Development of a universal vaccine against malaria caused by *Plasmodium vivax*.

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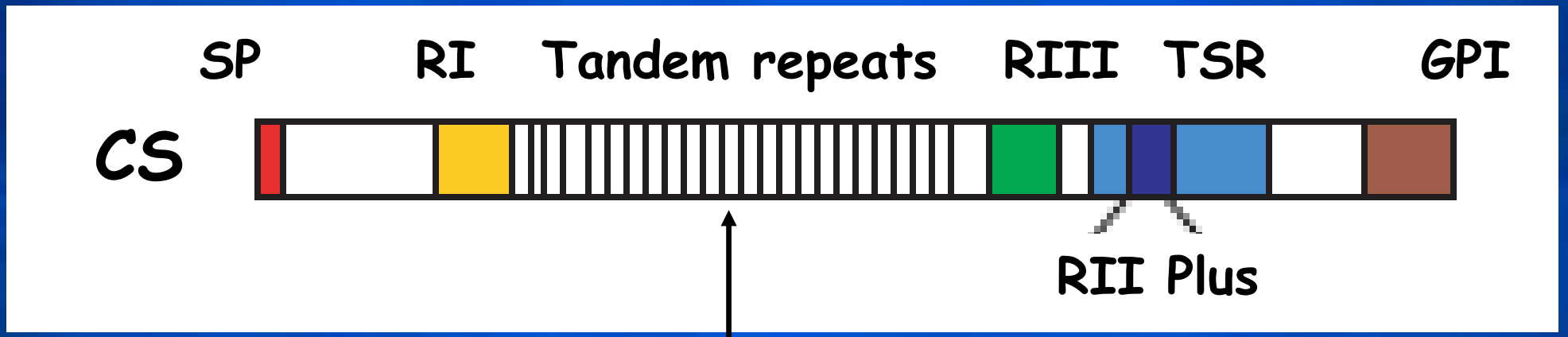
Sporozoite major surface antigen: CS protein



