P. vivax malaria vaccine; Key considerations

HERBERT OPI

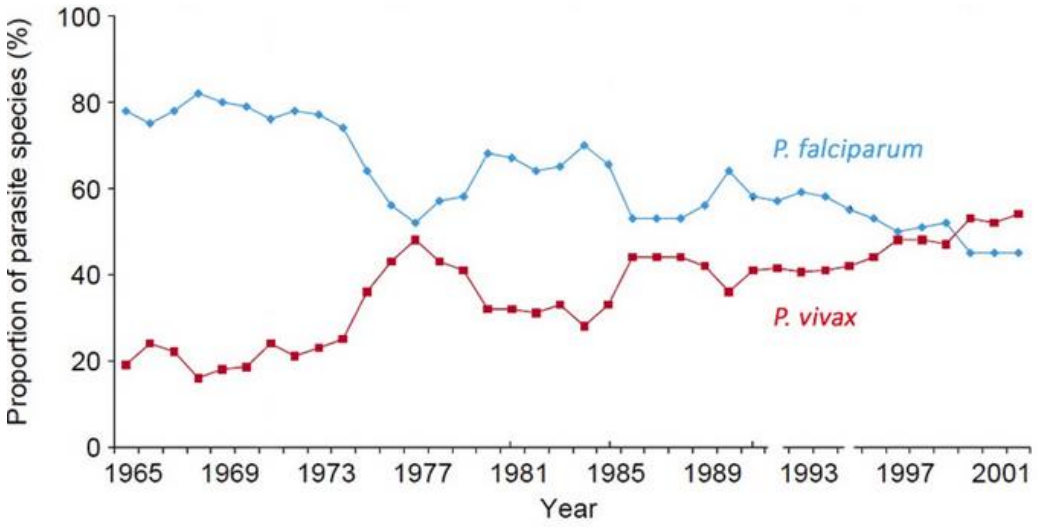
Burnet
reach for the many

P. vivax poses a significant challenge for malaria elimination

NEW TOOLS REQUIRED

Malaria control has stagnated in recent years

THAILAND

