

Mapping gametocyte development in in vitro–cultured *Plasmodium knowlesi* by single-cell RNA sequencing

Authors: Thafne Plastina Astro¹, Taís Baruel Vieira¹, Rafaela Xavier Espinosa¹, Erin Sauve², Pieter Monsiers², Gabriel Moraes Leal³, Tatiana Kugelmeier³, Anna Rosanas-Urgell², Anna Caroline Campos Aguiar¹, Daniel Youssef Bargieri⁴, Roberto Rudge de Moraes Barros¹

¹UNIFESP, Microbiology, Immunology and Parasitology, São Paulo, Brazil;

²Institute of Tropical Medicine of Antwerp, Antwerp, Belgium.

³Fiocruz, Salvador - Bahia, Brazil.

⁴USP, São Paulo, Brazil.

Introduction: Gametocytes are the sexual forms of malaria parasites responsible for transmission to mosquitoes of the genus *Anopheles*. Molecular characterization of these stages is essential for the development of transmission blocking strategies. Although *Plasmodium falciparum* gametocytes are well characterized, there are still gaps in knowledge about the biology of gametocytes from other species, such as *Plasmodium knowlesi*. **Objectives:** To molecularly characterize *P. knowlesi* gametocytes throughout their development and to investigate events associated with sexual commitment. **Methods:** *P. knowlesi* parasites cultured in vitro were analyzed by single-cell RNA sequencing. 47,008 cells of the H cell line with high-quality sequences were included in the transcriptomic analyses. Transcription of the transcription factors AP2-G and AP2-G2 was determined as a characteristic of sexual commitment. Cells with active transcription of these genes were isolated and characterized. A comparison was also made between cells of the H lineage (which produces gametocytes) and published data from the H1A1 lineage (incapable of producing sexed forms). **Results:** It was possible to identify and characterize populations of young and mature gametocytes, revealing distinct transcriptional profiles throughout sexual development. The analysis showed transcriptional alterations associated with sexual impairment, identifying important new transcriptional networks. The comparison between the H and H1A1 lineages revealed consistent transcriptional differences, especially in genes related to protein export and erythrocyte remodeling. **Conclusion:** This study identified transcriptional alterations during sexual differentiation of *P. knowlesi*, different from those observed in *P. falciparum*, reinforcing its potential as a model for the study of human malaria and contributing to the identification of targets for transmission blocking strategies for this species and for *P. vivax*.