Target of protective antibodies

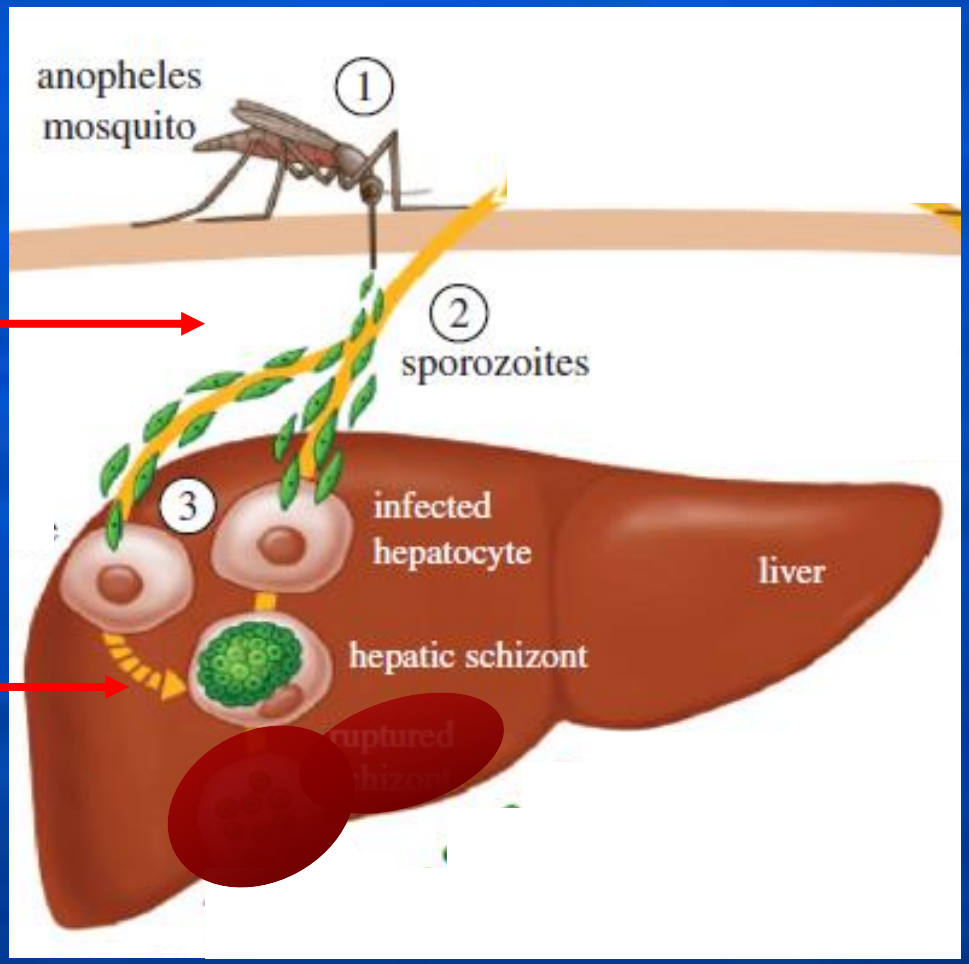
Target of protective T cells

Plasmodium sp. life cycle

Immune mechanisms

Antibodies to CS

CD4 and CD8 T cells



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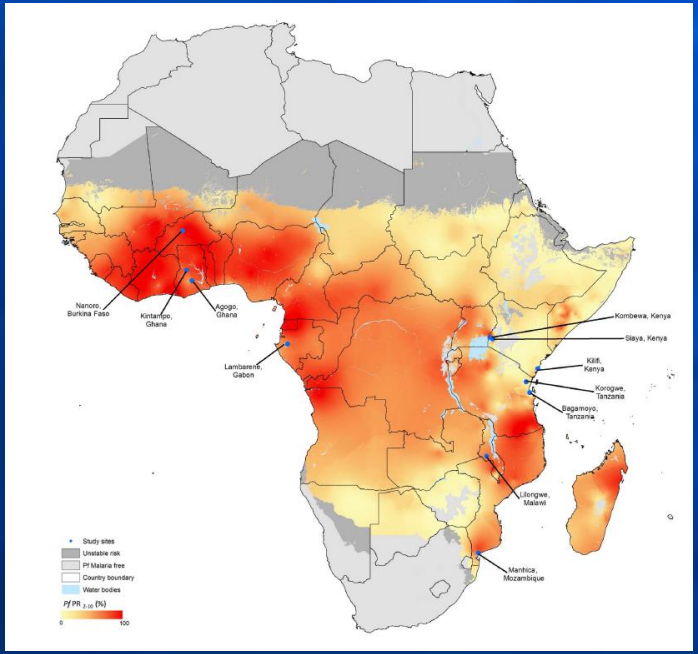
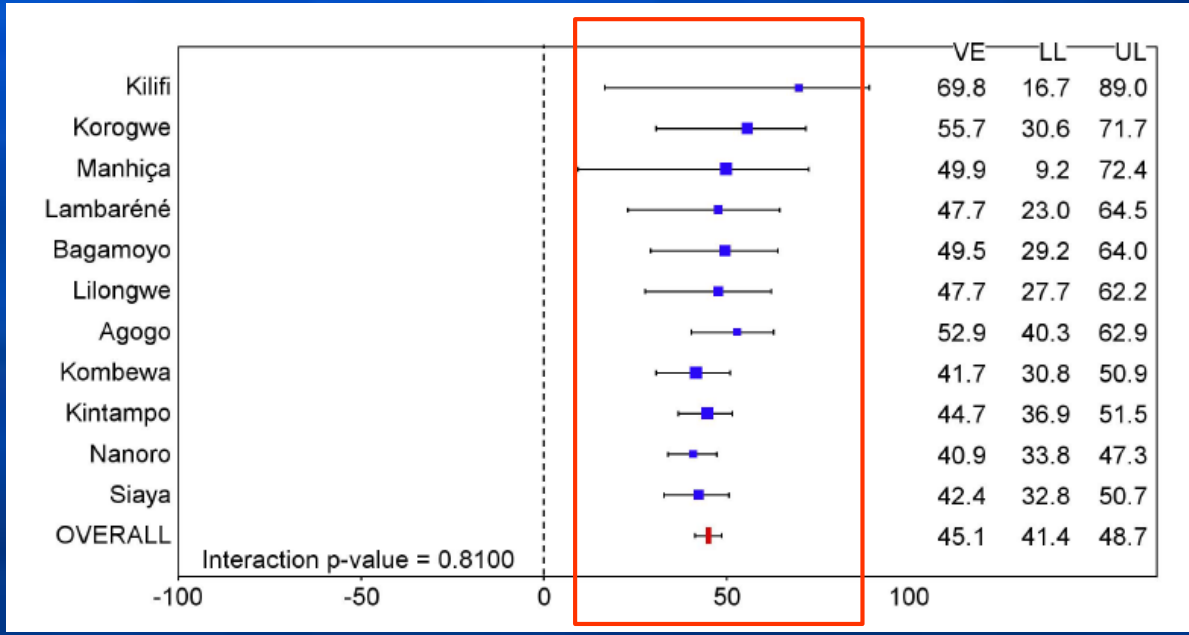
PLOS | MEDICINE

Efficacy and Safety of the RTS,S/AS01 Malaria Vaccine during 18 Months after Vaccination: A Phase 3 Randomized, Controlled Trial in Children and Young Infants at 11 African Sites

The RTS,S Clinical Trials Partnership¶*

August 2014





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PLoS NEGLECTED TROPICAL DISEASES

The International Limits and Population at Risk of *Plasmodium vivax* Transmission in 2009

Carlos A. Guerra[1*], Rosalind E. Howes[1], Anand P. Patil[1], Peter W. Gething[1], Thomas P. Van Boeckel[1,2], William H. Temperley[1], Caroline W. Kabaria[3], Andrew J. Tatem[4,5], Bui H. Manh[6], Iqbal R. F. Elyazar[7], J. Kevin Baird[7,8], Robert W. Snow[3,9], Simon I. Hay[1*]

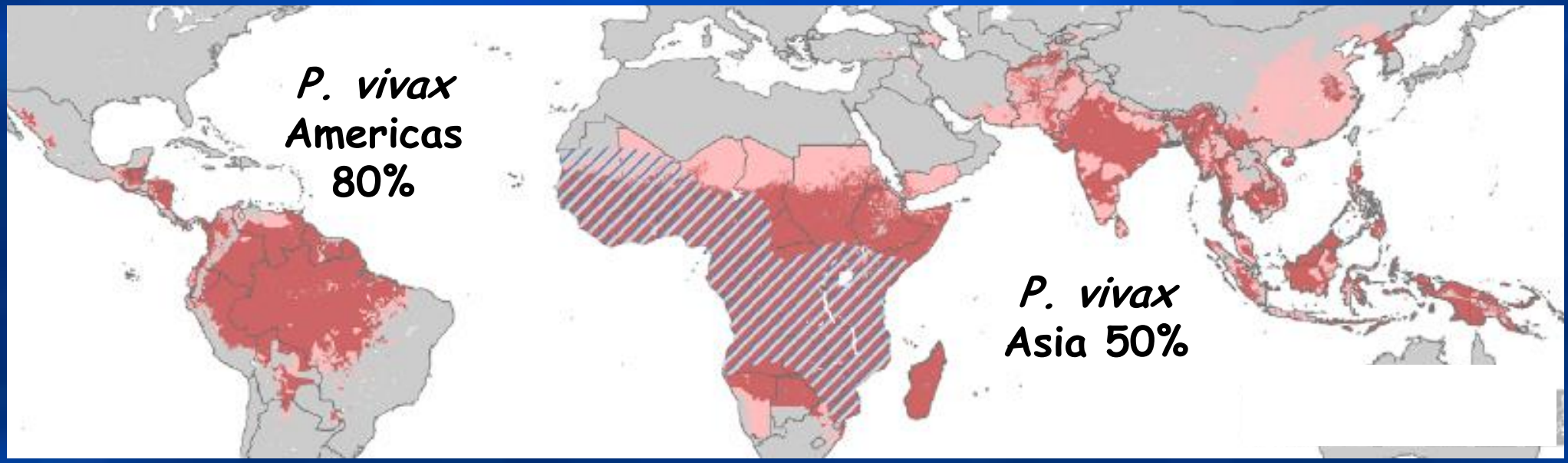
2.85 billions at risk

132-391 M cases per year

P. falciparum

P. vivax

Duffy negative ind.



CS Protein of *Plasmodium vivax*

VK210 = $(DRADGQPAG)_2$ $(DRAAGQPAG)_2$ DRADGQPAGD

VK247 = $(ANGAGNQPG)_4$

P. vivax-like = $(APGANQEGGAA)_3$?

Universal vaccine = immunity to the all three allelic forms.

