Indo-Dutch Workshop
on
Life Science and Health
Trivandrum, January 21-23, 2010

Bart Faber

Biomedical Primate Research Center (BPRC)

Rijswijk The Netherlands

The Biomedical Primate Research Centre

- Independent, not for profit research foundation
- Largest non-human primate research facility in Europe
- Mission - For many common deadly diseases there is still a desperate need for effective medicines. BPRC is a scientific research institute that exists to perform vital research that contributes to the identification and development of these new medicines. BPRC is committed to using non-human primates for this critical research only where there are no suitable alternatives. Research is also conducted to identify and develop alternatives to the use of non-human primates (reduction, refinement and replacement)
- Science- Applied, exploratory and fundamental
- Funding-
  - Core Dutch government support
  - competitive peer reviewed grants (Europe)
  - Strategic NGO as well as commercial collaborations

BPRC 2010

www.bprc.nl

BPRC Research Departments

- Parasitology
  - Malaria — drug & vaccine testing & development
  - Tuberculosis — vaccine testing (expand to drug testing?)
- Immunobiology
  - Autoimmune diseases (MS, RA) — drug testing, fundamental research
  - Organ transplantation — fundamental research
  - Neurodegenerative diseases
- Virology
  - HIV/SIV — vaccine testing
  - Hepatitis C — vaccine testing
- Comparative Immunogenetics — fundamental research
- Alternatives

