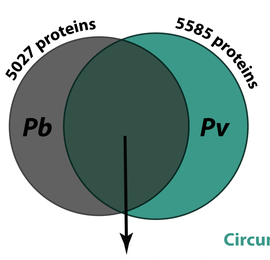
Characterization of a new malaria vaccine candidate against *Plasmodium vivax* using genetically modified rodent malaria parasites

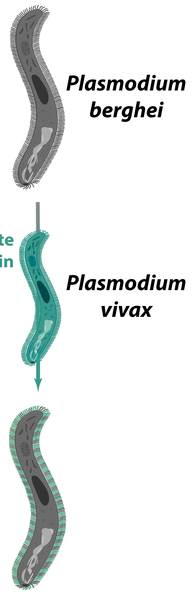
Diana Moita[1], Teresa Maia[1], Miguel Duarte[1], Carolina M. Andrade[1], Ankit Dwivedi[2], Joana C. Silva[2], Lilia González-Céron[3], Chris J. Janse[4], Shahid M. Khan[4], António M. Mendes[1], Miguel Prudêncio[1]

[1] Instituto de Medicina Molecular, João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[2] Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA
[3] Centro Regional de Investigación em Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México
[4] Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands

Introduction

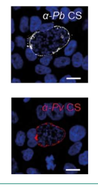
Malaria, an infectious disease caused by *Plasmodium* parasites, is the most prevalent parasitic infection worldwide. Despite major efforts, there is still no effective vaccine against any of the human-infective *Plasmodium* parasites, of which *P. vivax* (*Pv*) constitutes the most geographically widespread. Recently, our lab developed a new whole-sporozoite (Wsp) vaccine based on the use of transgenic rodent *P. berghei* (*Pb*) parasites as a platform to deliver immunogens of human-infective *Plasmodium* species. Since our *in silico* data predict that >60% of CD8+ T cell epitopes encoded in both *Pv* and *Pb* proteomes are shared between these two parasites, we generated a new genetically modified *Pb* expressing the highly immunogenic circumsporozoite protein (CS) from *Pv* (*Pv*CS) to be used as a vaccine candidate against *Pv* malaria. Therefore, we aim to fully characterize the infectivity and development of the vaccine candidate *Pb*(*Pv*CS@UIS4) throughout the *Plasmodium* life cycle and to unveil the immune responses elicited by immunization with this transgenic parasite.





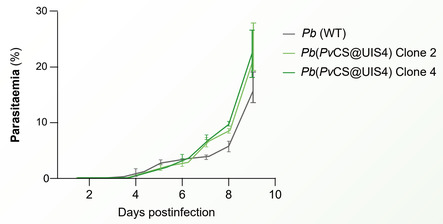
Pb(PvCS@UIS4)

Plasmodium vivax CS gene **inserted** in a neutral locus fused to the promoter sequence of *Pb*UIS4

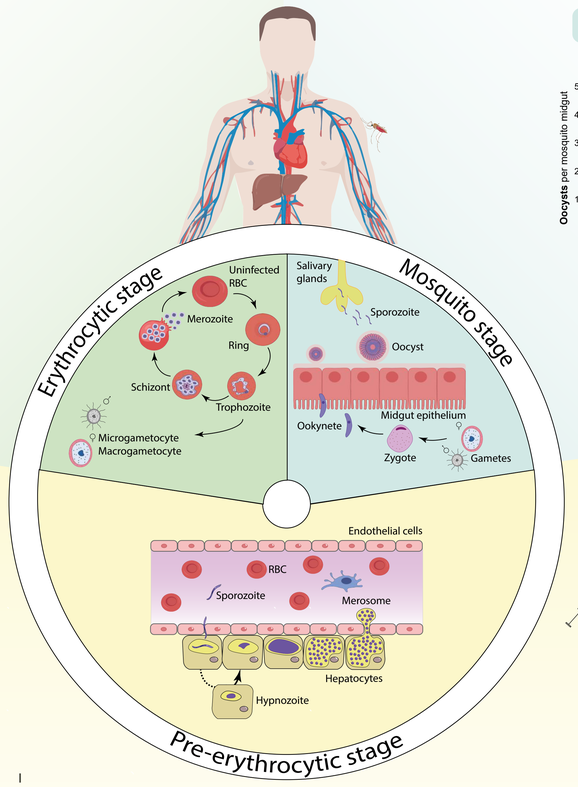


Results

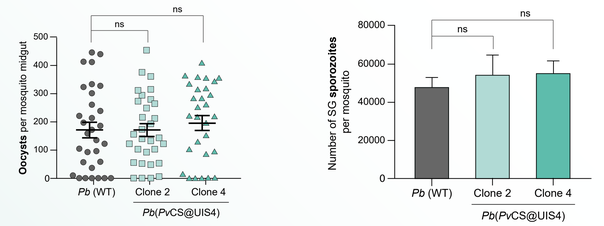
Blood stage development of *Pb*(*Pv*CS@UIS4)



The presence of the *Pv*CS protein on the transgenic *Pb*(*Pv*CS@UIS4) parasite does not influence the parasite's ability to **multiply assexually** within **red blood cells**.