His_6-PvCS-VK210

VK210- $(DRADGQPAG)_2$ $(DRAAGQPAG)_2$ DRADGQPAGD,

```
1-   MGSSHHHHHH SSGLVPRGSH MTHCGHNVDL SKAINLNGVN FNNVDASSLG AAHVGQSASR
61-  GRGLGENPDD EEGDAKKKKD GKKAEPKNPR ENKLKQPGDR ADGQPAGDRA DGQPAGDRAD
121- GQPAGDRADG QPAGDRAAGQ PAGDRADGQP AGDRADGQPA GDRADGQPAG DRADGQPAGD
181- RAAGQPAGDR AAGQPAGDRA DGQPAGDRAA GQPAGDRADG QPAGDRAAGQ PAGDRADGQP
241- AGDRAAGQPA GDRAAGQPAG DRAAGQAAGD RAAGQAAGGN AGGQGQNNEG ANAPNEKSVK
301- EYLDKVRATV GTEWTPCSVT CGVGVRVRRR VNAANKKPED LTLNDLETDV CT
```

His_6-PvCS-VK247

VK-247- $(ANGAGNQPG)_4$

```
 1-  MGSSHHHHHH SSGLVPRGSH MTHCGHNVDL SKAINLNGVN FNNVDASSLG AAHVGQSASR
61-  GRGLGENPDD EEGDAKKKKD GKKAEPKNPR ENKLKQPGAN GAGNQPGANG AGNQPGANGA
121- GNQPGANGAG NQPGANGAGN QPGANGAGNQ PGANGAGNQP GANGAGNQPG ANGAGNQPGA
181- NGAGNQPGAN GAGNQPGANG AGNQPGANGA GNQPGANGAG NQPGANGAGN QPGANGAGNQ
241- PGANGAGNQP GANGAGNQPG ANGAGNQPGA NGAGNQPGGN AGGQGQNNEG ANAPNEKSVK
300- EYLDKVRATV GTEWTPCSVT CGVGVRVRRR VNAANKKPED LTLNDLETDV CT
```

His_6-PvCS-Vivax-like

(Vivax-like- APGANQEGGAA)$_3$

```
1-   MGSSHHHHHH SSGLVPRGSH MTHCGHNVDL SKAINLNGVN FNNVDASSLG AAHVGQSASR
61-  GRGLGENPDD EEGDAKKKKD GKKAEPKNPR ENKLKQPGAP GANQEGGAAA PGANQEGGAA
121- APGANQEGGA AAPGANQEGG AAAPGANQEG GAAAPGANQE GGAAAPGANQ EGGAAAPGAN
181- QEGGAAAPGA NQEGGAAAPG ANQEGGAAAP GANQEGGAAA PGANQEGGAA APGANQEGGA
241- AAPGANQEGG AAAPGANQEG GAAAPGANQE GGAADRAAGQ AAGGNAGGQG QNNEGANAPN
300- EKSVKEYLDK VRATVGTEWT PCSVTCGVGV RVRRRVNAAN KKPEDLTLND LETDVCT
```

His_6-PvCS-All-CS-epitopes

All-CS-epitopes

```
1-   MGSSHHHHHH SSGLVPRGSH MTHCGHNVDL SKAINLNGVN FNNVDASSLG AAHVGQSASR
61-  GRGLGENPDD EEGDAKKKKD GKKAEPKNPR ENKLKQPGPG DRADGQPAGD RADGQPAGDR
121- AAGQPAGDRA AGQPAGDRAD GQPAGDRADG QPAGDRADAP GANQEGGAAA PGANQEGGAA
181- APGANQEGGA AAAPGANQEG GAAAPGANQE GGAAAPGANQ EGGAAAANGA GNQPGANGAG
241- NQPGANGAGN QPGANGAGNQ PGANGAGNQP GDRAAGQAAG GNAGGQGQNN EGANAPNEKS
300- VKEYLDKVRA TVGTEWTPCS VTCGVGVRVR RRVNAANKKP EDLTLNDLET DVCT
```

Bacterial recombinant CS proteins of *Plasmodium vivax*

Synthetic genes:
codon optmized

↓

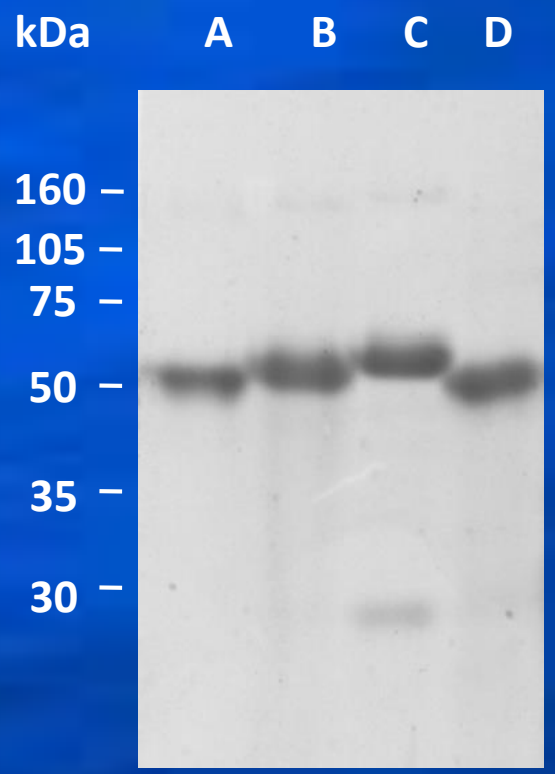
Cloning pET28b

↓

Expression BL-21

↓

Purification
(affinity and FPLC)



A - His_6-PvCS-VK210
B - His_6-PvCS-VK247
C - His_6-PvCS-Vivax-like
D - His_6-PvCS-All-CS-epitopes

Yeast recombinant CS proteins of *P. vivax*

Synthetic genes:
codon optmized

↓

Cloning pPIC9K

↓

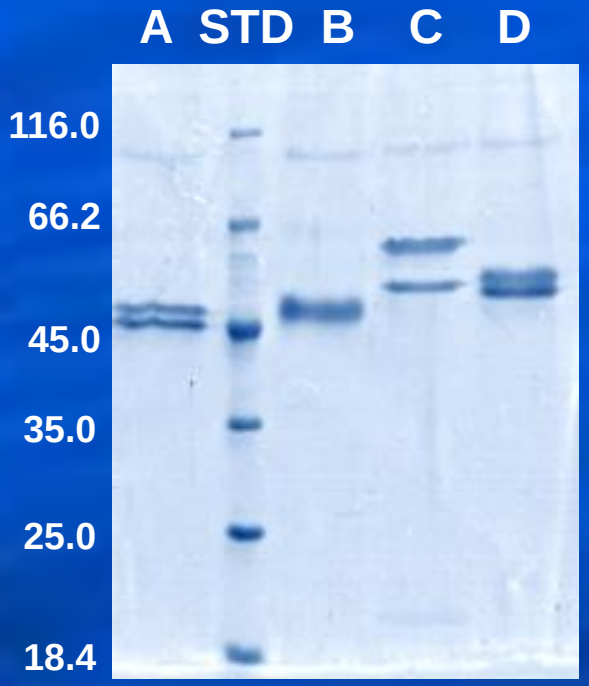
Expression *P. pastoris*

↓

Secretion

↓

Purification
(affinity and FPLC)