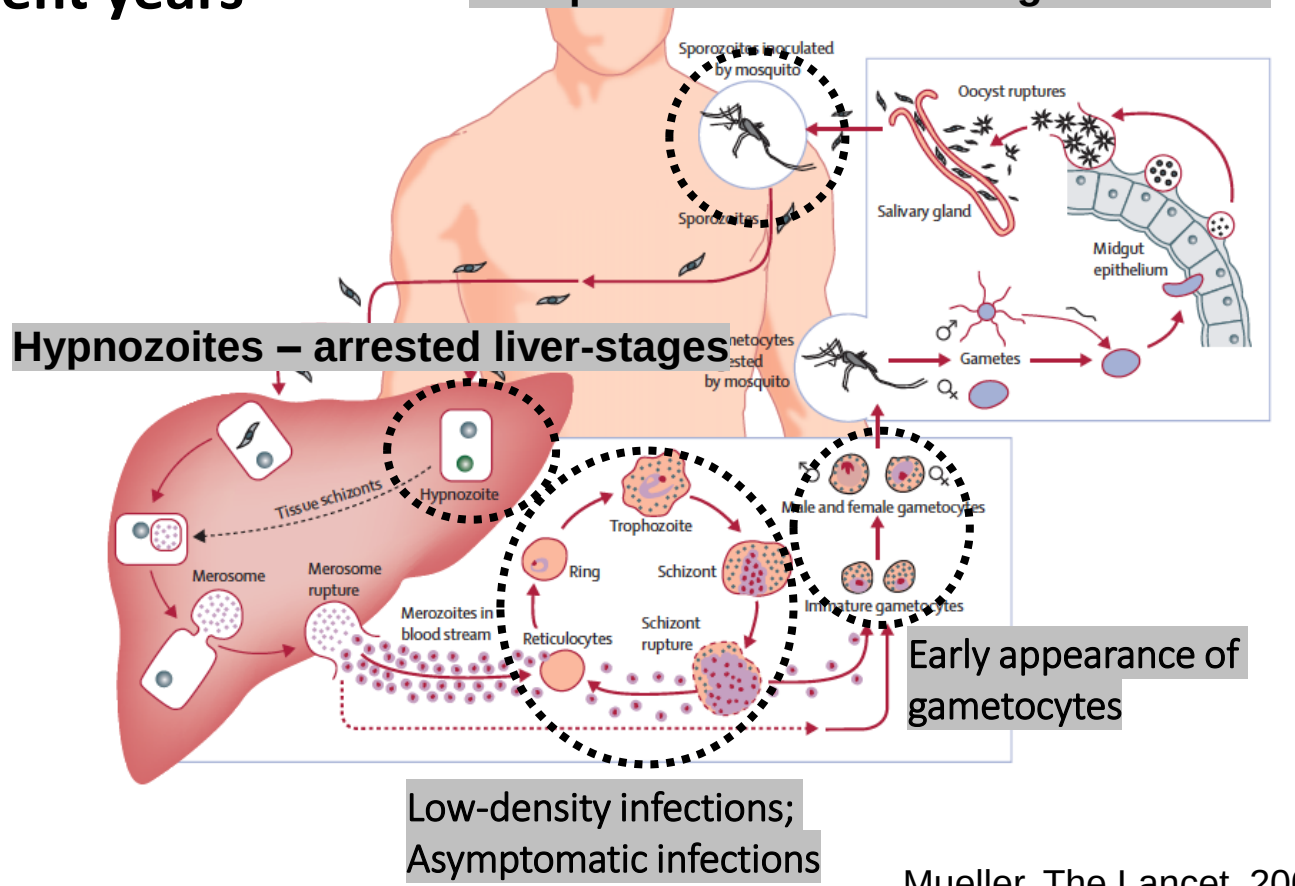


Increasing proportion of *P. vivax* infections as transmission declines

Sattabongkot, Trends Parasitol, 2004

Mosquitoes – outdoor biting and earlier

Hypnozoites – arrested liver-stages



Early appearance of gametocytes