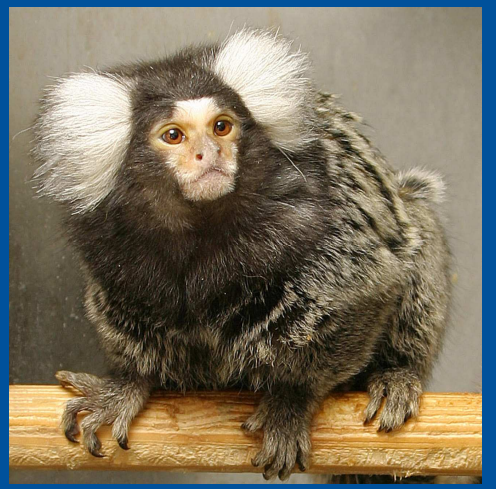
BPRC non-human primates

- >1100 macaque monkeys
  - Primarily rhesus, some cynomolgus

Models: malaria, RA, MS, Tuberculosis, parkinson's

- 200 new world monkeys
  - Common marmoset

Models: malaria, RA, MS

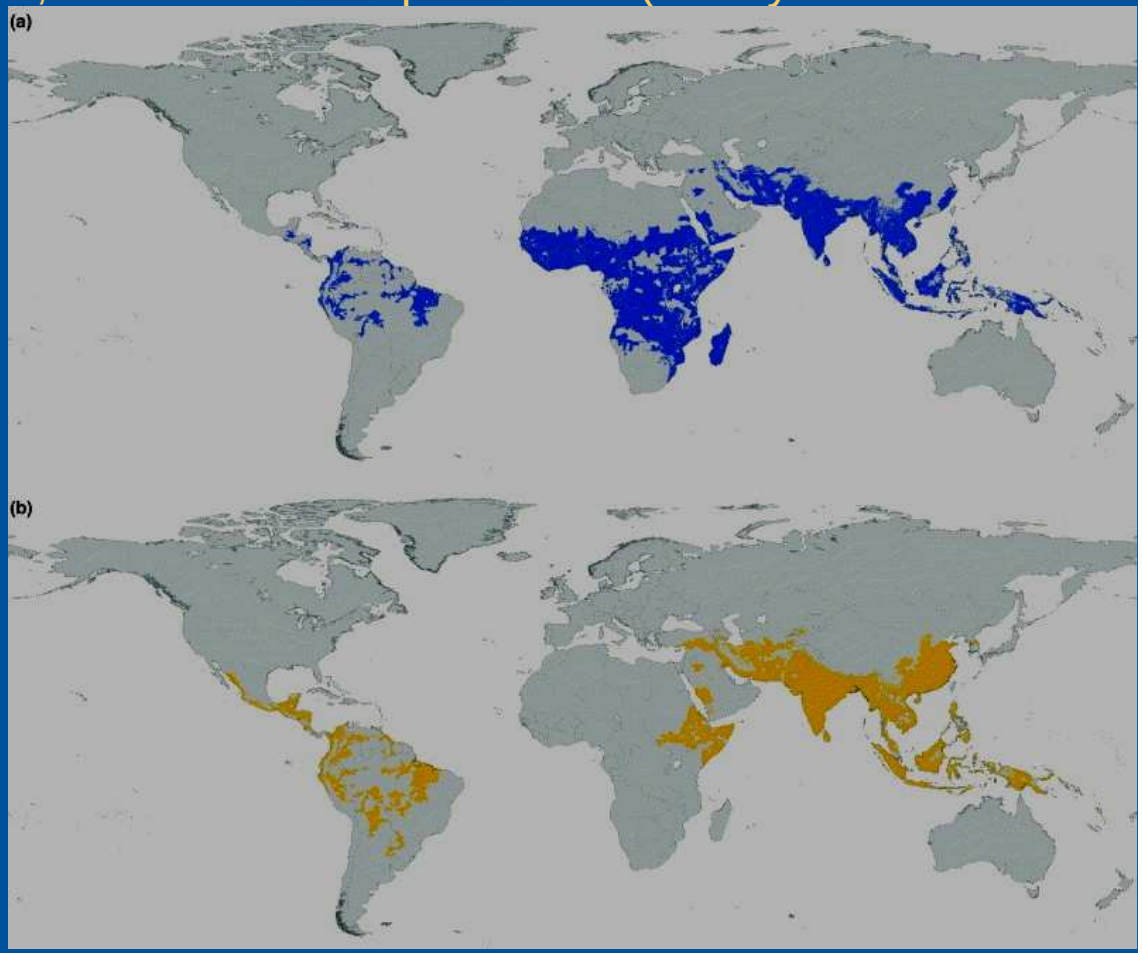


Malaria

- 4 human parasites, 2 important-
- P. falciparum: 300-500 million cases; 1 million deaths per annum (mainly children < 5 yrs in Sub-Saharan Africa)
- P. vivax ‘benign malaria’ (not many deaths)

