P. vivax malaria vaccine; Key considerations

HERBERT OPI
*P. vivax* poses a significant challenge for malaria elimination

**NEW TOOLS REQUIRED**

Malaria control has stagnated in recent years

**THAILAND**

- Increasing proportion of *P. vivax* infections as transmission declines

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- Mosquitoes – outdoor biting and earlier
- Hypnozoites – arrested liver-stages
- Early appearance of gametocytes
- Low-density infections; Asymptomatic infections

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Sattabongkot, Trends Parasitol, 2004

Mueller, The Lancet, 2009

Slide courtesy of Rhea Longley
**P. vivax** malaria vaccine lags significantly behind

WHO has recommended the development of highly efficacious vaccines for both *P. vivax* and *P. falciparum*

* Targets and mechanisms of action of *P. vivax* immunity poorly understood
Strategies to develop efficacious and long-lasting malaria vaccines

Most current lead candidates selected before the era of malaria genomics and proteomics

Lead candidates

Novel Approaches

Assays for vaccine evaluation

Vaccine platforms

Overcoming antigen polymorphisms and vaccine escape by parasite

Identifies strategies for optimal induction and maintenance of protective responses

In vitro and animal models

Functional protective immunity

Pre-clinical evaluation

Correlates of protection

Clinical evaluation in humans

Controlled human malaria infection trials

Field trials

Refinement

Prioritize candidates

Construct design

Combinations

Delivery platforms and adjuvant

Dosing and regimens

Candidate selection

Antigen discovery

Existing candidates

Beeson et al, Science Translational Med, 2019
Multi-stage vaccines: Targeting sporozoites and blood stage parasites to improve efficacy and longevity

**Target Sporozoites:** Prevent infection

**Pros:**
- Proven approach to achieve significant efficacy

**Key challenges:**
- Difficult to achieve high levels of efficacy and longevity
- Need to induce and maintain high antibody levels

**Target Merozoites:** Clear parasites and prevent illness

**Pros:**
- Can harness immune recall responses, good for longevity

**Key challenges:**
- Concept established, but limited success in vaccine trials
- Many antigens - which are optimal targets?

We have multiple strategies to overcome these challenges – e.g. functional antibody profiling, immune longevity
Antibodies play an important role in immunity to malaria

Beyond antibody magnitude and neutralization – antibody Fc interactions

- Both antibody magnitude and neutralization are poor correlates of protection from malaria
- GIA current gold standard for blood-stage vaccines
- A lot of candidates selected via this pathways have failed in efficacy trials
- Some candidates with limited neutralizing activity show promising efficacy and vice versa

Beyond antibody magnitude and neutralization – antibody Fc interactions

**Complement fixation**
- Mediates:
  - Phagocytosis
  - Lysis
  - Enhanced neutralization

**Fcγ Receptors**
- On resting cells:
  - FcγRI - monocytes
  - FcγRIIa - monocytes and neutrophils
  - FcγRIII - neutrophils and NK cells

- Mediate:
  - Phagocytosis
  - Antibody dependent cellular cytotoxicity (ADCC)
  - Antibody dependent cellular inhibition (ADCI)
  - Antibody dependent respiratory burst (ADRB)

*P. vivax* antibody functions poorly understood

Opi, D.H. *et al.*
Expert Review Vaccines, 2021
Our Approaches to Quantify Antibody Function

Developed methods to quantify the ability of antibodies to interact with complement and Fcγ receptors

Examined four functional antibody responses
Complement fixation & FcγRI, FcγRIIa & FcγRIII binding
Our Approaches to Quantify Antibody Function

Adapted to automated high-throughput platform at Burnet Institute (test up to 10,000 samples)
Quantifying antigen-specific functional antibodies to *P. vivax*

- Are *P. vivax* antigens targets of functional antibody responses of **complement (C1q) fixation** and **FcγR binding**?

- Are these functional antibody responses **associated with protection** from clinical *P. vivax* malaria?

- What are the **kinetics** (acquisition and maintenance) of *P. vivax* functional antibody responses?
Selected 30 *P. vivax* proteins

- Good serological markers
- Immunogenic and/or
- Known associations with protection

**Blood-stage proteins:**

- AMA1, MSP7 (x4), RBP2b, RAMA, MSP8, MSP3A, MSP3b, RON2, MSP1-19, MSP5

**Pre-erythrocytic proteins:**

- CSP247, CSP210, TRAP, SIAP2

**Other:**

- Hypothetical proteins x3, 1x “unspecified protein”, 2x “exported proteins”, PTEX150, SSA s16, Pv-fam-a x5
P. vivax antigens are targets of functional antibodies

Longitudinal cohort of ~200 children (1-3yrs old) in East Sepik Province in **PNG vs naïve controls**
Functional antibodies targeting *P. vivax* associated with protection

