Development of Monoclonal Antibodies Against the Infectious Forms of *Plasmodium vivax*

Mariângela de Oliveira Silva[1], Natalia Soares Ferreira[1], Zijun Wang[2], Marcilio Jorge Fumagalli[2], Anna Gazumyan[2], Marcio Massao Yamamoto[1], Tatiane Rodrigues de Oliveira[1], Kenya Elizana de Souza Abreu[1], Maisa da Silva Araújo[3], Irene da Silva Soares[1], Rogerio Amino[4], Michel C. Nussenzweig[2], Silvia Beatriz Boscardin[1]

1 Universidade de São Paulo, Brazil
2 Rockefeller University, USA
3 Fiocruz Rondônia, Brazil
4 Institut Pasteur, France

Plasmodium vivax is the most prevalent malaria-causing species outside the African continent. While less fatal than *P. falciparum,* it contributes substantially to malaria-related illness and can lead to severe complications. To date, there is no available vaccine against this species, and solutions for prevention and treatment of infection are extremely relevant. Human monoclonal antibodies (hmAbs) targeting the circumsporozoite protein (CSP) have shown potential protective effects against *P. falciparum*. However, the development of such antibodies specifically against *P. vivax* has been less explored. In this study, we report the isolation of human monoclonal antibodies specific to the *P. vivax* CSP (PvCSP) from individuals naturally infected in an endemic region of Brazil. Memory B cells were sorted using a hybrid PvCSP protein containing repeat domains from the three PvCSP variants (VK210, VK247, and *P. vivax-like)*, collectively referred to as PvCSP-all epitopes. A total of 80 paired heavy and light chain antibody sequences were obtained from six donors. Sequence analysis revealed expanded clones of CSP-specific memory B cells in almost all individuals. V gene analysis showed preferential usage of certain gene segments, with a notable overexpression of IGHV3-33, IGLV3-10, and IGKV2-28 family genes. Thirty-eight representative antibody sequences were selected for expression and further characterization. ELISA-based binding assays showed that nearly all antibodies bound to the PvCSP-All-epitopes construct, with most of them displaying cross-reactivity to multiple variants and some showing allele-specific recognition. Preliminary

functional analyses indicated that some antibodies were able to recognize *P. vivax* sporozoites and induce parasite death. Although these antibodies will still be further evaluated for their protective effect *in vivo*, they already demonstrate potential as valuable tools for antibody-based interventions and may contribute to reducing the global burden of *P. vivax* malaria.