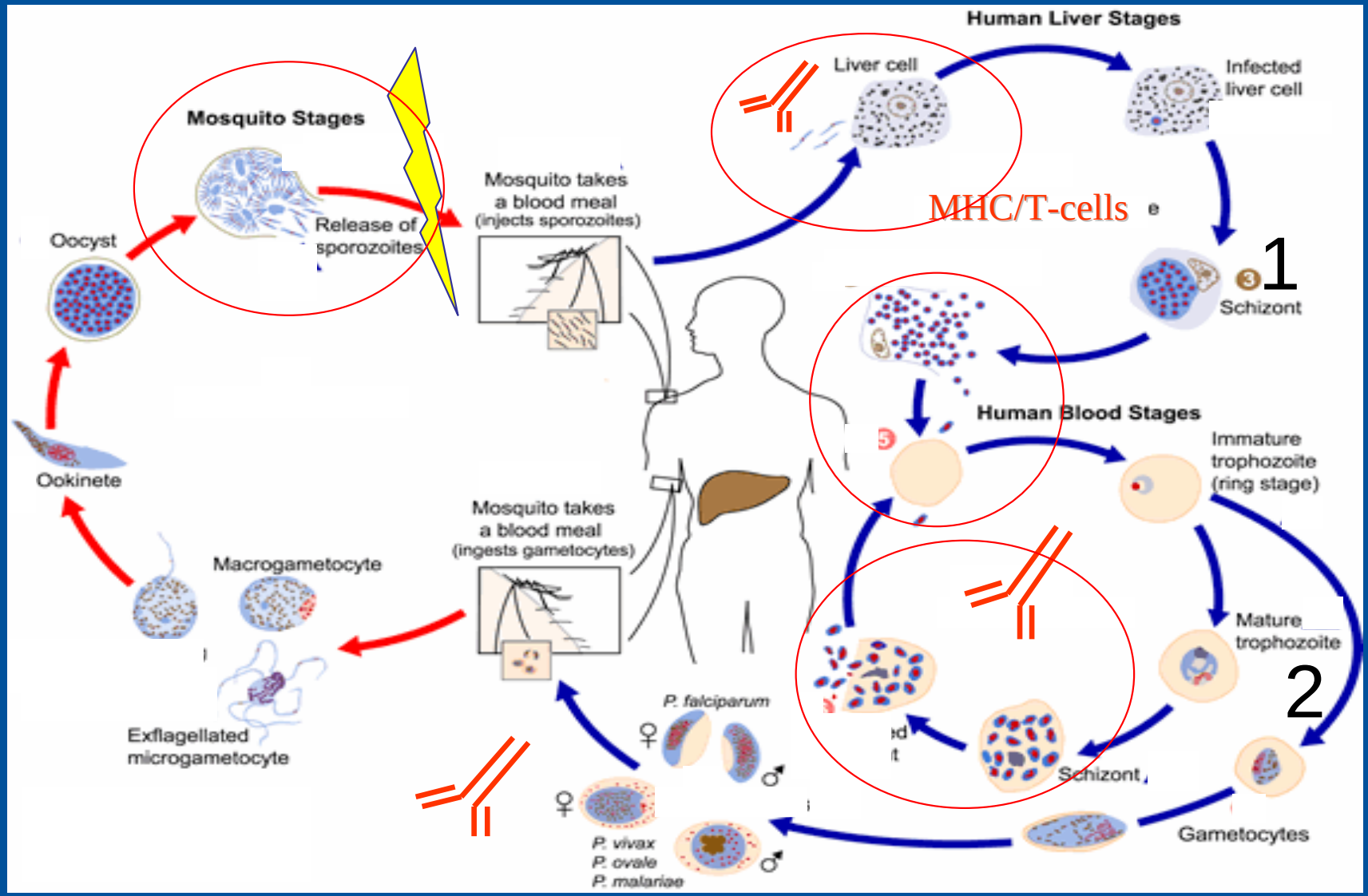
--Duffy receptor--

--hypnozoites--

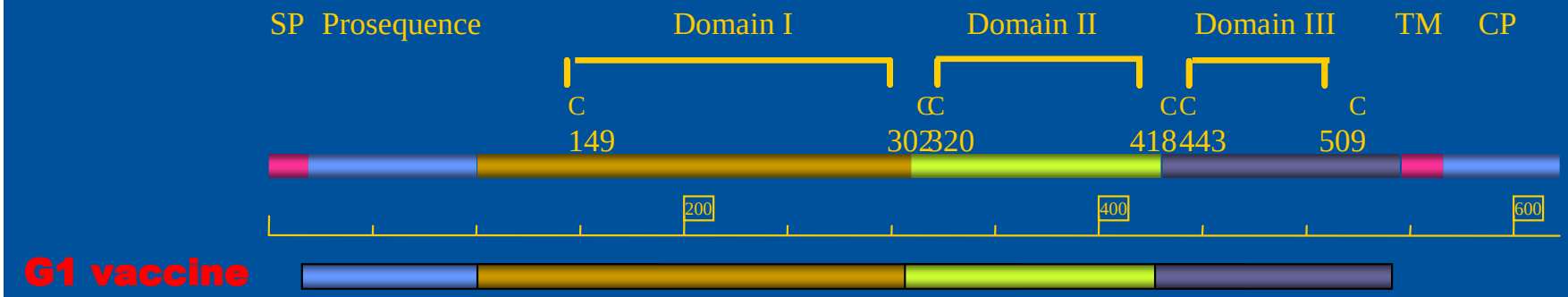


Production and PoP for PvAMA1 as a vaccine was published.
No interest from both commercial nor public funding agencies!
Crystals made and structure published (2005)

Pv-Pf-AMA1 in the life cycle



Pf-AMA1: Apical Membrane Antigen



PfAMA1

622 aa, 83 kDa, processed to 66 kDa

Expressed on merozoites

Involved in erythrocyte invasion

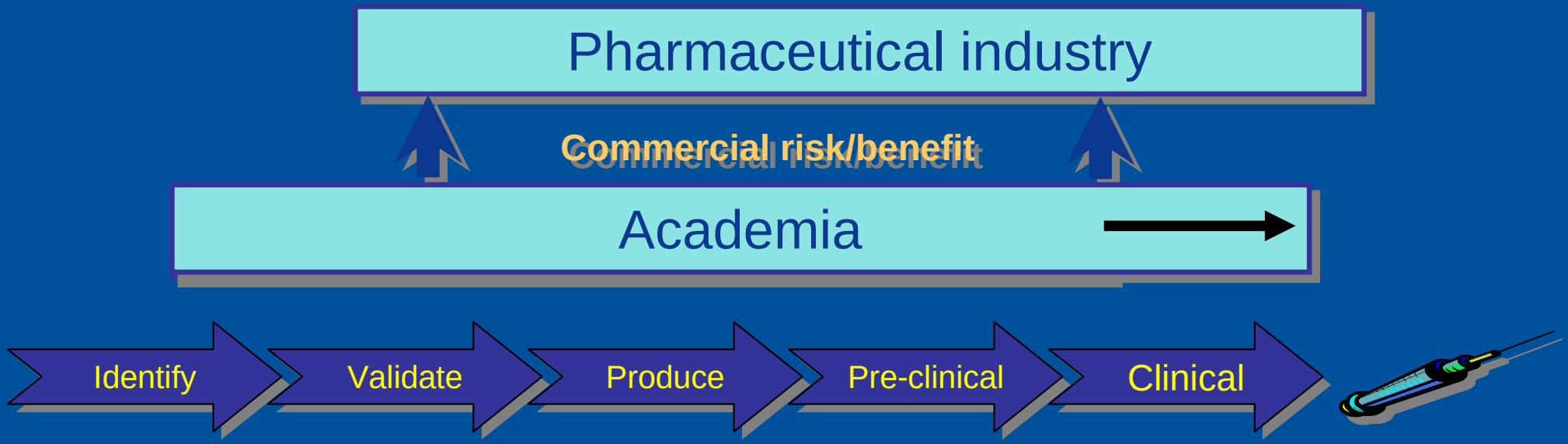
Single gene, allelic variation at 64 polymorphic aa positions