A- yPvCS-VK210-His$_6$

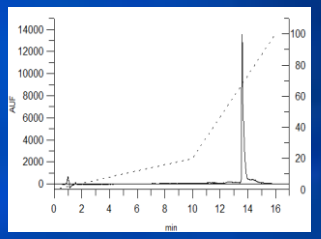
B- yPvCS-VK247-His$_6$

C- yPvCS-VL-His$_6$

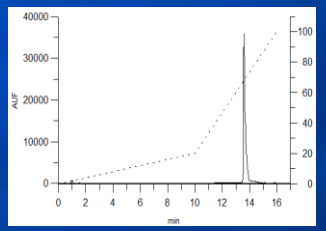
D- yPvCS-All-CS-epitopes His$_6$

HPLC analysis

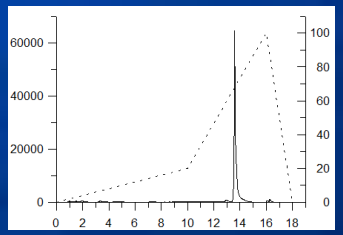
A- yPvCS-VK210-His$_6$



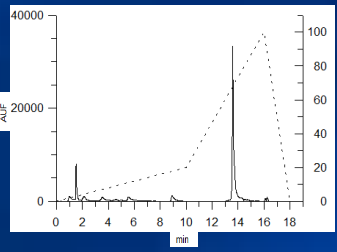
B- yPvCS-VK247-His$_6$



C- yPvCS-VL-His$_6$



D- yPvCS-All-CS-epitopes His$_6$



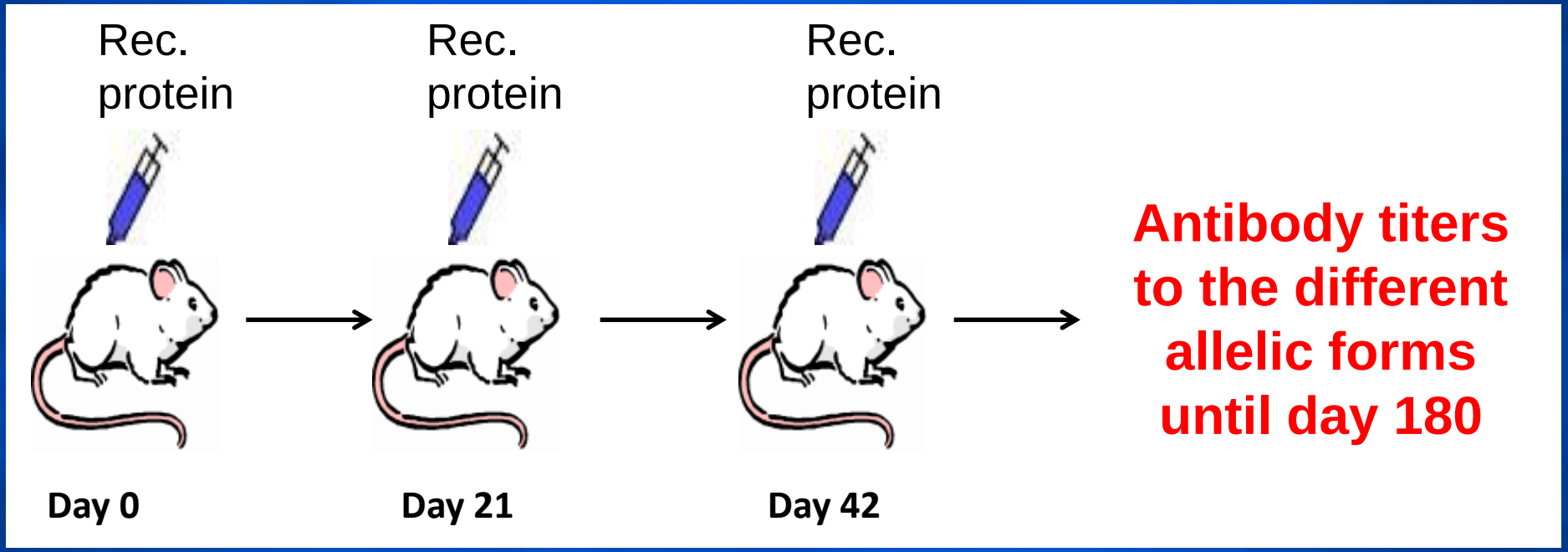
Universal vaccine = immunity to the all three allelic forms.

Three rec. proteins X His_6PvCS-All-CS-epitopes

Poly (I:C)
(TLR-3 agonist)

Titers and specificity

C57Bl/6 mice immunization protocol with the recombinant proteins



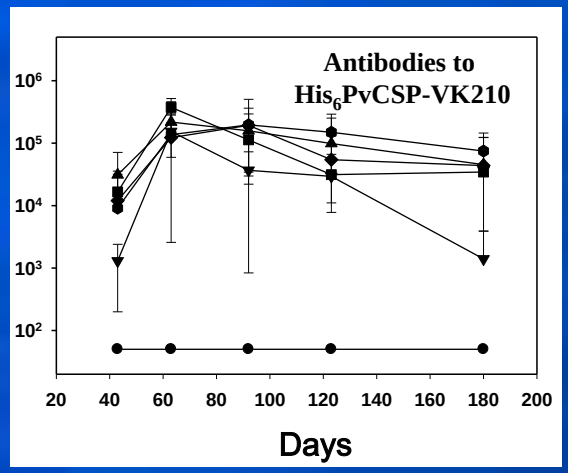
Gr. 1	Adjuvant alone	
Gr. 2	His_6PvCS-VK210	1 μg/mouse/dose
Gr. 3	His_6PvCS-VK247	1 μg/mouse/dose
Gr. 4	His_6PvCS-Vivax-like	1 μg/mouse/dose
Gr. 5	Three rec. proteins	3 μg/mouse/dose
Gr. 6	His_6PvCS-All-CS-epitopes	3 μg/mouse/dose