Low-density infections;
Asymptomatic infections

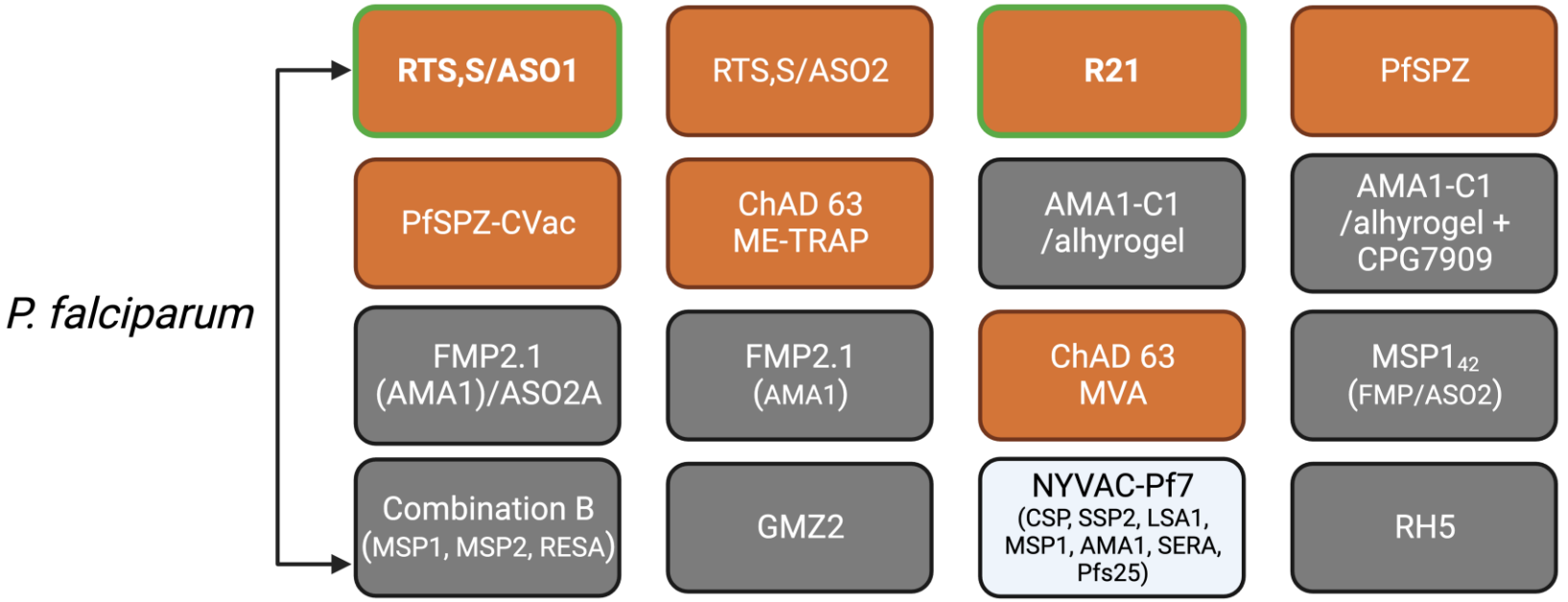
Mueller, The Lancet, 2009

+ DIFFICULT TO TREAT

Slide courtesy of Rhea Longley

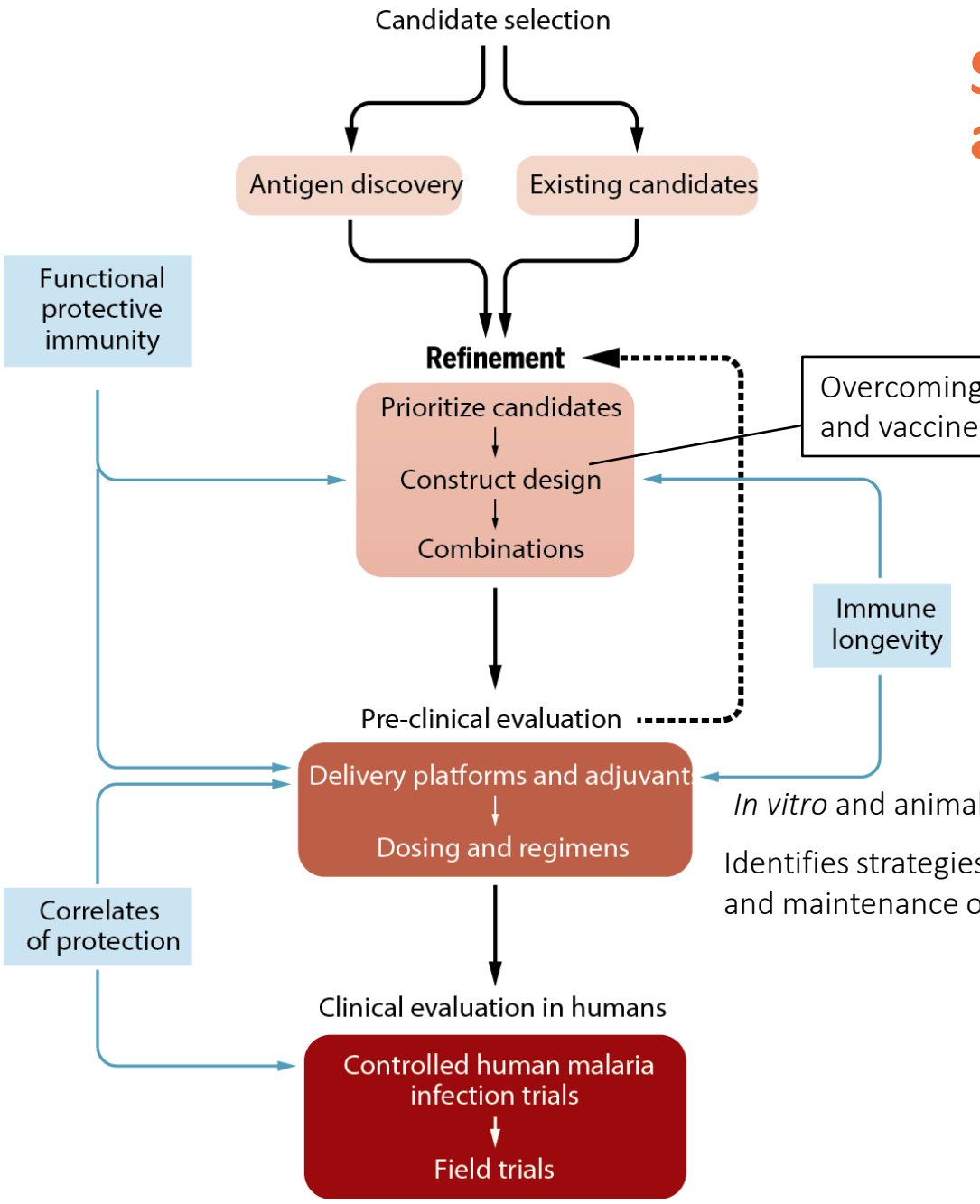
P. vivax malaria vaccine lags significantly behind