Incidence Rate Ratios (95% CI)

Incidence Rate Ratios (95% CI)
Few antigens elicit poly-functional protective responses
Increased protection with multiple antigens and antibody functions

**Antigen combinations (Complement fixation)**
- Reduced risk
- PvRBP2b
- PvRON2
- PvMSP5
- Top 3 antigen combinations

**Incidence Rate Ratios (95% Confidence Interval)**

**Antibody functions combinations (MSP5)**
- Reduced risk
- FcγRIII
- FcγRIIa
- C1q
- FcγRI
- FcγRs combinations
- FcγRs & C1q combinations

**Incidence Rate Ratios (95% Confidence Interval)**
Functional antibodies more strongly associated with protection than GIA (*P. falciparum*)

Reiling, L. *et al.* Nature Comm, 2019

Nkumama, I.N. *et al.* bioRxiv, 2022
Studies on antibody decay to achieve long-lived immunity

Vaccine half-lives in a malaria endemic population
- Measles: 457 years
- Tetanus: 7-12 years (dependent on number of doses)

Longitudinal observational cohort following clinical infection in Thailand

\textit{P. vivax} functional antibodies wane rapidly

Some antigens and individuals have better longevity
Studies on antibody decay to achieve long-lived immunity

RTS,S for *P. falciparum* in children

**Induction of functional antibodies**

**Rapid decay of functional antibodies during follow-up**

Two vaccine cohorts studied: C1 and C2

Corresponds with loss of vaccine efficacy

Antibodies to *P. vivax* antigens mediate opsonic-phagocytosis

*Opsonic phagocytosis by THP-1 monocyte line of antigen-coated beads*
Multifunctional antibody profiling in vaccine evaluation and development

A complex parasite requiring a complex approach

Burnet mRNA Vaccine Program

- Focus on 3 diseases: malaria (*P. vivax* and *P. falciparum*), Hepatitis C, COVID-19,
- Developing existing lead candidates using mRNA vaccine platform
- Ongoing development and refinement of other candidates and novel approaches
- Strategies to increase potency of immune responses and immune longevity

- 12 infectious diseases research groups feed into our Burnet Vaccine program

- Funding from Victorian Government and Burnet Institute, and other grants
- Related funding from various agencies for vaccine discovery and design
Malaria mRNA vaccine development

Our Approach:
- Design the antigens
- Partners include Monash Institute of Pharmaceutical Sciences
- mRNA platform (synthesis & formulation)

Challenges in malaria:
- Poor immunogenicity of many malaria proteins
- *Plasmodium* protein expression in mammalian cells
  - Amount of protein
  - Confirmation and presentation
- Longevity of immunity

Malaria mRNA vaccines:
- *P. vivax* antigens (and *P. falciparum*)
  - Liver and Blood stages
  - Establish and evaluate the platform

Malaria mRNA vaccine development

mRNA (MIPS) malaria vaccine constructs:
• Confirmed expression in Human HEK293 cells
• Formulation of single mRNA constructs in Lipid Nanoparticles

Mice immunisation studies:
• Vaccine schedule and immunogenicity
• Longevity of immunity

Ongoing work:
• Assess other *P. vivax* antigens
  • Liver and Blood stages
• Explore multi-antigen formulations
• Assess different construct designs

Future work
• Human trials
Industry Best Practices – Quality Management System

Suite of carefully selected elements of ISO9001 quality management system to improve data reliability, reproducibility and alignment with requirements of future industry partners, and regulators.
### Expertise
- Independent evaluation of immunogenicity
- Neutralization assays for functional antibodies
- Antibody specificity using competition assays
  - HCV, HIV, COVID-19
- Live virus assays
  - SARS-CoV-2, HIV and HCV
- Malaria culture and antibody effector assays

### Compliance
- PC2 and PC3
- ISO 9001 Quality Management System

### Partnerships
- DFAT accredited NGO
- Strong partnerships are key to Burnet Impact
- Global reach with international offices and partners
- Ability to co-fund
- Access to end-user group cohorts for clinical trials - globally

### Vaccine implementation
- Health system strengthening
- Optimization of vaccination strategies
Achieving higher vaccine efficacy

Targeting key epitopes, maximizing functional responses

**Improved vaccine design through knowledge of key functional epitopes**
- Structure-based vaccine design
- Reduce vaccine escape polymorphisms

**Exploiting multiple antibody functions to improve protective immunity**
- Direct neutralization activity
- Antibody interactions with complement system
- Roles for antibody-immune cell interactions (monocytes, neutrophils, NK cells)

**Combining multiple antigens**
- Increase functional activity, or induce multiple functional activities
- Target multiple stages
- Reduce vaccine escape
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