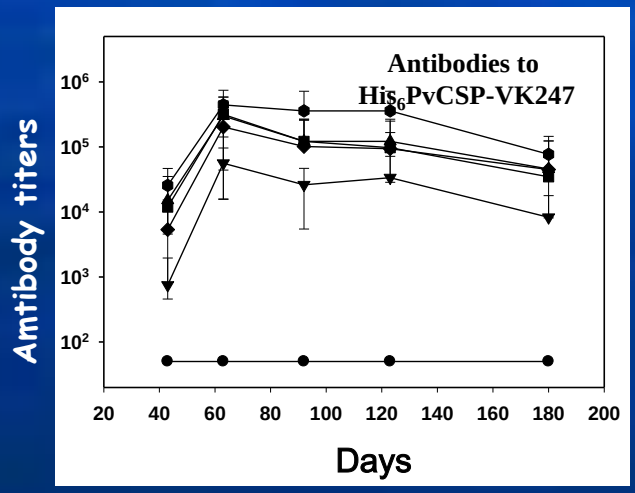
Adjuvants

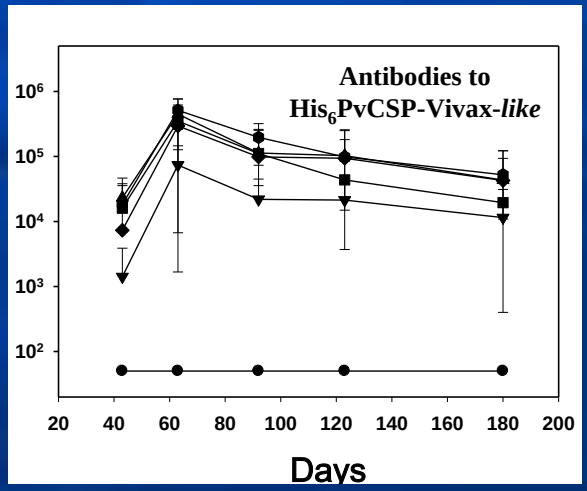
Poly (I:C)
(TLR-3 agonist)

Antibody immune response to the three different allelic forms of the *P. vivax* CS protein

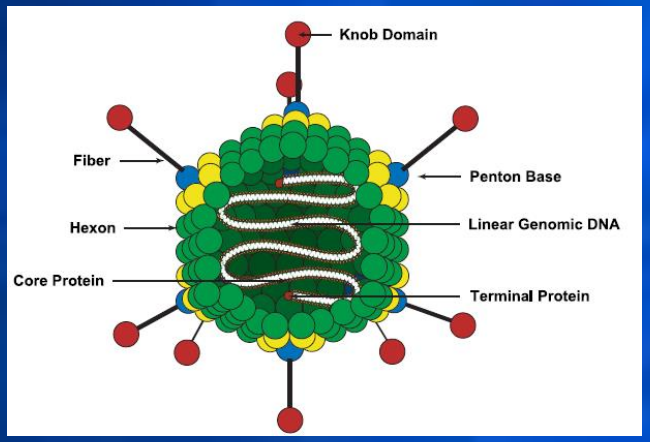
- G1: Adjuvant
- G2: yPvCSP-VK210 / Adjuvant
- G3: yPvCSP-VK247 / Adjuvant
- G4: yPvCSP-Vivax-like / Adjuvant
- G5: yPvCSP-All-CS-epitopes / Adjuvant
- G6: Protein Mix /Adjuvant