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

In vitro and animal models

Identifies strategies for optimal induction and maintenance of protective responses

Lead candidates

Novel Approaches

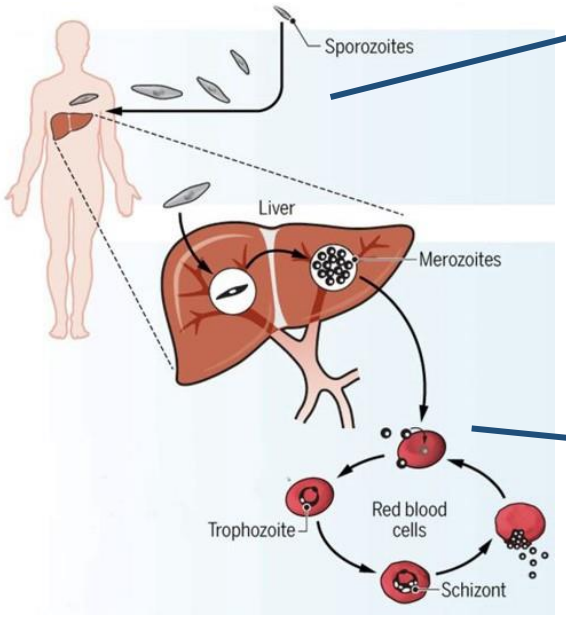
Assays for vaccine evaluation

Vaccine platforms

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:

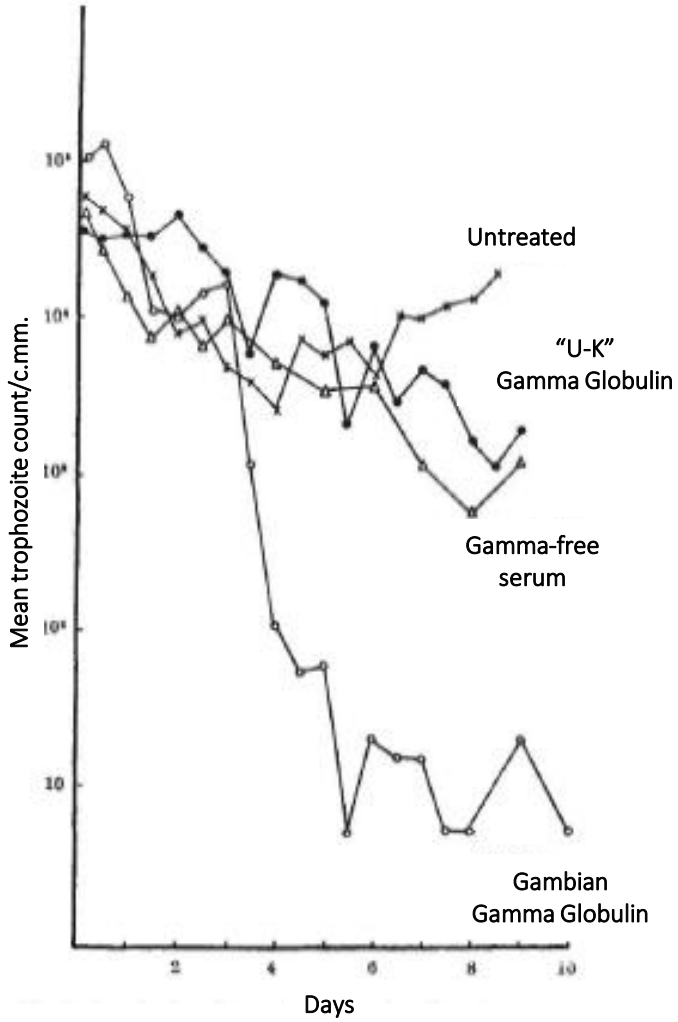
- Can harness immune recall responses, good for longevity