G1 vaccine

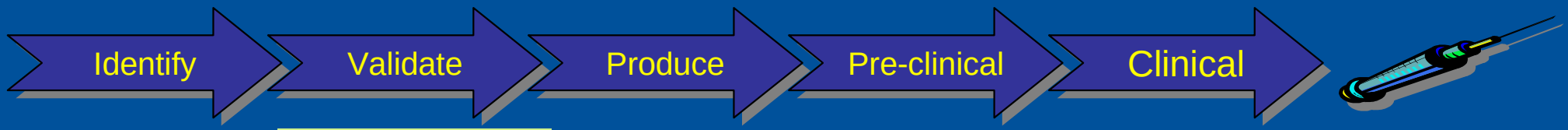
Pro-domain-DI-DII-DIII, 60 kDa

Expressed in *Pichia pastoris* with high yield & correct conformation

Vaccine development



Malaria project outlines



PkAMA1 - *BPRC*

STREP MeV - *EC*

BioMalPar - *EC*

Polymorphism - *EMVI*

Proteo/Genomics - *NWO*

G1 and G2 AMA1 vaccines - *EMVI*

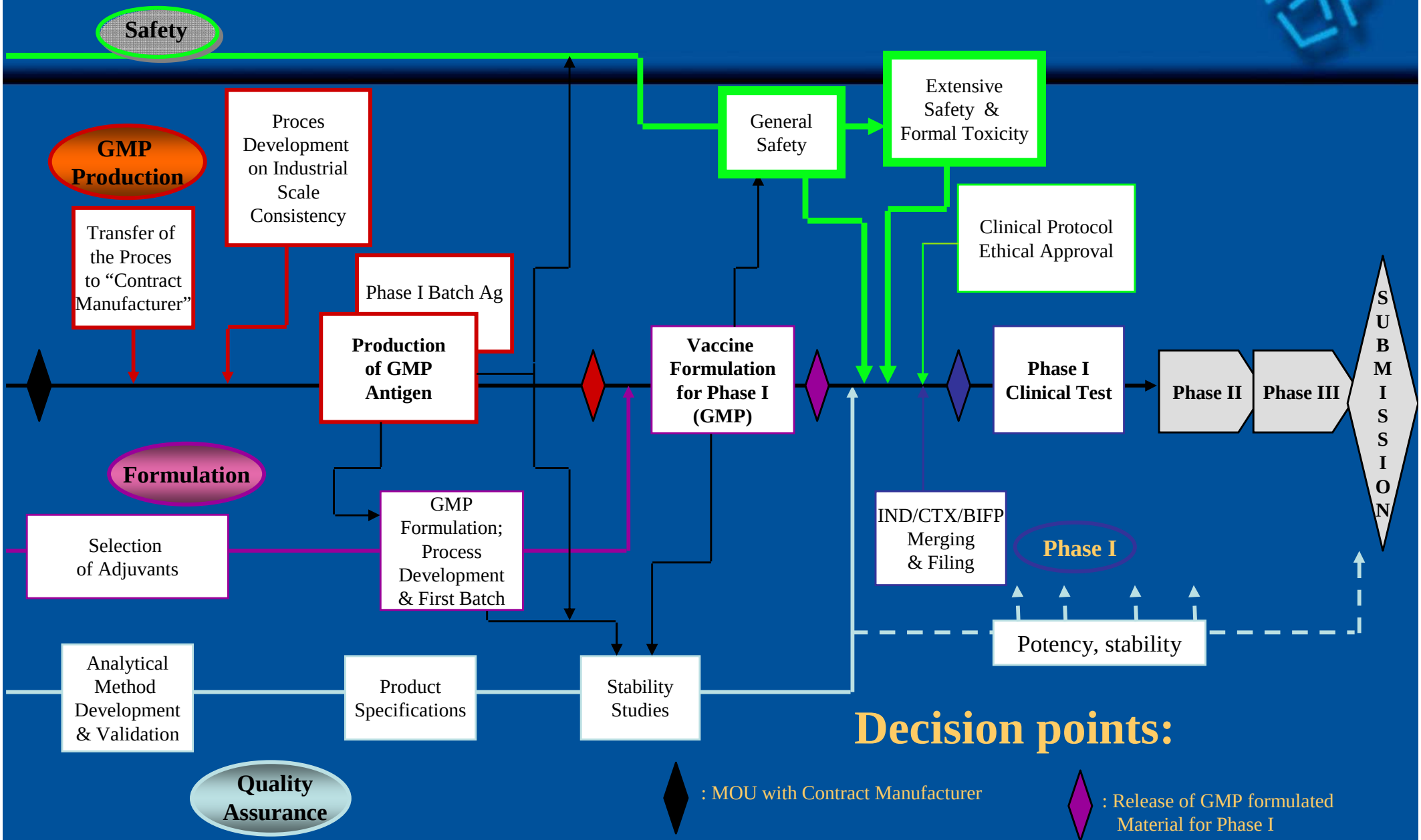
New drugs - *Novartis/Wellcome/MMV*

Adjuvant platform - *EMVI/MVI*

EMVDA Fusions and assays - *EC*

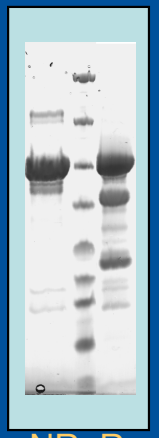
ANTIMAL - *EC*

P. falciparum model vaccine, Project Plan



Phase Ia in Nijmegen, The Netherlands: summary

Product



NR R

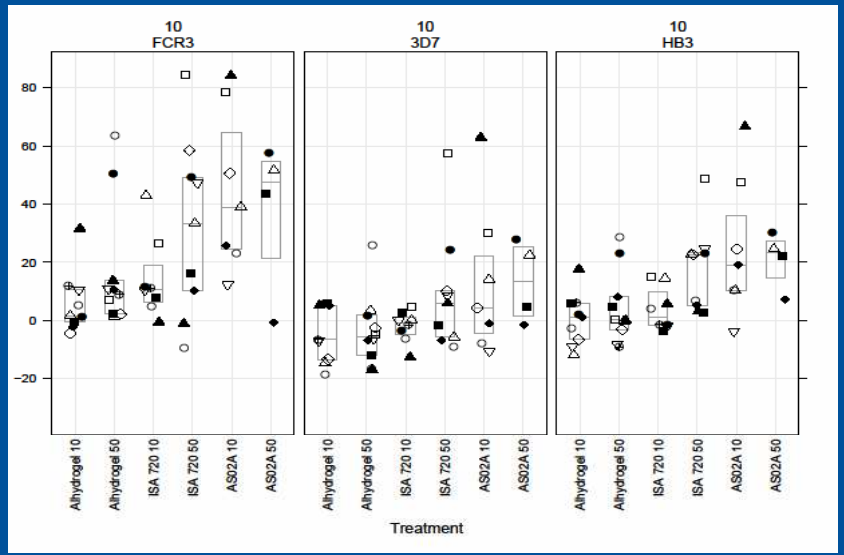
Safety

Adjuvant	Alhydrogel		Montanide ISA 720		AS02A		Total
Dose	10µg	50µg	10µg	50µg	10µg	50µg	
N	10	10	10	9	9	8	56
Total	8 (80.0 %)	10 (100.0 %)	9 (90.0 %)	9 (100.0 %)	9 (100.0 %)	8 (100.0 %)	53 (94.6 %)
LOCAL							
Pain	8 (80.0 %)	10 (100.0 %)	8 (80.0 %)	9 (100.0 %)	9 (100.0 %)	8 (100.0 %)	52 (92.9 %)