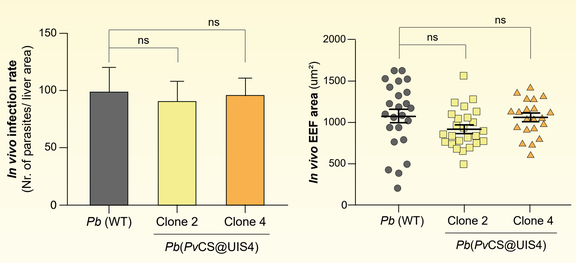


Mosquito stage development of *Pb*(*Pv*CS@UIS4)

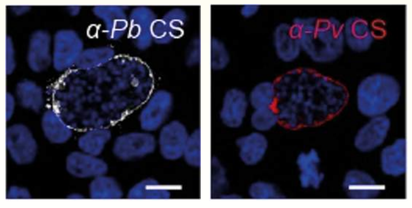


Pb(*Pv*CS@UIS4)'s ability to complete **oocyst development** and to **form** and **release sporozoites** is similar to that of the wild-type (WT) parasite control.

Pre-erythrocytic stage development of *Pb*(*Pv*CS@UIS4)

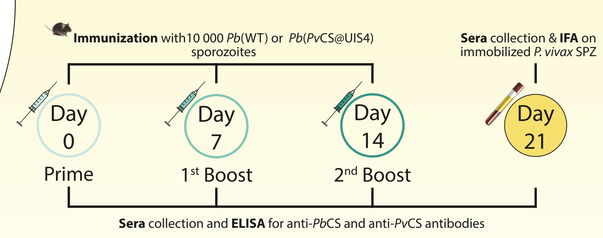


Immunofluorescence microscopy of EEFs

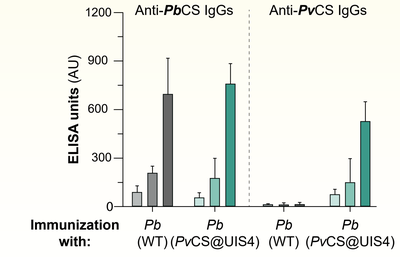


Pb(*Pv*CS@UIS4) parasites **express *Pv*CS** in addition to its endogenous *Pb*CS and are able to **infect and develop** inside **mouse hepatocytes** to the same extent as the control *Pb* (WT).

Immune response elicited by mice immunization with *Pb*(*Pv*CS@UIS4)

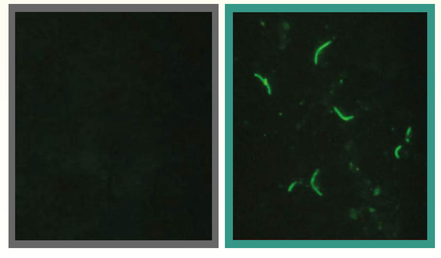


ELISA for anti-*Pb*CS and anti-*Pv*CS antibodies



Immunization of mice with *Pb*(*Pv*CS@UIS4) parasites elicits the production of increasing titers of **antibodies** against *Pb*CS and *Pv*CS.

IFA on immobilized *P. vivax* SPZ



Immunization of mice with *Pb*(*Pv*CS@UIS4) elicits the production of **antibodies** capable of **recognizing** and **binding** to *P. vivax* sporozoites.

Conclusion

Altogether, these results demonstrate that the insertion of the *Pv*CS gene in *Pb* does not have an impact on the parasite's fitness throughout its life cycle supporting its potential use as an immunization agent. Importantly, immunization of rodents with the vaccine candidate generates antibodies that efficiently recognize and bind to *Pv* sporozoites. Considering the lack of efficient strategies to tackle *Pv*, this study represents a crucial step in the development of a new Wsp vaccine candidate against this so often neglected parasite species.

Future steps

- Assess the functionality of the antibodies elicited by mice immunization through an Inhibition of Sporozoite Invasion assay
- Evaluate the protective efficacy of the vaccine candidate against a sporozoite challenge
- Uncover the main mediators of the elicited protection (*Pv*CS and/or *P. berghei* heterologous epitopes)

Prudêncio Antimalarial strategies Lab
Faculdade de Medicina Lisboa
Instituto de Medicina Molecular | João Lobo Antunes
FCT Fundação para a Ciência e a Tecnologia