Key challenges:

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

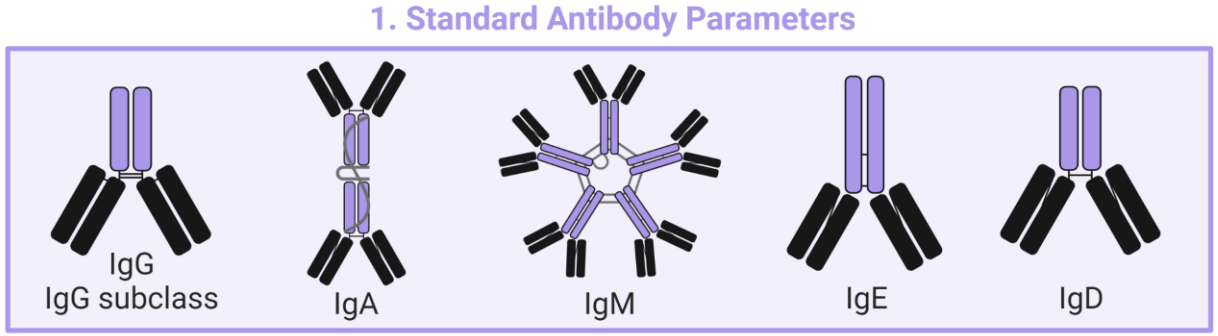
We have multiple strategies to overcome these challenges – e.g. functional antibody profiling, immune longevity

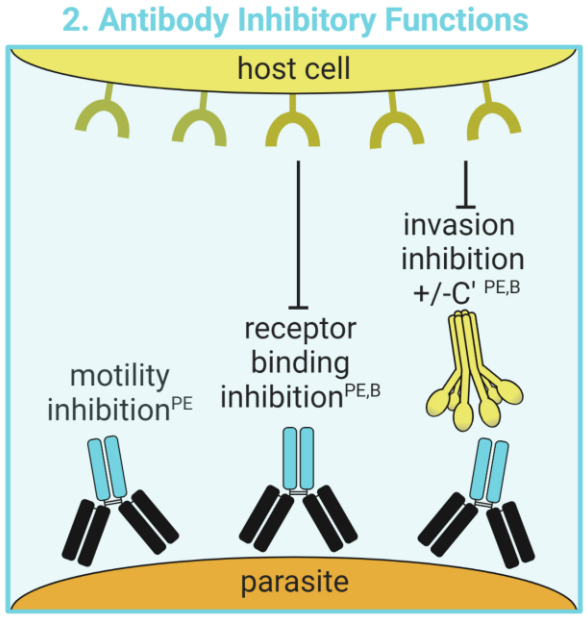
Antibodies play an important role in immunity to malaria