Erythema	-	-	2 (20.0 %)	2 (22.2%)	4 (44.4%)	6 (75.0%)	14 (25%)
Swelling	-	-	1 (10.0 %)	-	3 (33.3 %)	1 (12.5 %)	5 (8.9 %)
Induration	-	-	1 (10.0 %)	2 (22.2 %)	-	-	3 (5.4 %)
Sterile abscess	-	-	-	2 (22.2 %)	-	-	2 (3.6 %)
SYSTEMIC							
Headache	1 (10.0 %)	-	2 (20.0 %)	-	6 (66.7 %)	7 (87.5 %)	16 (28.6 %)
Malaise	-	-	-	1 (11.1 %)	6 (66.7 %)	7 (87.5 %)	14 (25.0 %)
Fever	-	-	-	-	5 (55.6 %)	5 (62.5 %)	10 (17.9 %)
Myalgia	-	-	-	-	4 (44.4 %)	2 (25.0 %)	6 (10.7 %)
Nausea	1 (10.0 %)	-	-	-	1 (11.1 %)	2 (25.0 %)	4 (7.1 %)
Fatigue	-	-	-	-	-	2 (25.0 %)	2 (3.6 %)
Arthralgia	-	-	-	-	1 (11.1 %)	-	1 (1.8 %)
Abdominal pain	-	-	-	-	1 (11.1 %)	-	1 (1.8 %)

Local: Alhydrogel best, some problems with AS02A, more with Montanide720

Systemic: Alhydrogel best, as Montanide, AS02A has some problems

Immunogenicity



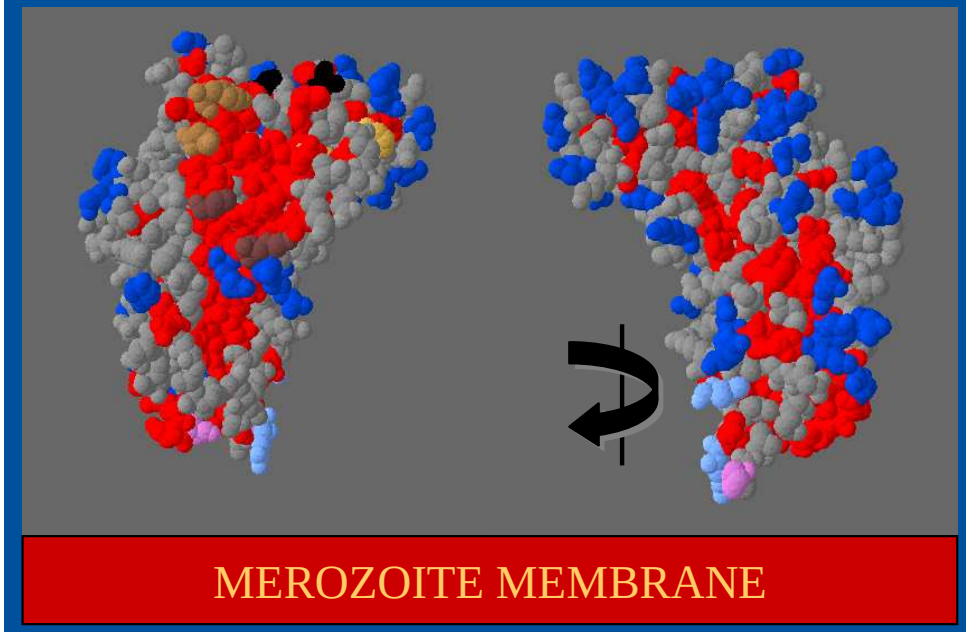
Allele-specific response much better than responses to other allele.

