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

Diana Moita¹, Teresa Maia¹, Miguel Duarte¹, Carolina M. Andrade¹, Ankit Dwivedi², Joana C. Silva², Lila González-Céron³, Chris J. Janse³, Shahid M. Khan⁴, António M. Mendes¹, Miguel Prudêncio¹

¹Instituto de Medicina Molecular, João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
²Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México
³Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA
⁴Department 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 (PvCS) 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(PvCS@UIS4) throughout the *Plasmodium* life cycle and to unveil the immune responses elicited by immunization with this transgenic parasite.

**Results**

**Blood stage development of Pb(PvCS@UIS4)**

The presence of the PvCS protein on the transgenic Pb(PvCS@UIS4) parasite does not influence the parasite’s ability to multiply asexually within red blood cells.

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

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

**Mosquito stage development of Pb(PvCS@UIS4)**

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

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

IFA on immobilized *P. vivax* SPZ

**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 (PvCS and/or *P. berghei* heterologous epitopes)

**Conclusion**

Altogether, these results demonstrate that the insertion of the PvCS 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 Pb 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.