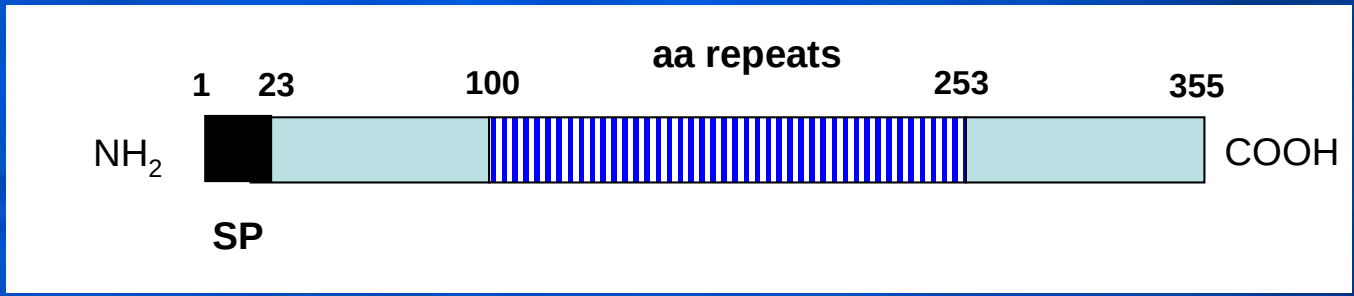


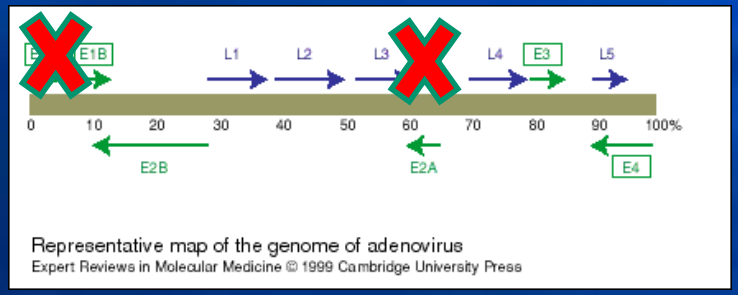




Replication deficient adenovirus
Human type 5
Simian C68







Representative map of the genome of adenovirus
Expert Reviews in Molecular Medicine © 1999 Cambridge University Press

```
1-  mgmqvqsiql fllllwvpgs rgthcghnvd lskainlngv nfnnvdassl gaahvgqsas
61- rgrglgenpd deegdakkkk dgkkaepknp renklkqpgp gdradgqpag dradgqpagd
121-raagqpagdr aagqpagdra dgqpagdrad gqpagdrada pganqeggaa apganqegga
181-aapganqegg aaaapganqe ggaaapganq eggaaapgan qeggaaaang agnqpganga
241-gnqpgangag nqpgangagn qpgangagnq pgdraagqaa ggnaggqgqn neganapnek
301-svkeyldkvr atvgtewtpc svtcgvgvrv rrrvnaankk pedltlndle tdvct
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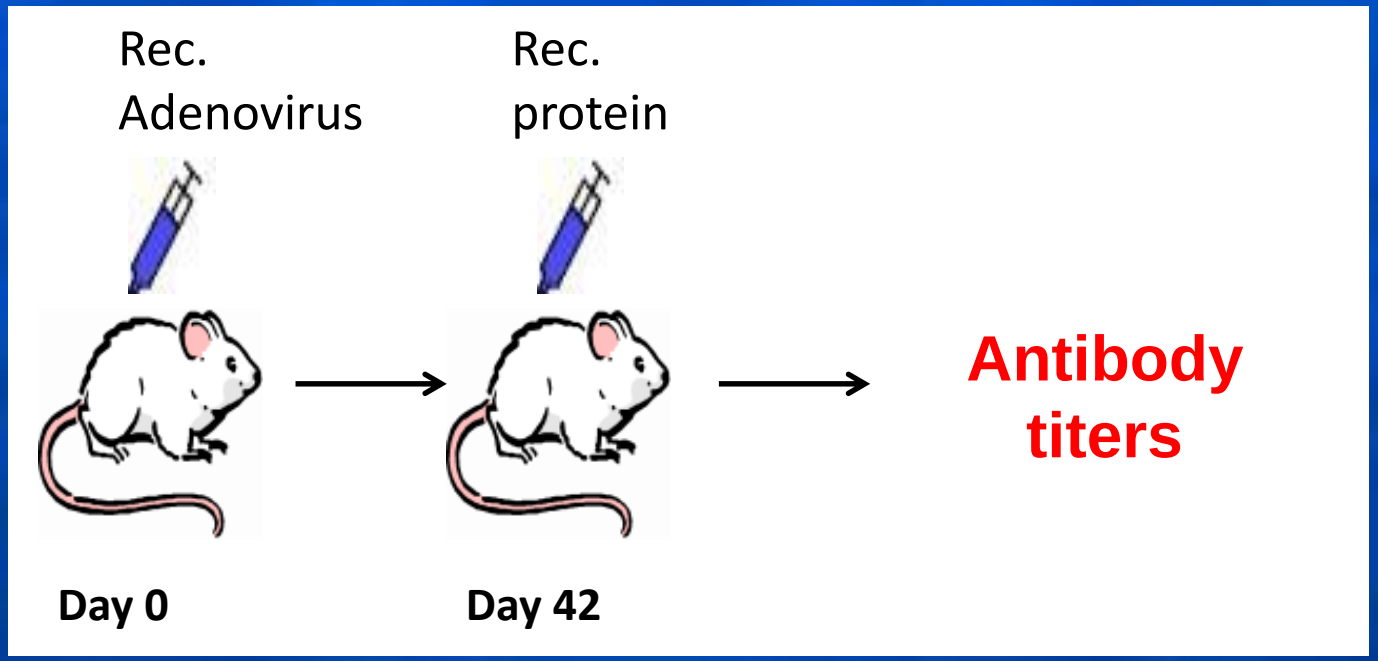
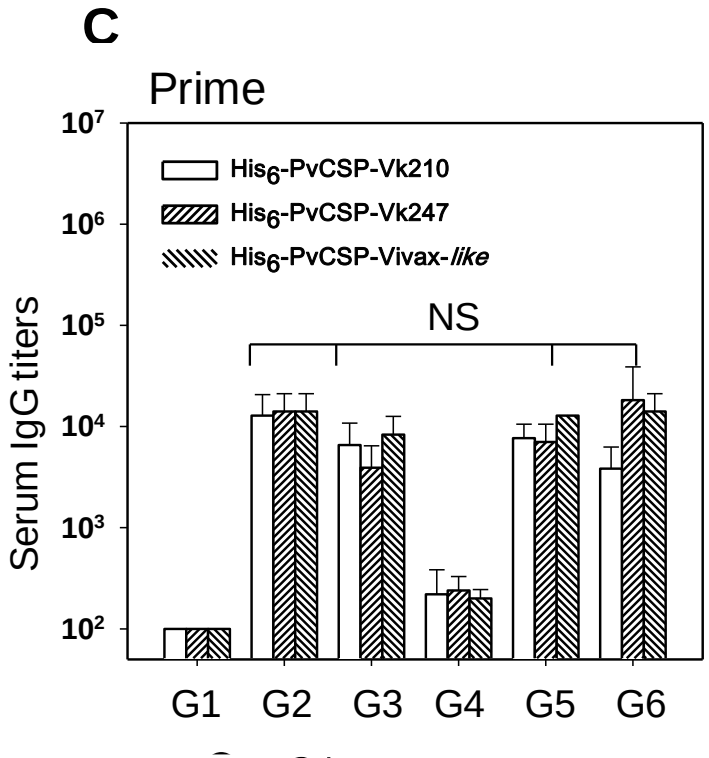
C57Bl/6 mice immunization protocol with the recombinant proteins



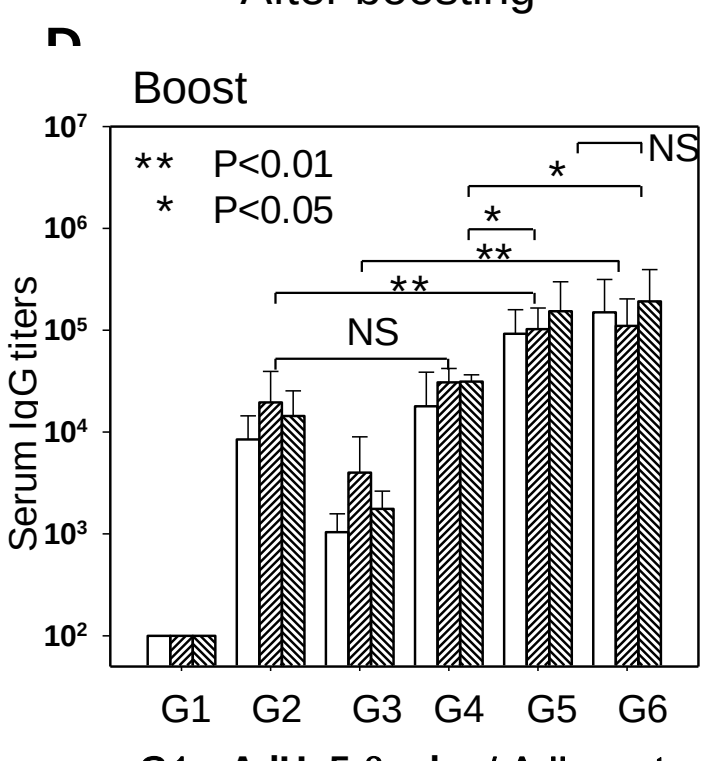
Heterologous prime-boost vaccination

After priming



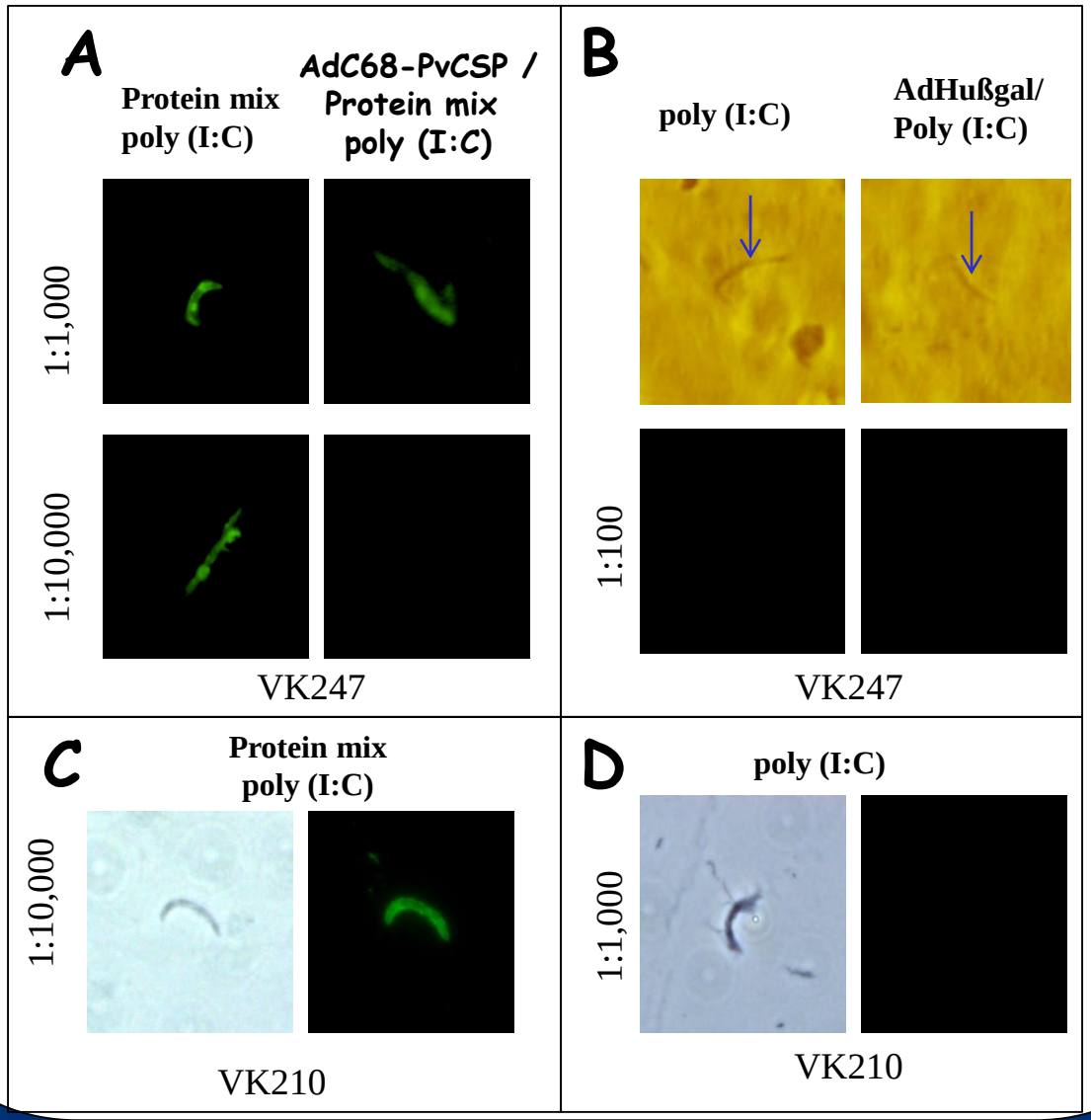
G1: **AdHu5-βgal**
G2: AdC68-PvCSP
G3: AdHu5-PvCSP
G4: AdHuβgal
G5: AdC68-PvCSP
G6: AdHu5-PvCSP

After boosting



G1: **AdHu5-βgal** / Adjuvant