- Need to improve vaccine

BPRC's strategies for improvement

Diversity Covering (DiCo) approach

Tackle 'polymorphic' issue



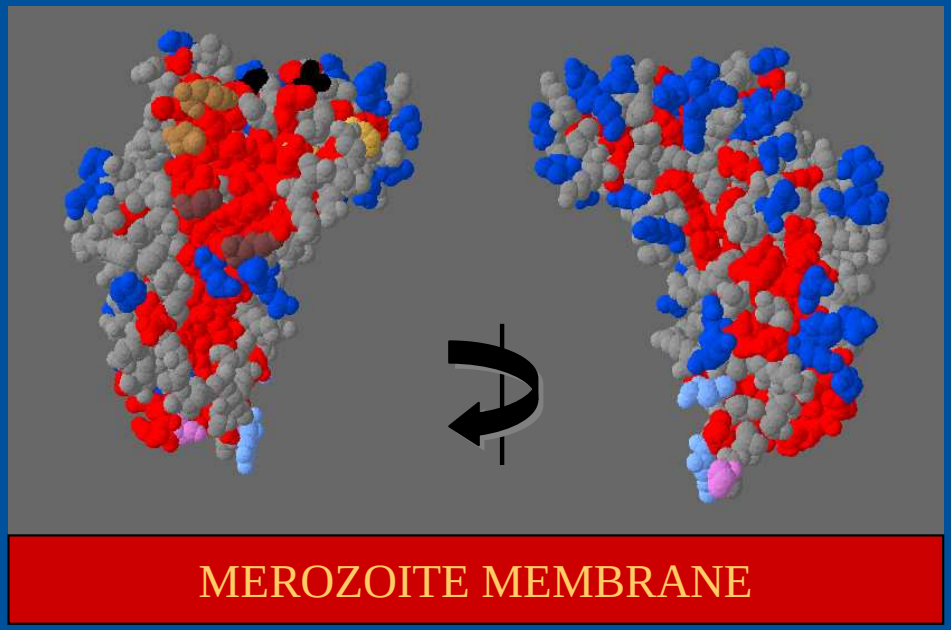
Blue: polymorphic; Red: conserved; Grey: neutral
Other colors used to mark non-resolved regions of the crystal

Pos	Options							Coverage		Seq1	Seq2	Seq3
162	N	77.2	K	22.8				100.0		Q	Q	K
167	T	73.8	K	26.2				100.0		T	K	T
172	G	45.5	E	54.5				100.0		E	G	E
173	N	85.7	K	13.8				85.7		N	N	N
175	Y	10.4	D	89.6				89.6		D	D	D
187	N	38.8	K	23.9	E	37.4		100.0		K	N	E
190	I	32.6	M	67.4				100.0		M	I	M
196	N	25.4	D	74.6				100.0		D	N	D
197	G	23.9	D	23.6	Q	32.6		80.1		Q	G	D
200	D	44.5	H	40.6				85.1		H	D	D
201	F	78.9	L	18.5				97.5		F	F	L
204	N	42.1	D	57.9				100.0		D	N	D
206	E	78.1	K	21.9				100.0		E	E	K
207	Y	83.7	D	16.3				83.7		Y	Y	Y
225	N	68.3	I	31.7				100.0		N	N	I
230	K	67.0	E	27.3				100.0		K	E	K
242	Y	51.4	D	48.6				100.0		D	Y	Y
243	N	20.2	E	17.1	K	62.6		100.0		K	N	E
267	Q	39.4	E	60.6				100.0		E	E	Q
282	K	67.0	I	33.0				100.0		K	K	I
283	L	29.9	S	70.1				100.0		S	L	S
285	E	21.1	Q	78.9				100.0		Q	E	Q
296	D	80.1	H	19.9				100.0		D	H	D
300	E	27.5	K	72.5				100.0		K	E	K
308	E	64.3	Q	35.1				99.4		E	E	Q
332	N	82.5	I	17.5				100.0		N	N	I
393	H	68.9	R	30.5				99.4		H	R	H
404	R	57.2	T	42.8				100.0		R	R	T
405	E	49.1	K	50.9				100.0		K	K	E
407	Q	84.4	H	15.6				84.4		Q	Q	Q
435	I	78.4	N	20.4				98.8		I	I	N
439	H	59.0	N	38.6				97.6		H	N	H
448	N	18.6	D	81.4				100.0		D	N	D
451	M	41.3	K	58.7				100.0		K	M	K
485	K	58.1	I	41.9				100.0		K	K	I
493	D	78.4	A	21.6				100.0		D	D	A
496	M	43.7	I	56.3				100.0		I	M	I
503	R	41.3	N	58.1				99.4		N	R	N
512	R	47.3	K	52.7				100.0		K	R	K
544	N	33.6	K	66.4				100.0		K	N	K

Strategy for improvement

Diversity Covering (DiCo) approach

Tackle 'polymorphic' issue



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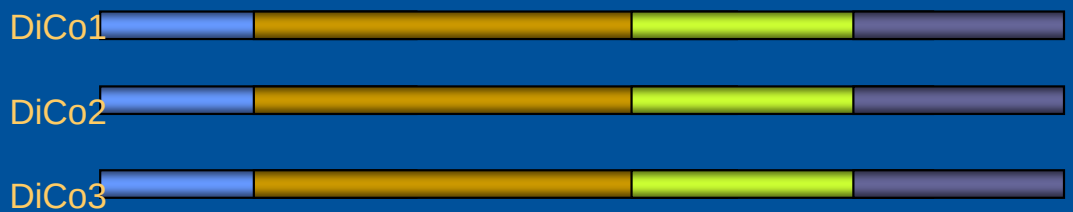