Cohen, S. *et al.* Nature, 1961

Beyond antibody magnitude and neutralization – antibody Fc interactions





Both antibody magnitude and neutralization are poor correlates of protection from malaria

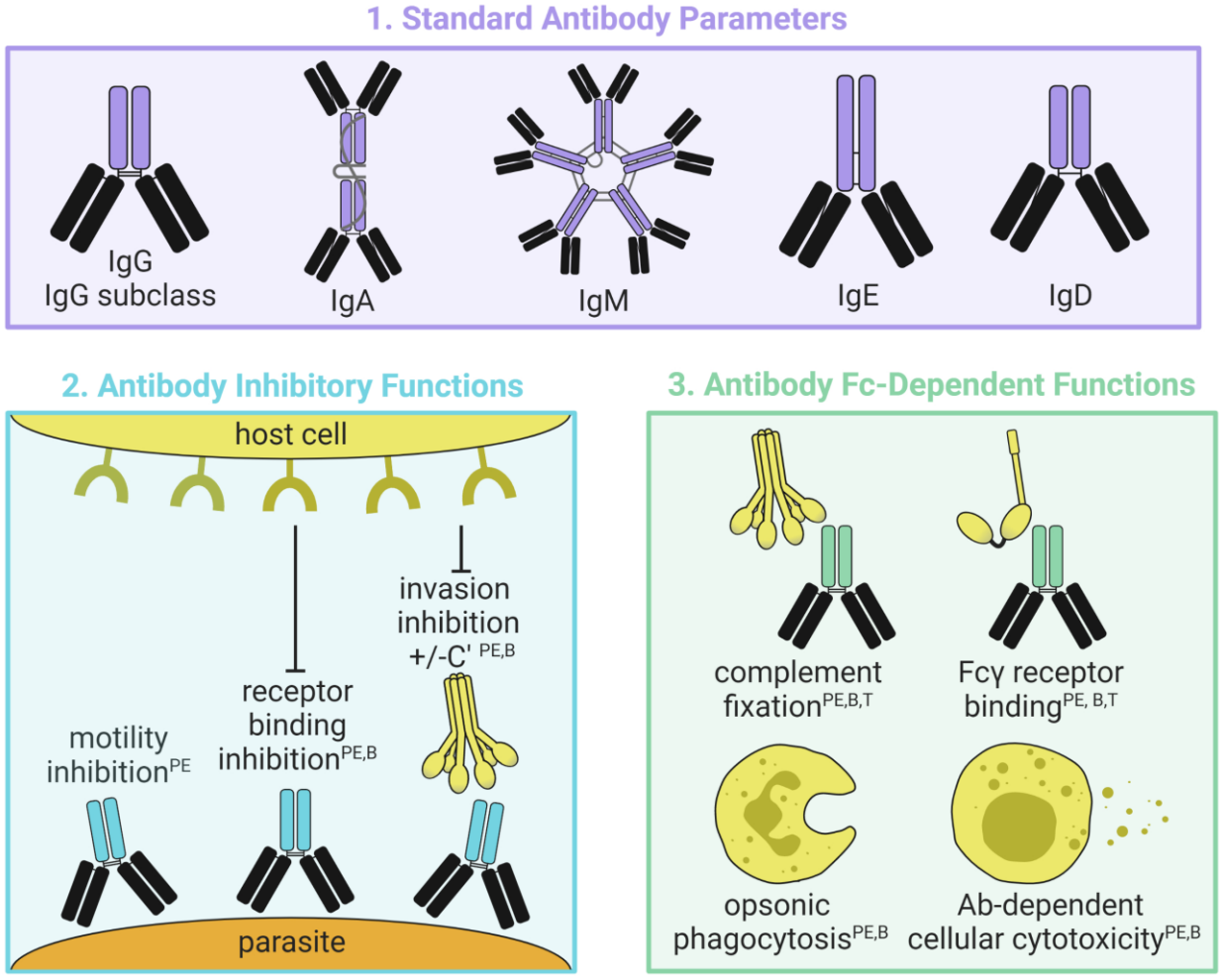
GIA current gold standard for blood-stage vaccines