G2: AdC68-PvCSP / Adjuvant
G3: AdHu5-PvCSP / Adjuvant
G4: AdHuβgal / Protein Mix
G5: AdC68-PvCSP / Protein Mix
G6: AdHu5-PvCSP / Protein Mix

Parasite recognition



Experimental challenge

Rec. protein

Rec. protein

Challenge *P. berghei* transfected with PvCS

Day 0

Day 14

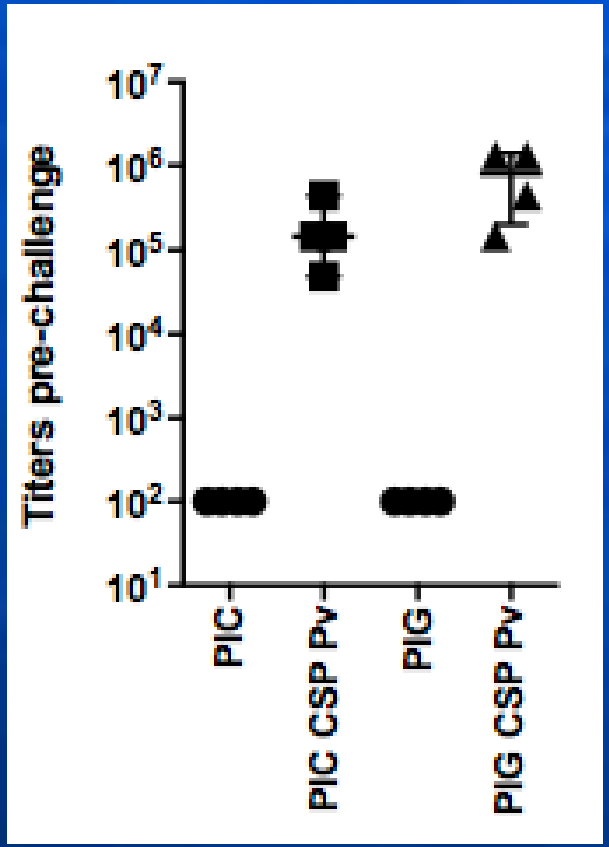
Day 28

Parasitemia

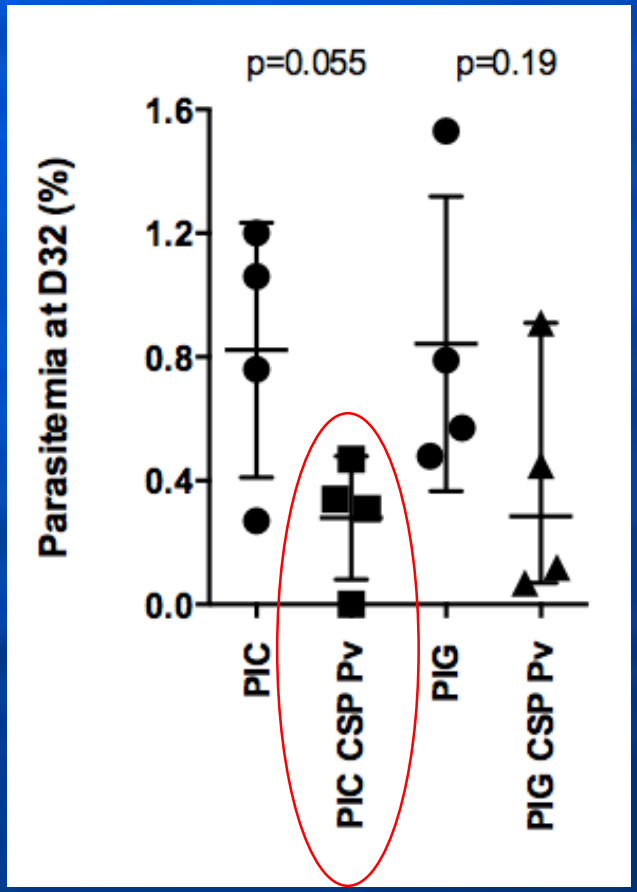
Antibody titers

Dr. Rogerio Amino
Institut Pasteur

Antibody titers



Parasitemia



Conclusions

Three rec. proteins
Poly (I:C)

=

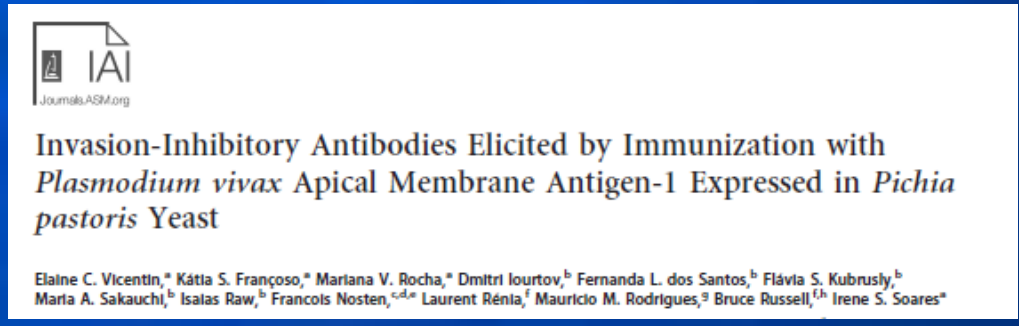
His_6PvCS-All-CS-epitopes
Poly (I:C)

Heterologous prime-boost vaccination rec. adenovirus followed by protein works well in the mouse model using the *P. vivax* CS protein.

Patent
Genetically modified sequences encoding *Plasmodium vivax* antigens
WO2010 127420

2014

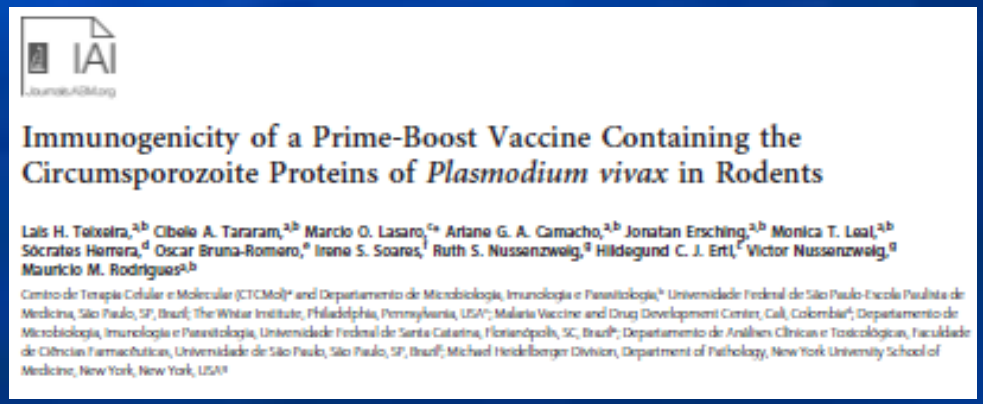
Plasmodium vivax vaccine compositions
USPTO 275220201WO1



IAI
Journals.ASM.org

Invasion-Inhibitory Antibodies Elicited by Immunization with *Plasmodium vivax* Apical Membrane Antigen-1 Expressed in *Pichia pastoris* Yeast

