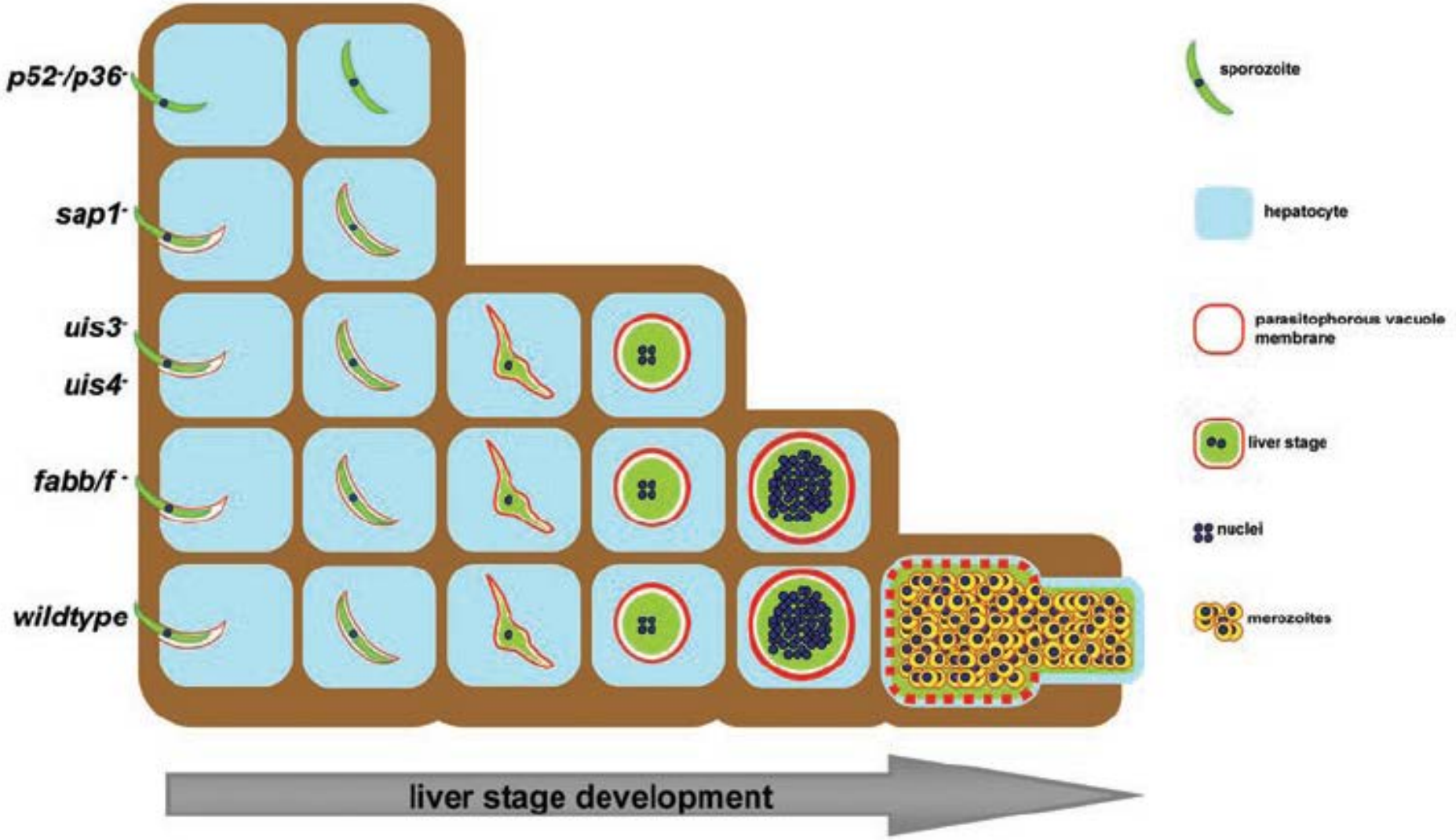
*The period (in days) between sporozoites infection and the detection of erythrocytic stages in blood smears.
ND: not done

Aly, et al, 2008_Molecular Microbiology

sap1(-) as potent GAP vaccine candidate

Parasite	Primary Dose	Boosts (Date given)	WT Challenge Dose / Months After Last Boost	Protection
Pysap1(-)	10,000 iv	(days 14, 28)	10,000 / 9 months	COMPLETE (6/6)
Pysap1(-)	1000 iv	(days 14, 28)	10,000 / 3 months	COMPLETE (5/5)
Pysap1(-)	10,000 iv	(days 14, 28, 42)	MB^+ / 12 months	COMPLETE (5/5)
Pysap1(-)	20,000 iv	(days 14, 28, 42)	20,000 / 3 months (Swiss Webster)	COMPLETE (5/5)
Pysap1(-)	100,000 subcutan.	(days 14, 28)	10,000 / 2 months	COMPLETE (5/5)

Genetically Attenuated Parasites (GAP) Vaccine



Thank you all for Listening

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