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175	Y	10.4	D	89.6				89.6	D	D	D
187	N	38.8	K	23.9	E	37.4		100.0	K	N	E
190	I	32.6	M	67.4				100.0	M	I	M
196	N	25.4	D	74.6				100.0	D	N	D
197	G	23.9	D	23.6	Q	32.6		80.1	Q	G	D
200	D	44.5	H	40.6				85.1	H	D	D
201	F	78.9	L	18.5				97.5	F	F	L
204	N	42.1	D	57.9				100.0	D	N	D
206	E	78.1	K	21.9				100.0	E	E	K
207	Y	83.7	D	16.3				83.7	Y	Y	Y
225	N	68.3	I	31.7				100.0	N	N	I
230	K	67.0	E	27.3				100.0	K	E	K
242	Y	51.4	D	48.6				100.0	D	Y	Y
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267	Q	39.4	E	60.6				100.0	E	E	Q
282	K	67.0	I	33.0				100.0	K	K	I
283	L	29.9	S	70.1				100.0	S	L	S
285	E	21.1	Q	78.9				100.0	Q	E	Q
296	D	80.1	H	19.9				100.0	D	H	D
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435	I	78.4	N	20.4				98.8	I	I	N
439	H	59.0	N	38.6				97.6	H	N	H
448	N	18.6	D	81.4				100.0	D	N	D
451	M	41.3	K	58.7				100.0	K	M	K
485	K	58.1	I	41.9				100.0	K	K	I
493	D	78.4	A	21.6				100.0	D	D	A
496	M	43.7	I	56.3				100.0	I	M	I
503	R	41.3	N	58.1				99.4	N	R	N
512	R	47.3	K	52.7				100.0	K	R	K
544	N	33.6	K	66.4				100.0	K	N	K

Malaria vaccine for India

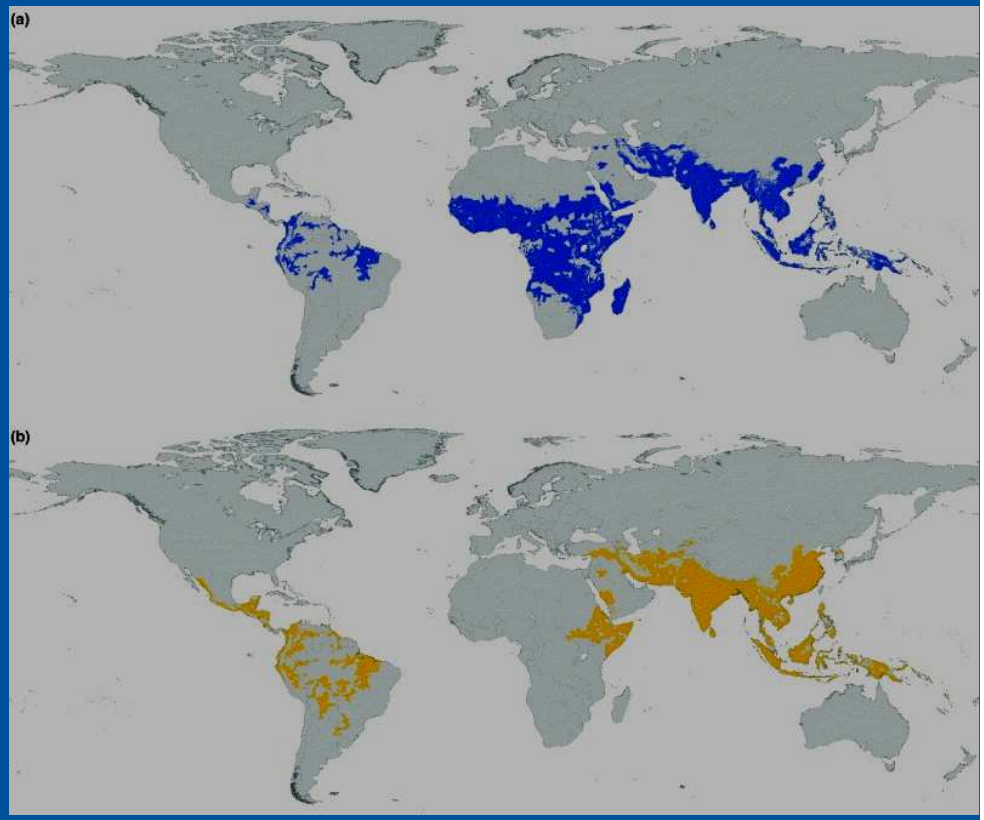
1. Partly active vaccine against P. falciparum has been developed:

May give P. vivax opportunity to expand

2. For Eradication of malaria, for all 4 species vaccines are necessary
3. P. vivax is not so benign as thought

Challenges for a vivax vaccine

1. Prevention of transmission (a TBV?)
2. Prevention/killing of sleeping forms (hypnozoites)
3. Reports claim P. vivax can invade human via non Duffy pathway