Elaine C. Vicentin,[a] Kátia S. Françoso,[a] Mariana V. Rocha,[a] Dmitri Iourtov,[b] Fernanda L. dos Santos,[b] Flávia S. Kubrusly,[b] Maria A. Sakauchi,[b] Isalas Raw,[b] Francois Nosten,[c,d,e] Laurent Rénia,[f] Mauricio M. Rodrigues,[g] Bruce Russell,[f,h] Irene S. Soares[a]



IAI

Immunogenicity of a Prime-Boost Vaccine Containing the Circumsporozoite Proteins of *Plasmodium vivax* in Rodents

Lais H. Teixeira,[a,b] Cibele A. Tararam,[a,b] Marcio O. Lasaro,[c,e] Ariane G. A. Camacho,[a,b] Jonatan Ersching,[a,b] Monica T. Leal,[a,b] Sócrates Herrera,[d] Oscar Bruna-Romero,[e] Irene S. Soares,[f] Ruth S. Nussenzweig,[g] Hildegund C. J. Ertl,[c] Victor Nussenzweig,[g] Mauricio M. Rodrigues[a,b]

Centro de Terapia Celular e Molecular (CTCMol)[a] and Departamento de Microbiologia, Imunologia e Parasitologia,[b] Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, SP, Brazil; The Wistar Institute, Philadelphia, Pennsylvania, USA[c]; Malaria Vaccine and Drug Development Center, Cali, Colombia[d]; Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil[e]; Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brazil[f]; Michael Heidelberger Division, Department of Pathology, New York University School of Medicine, New York, New York, USA[g]