IDENTIFICATION OF NOVEL PLASMODIUM VIVAX BLOOD-STAGE VACCINE TARGETS

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ABSTRACT

Identification of novel Plasmodium vivax *blood-stage vaccine targets*

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A vaccine targeting the illness-inducing blood stage of Plasmodium vivax *is hindered by major gaps in our knowledge of* P. vivax *biology, including critical events during merozoite invasion of erythrocytes. Only a single receptor-ligand interaction is currently known, and natural human immune responses to* P. vivax *during and after infection, which could provide clues for how to stimulate a protective immune response, have been the subject of only limited study. This lack of understanding of both the molecular details of invasion and the immunological responses during infection correlates with a relatively limited repertoire of potential* P. vivax *antigens being considered for vaccine development. A comprehensive and systematic approach is needed to advance our understanding of* P. vivax *invasion and to identify additional vaccine candidates.*

I first addressed this knowledge gap by investigating transcription in P. vivax *clinical isolates at the schizont stage, just prior to erythrocyte invasion. I purified RNA from schizont-enriched* P. vivax *isolates obtained directly from Cambodian patients with* P. vivax *malaria and sequenced the RNA using a strand-specific approach. These RNA-Seq data revealed hundreds of additional genes transcribed at this stage compared to previous microarray studies, and uncovered novel gene transcripts that enabled me to improve and annotate over 300 gene models in the reference genome. Comparisons of RNA-Seq data between clinical isolates revealed that genes related to host invasion were among the most variably-expressed genes. The data provided a basis to prioritize a list of targets consistently expressed during the late blood stages.*

I next produced a library of blood-stage P. vivax *proteins to use in functional and immuno-epidemiological studies. I selected candidates predicted to localize to the merozoite surface or invasive secretory organelles based on the* P. vivax *literature, expression during the schizont stage, and homology to* P. falciparum *vaccine candidates. I successfully expressed 37/39 full-length* P. vivax *proteins in a mammalian expression system and investigated their function using high-throughput screening methods. I screened all expressed proteins for their ability to bind reticulocytes and normocytes using a flow cytometry-based assay. I screened the complete* P. vivax *library against an*

*existing library of 40 erythrocyte surface proteins using an assay designed to detect low-affinity cell surface interactions in order to identify specific erythrocyte receptors. I also performed an intra-*P. vivax *library screen to identify parasite protein-protein interactions, which established that the interaction between surface proteins P12 and P41 known in* P. falciparum *is conserved in* P. vivax*, and detected several novel parasite protein-protein interactions. I then used surface plasmon resonance to biochemically confirm the intra-library interactions.*

Finally, I used this protein library to characterize immune responses in P. vivax*-exposed individuals. I screened the library using IgG from* P. vivax*-exposed individuals from Cambodia, the Solomon Islands, and Papua New Guinea (in collaboration), each with distinct transmission dynamics, in one of the first large-scale immunoepidemiological screens of a panel of full-length* P. vivax *merozoite proteins. Nearly all proteins were immunogenic in all 3 settings, confirming their utility in global immuno-epidemiological studies. We detected age-dependent associations for 12/34 proteins and strong correlations with clinical protection for 3 proteins, including a hypothetical protein of which little is known. Together, these data identified, characterized, and prioritized novel* P. vivax *vaccine targets for future pre-clinical testing in* ex vivo *assays.*

To Nick,

my love.

Thank you for supporting this adventure.

Let's have some more.

To Oscar,

my son.

You are magical.

Keep finding the moon.

To Elsie,

my daughter.

You are my best surprise.

Keep dancing to your own tune.

DECLARATION

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Acknowledgments, Appendix A, and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or is being concurrently submitted for any such degree, diploma, or other qualification at the University of Cambridge or any other University of similar institution. It does not exceed the prescribed 60,000-word limit (excluding bibliography, figures and appendices) for the Biology Degree Committee.

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As with raising my children, it takes a village to write a thesis.
Thank you to my wonderful village.

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LIST OF ABBREVIATIONS

6-cys	Six-cysteine
ACT	Artemisinin combination therapy
AMA1	Apical membrane antigen 1
Amp	Ampicillin
AVEXIS	Avidity-based extracellular interaction screening
BSG	Basigin
BLAST	Basic local alignment search tool
BSA	Bovine serum albumin
Ca2+	Calcium
COMP	Cartilage oligomeric matrix protein
CV	Column volume
DARC	Duffy antigen receptor for chemokines
DBL	Duffy binding-like
DBP	Duffy-binding protein
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DRM	Detergent-resistant membrane
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EBA	Erythrocyte-binding antigen
EBL	Erythrocyte binding-like
EDTA	Ethylenediaminetetraacetic acid
EGF	Epithelial growth factor
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
G6PD	Glucose-6-phosphate dehydrogenase
GMEP	Global Malaria Eradication Programme
GPI	Glycosylphosphatidylinositol
HBS	HEPES-buffered saline
HEK293	Human embryonic kidney 293
6-His	Hexa-histidine
HI-FBS	Heat-inactivated fetal bovine serum
HRP	Horseradish peroxidase
IDC	Intraerythrocytic development cycle
IgG	Immunoglobulin G
IRS	Indoor residual spraying
LB	Lysogeny broth/Luria broth
LLIN	Long-lasting insecticide-treated net
LM	Light microscopy

LMVR	Laboratory of Malaria and Vector Research
Mg2+	Magnesium
MolFOB	Molecular force of blood-stage exposure
MSP	Merozoite surface protein
MTRAP	Merozoite thrombospondin-related anonymous protein
MW	Molecular weight
NAI	Naturally-acquired immunity
NCBI	National Center for Biotechnology Information
NEB	New England Biolabs
NHS	National Health Service
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEI	Polyethylenimine
Pf	*Plasmodium falciparum*
Pv	*Plasmodium vivax*
PV	Parasitophorous vacuole
RBL	Reticulocyte binding-like
RCF	Relative centrifugal force
RDT	Rapid diagnostic test
RH	Reticulocyte-binding protein homologue
RNA	Ribonucleic acid
RON	Rhoptry neck protein
RPM	Revolution per minute
RPMI media	Roswell Park Memorial Institute media
RT	Room temperature
RU	Response units
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SNP	single-nucleotide polymorphism
SOC	super optimal broth with catabolite repression
SPR	Surface plasmon resonance
TBE	Tris-borate-EDTA
V	Volts
WHO	World Health Organization
WTSI	Wellcome Trust Sanger Institute

One letter code for amino acids

A	Alanine
C	Cysteine
D	Aspartate
E	Glutamate
F	Phenylalanine

G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Tryptophan
Y	Tyrosine