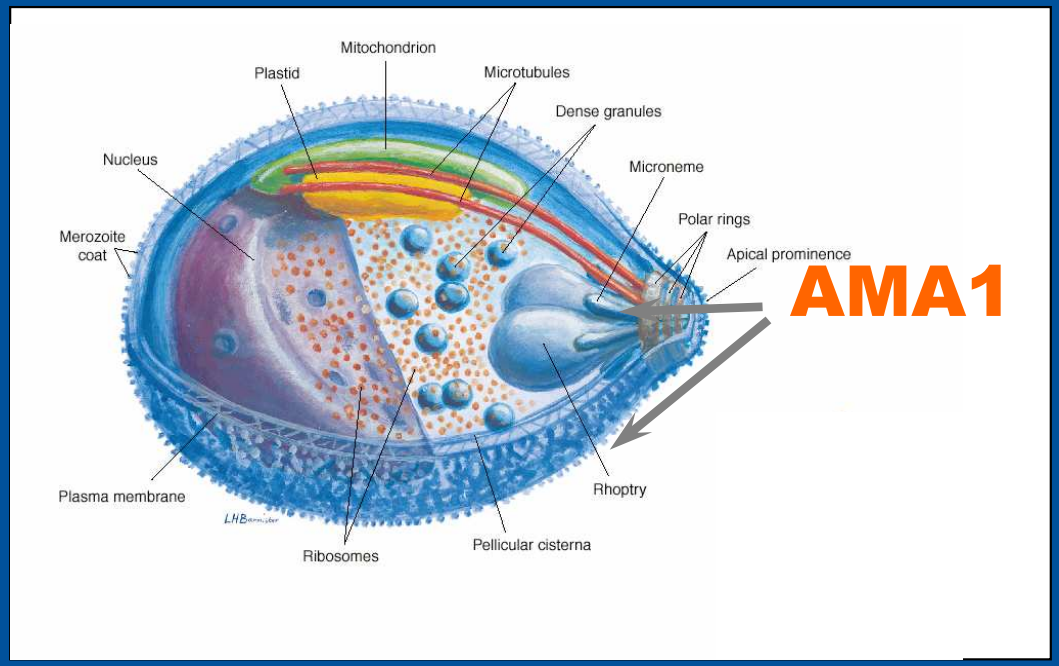
Plan for Life Sciences and Health programme

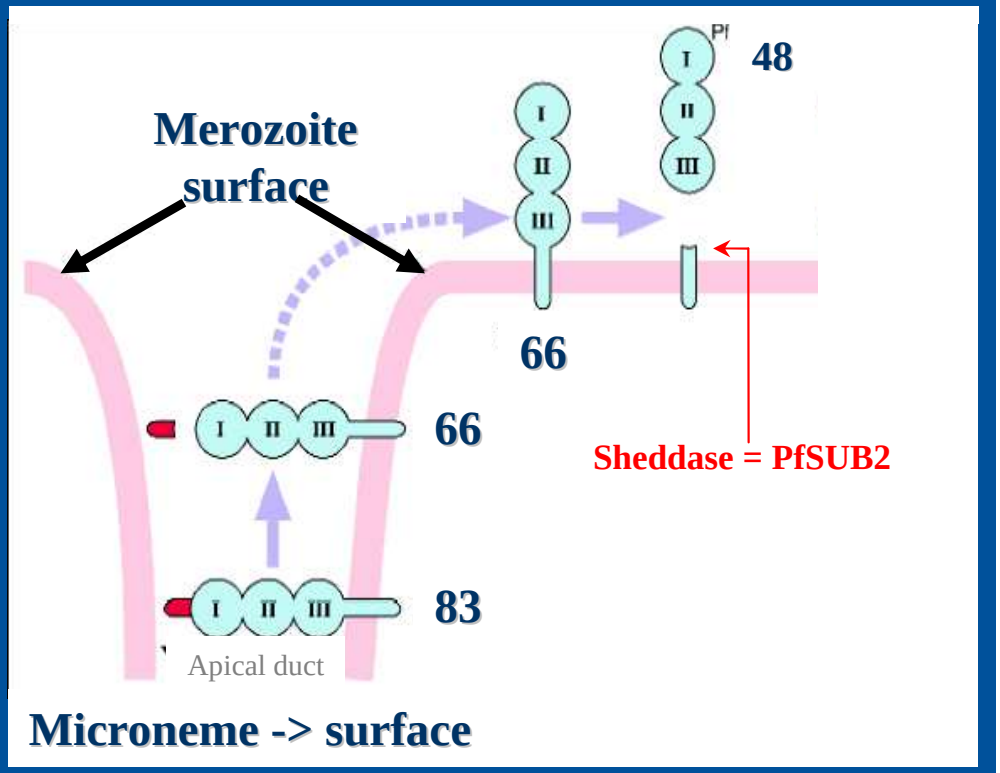
- Development of a protein vaccine against P. vivax malaria based on ICGEB product and BPRC's PvAMA1 (Diversity Covering) products
  - to tackle multiple pathways of invasion
  - to have additive/synergistic effects
  - to include a transmission blocking vaccine component

  And to be used together with the P. falciparum vaccine

A P. vivax vaccine will have great impact on the health of many people in India and elsewhere in the world!

Function of AMA-1: (trans)location and processing





Inhibition of invasion by antibodies

Antibodies inhibit re-orientation of the merozoite on the erythrocyte surface

Control

+ MoAb

+ MoAb