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

Opi, D.H. *et al.*
Expert Review Vaccines, 2021

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)

Opi, D.H. *et al.*
Expert Review Vaccines, 2021

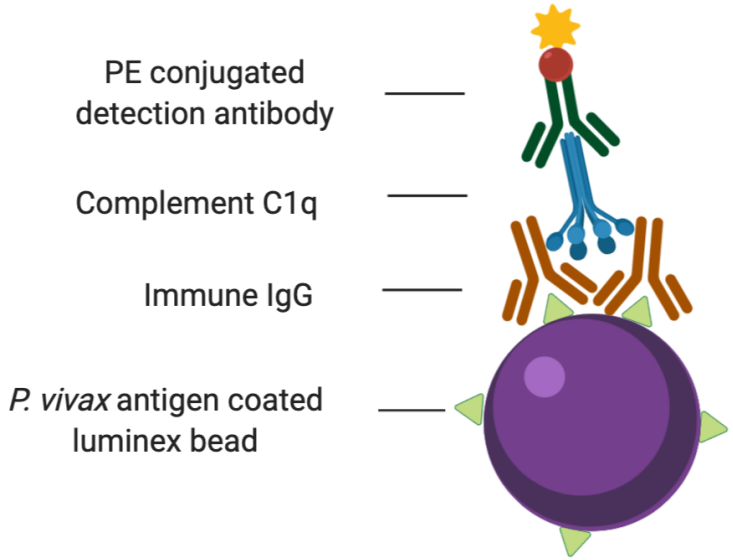
* *P. vivax* antibody functions poorly understood

Our Approaches to Quantify Antibody Function

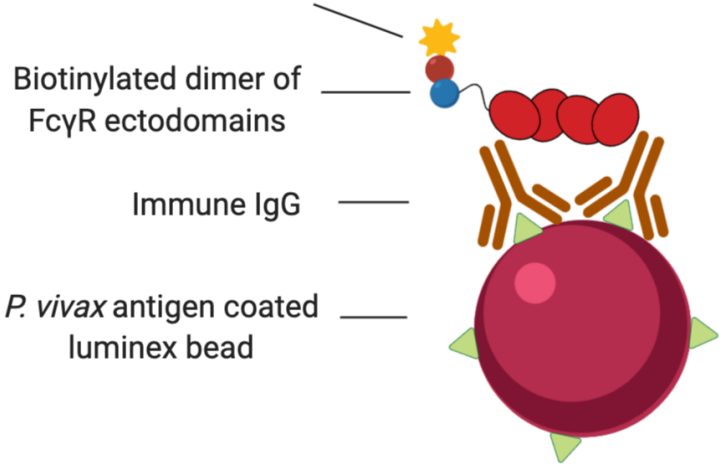
Developed methods to quantify the ability of antibodies to interact with complement and Fc*γ* receptors

Multiple luminex beads coated with different parasite proteins

Complement Deposition



Fcγ Receptor Binding

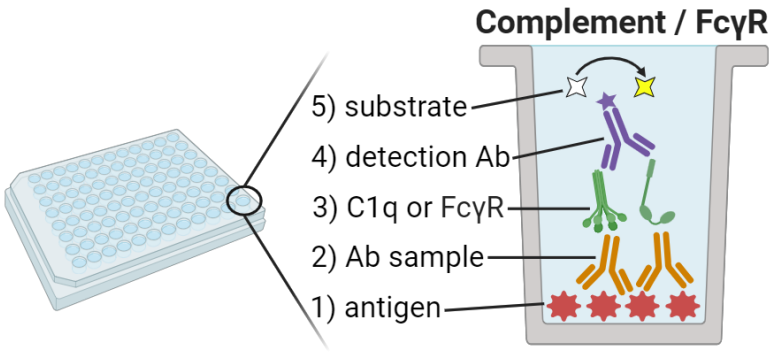


Examined **four** functional antibody responses

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)



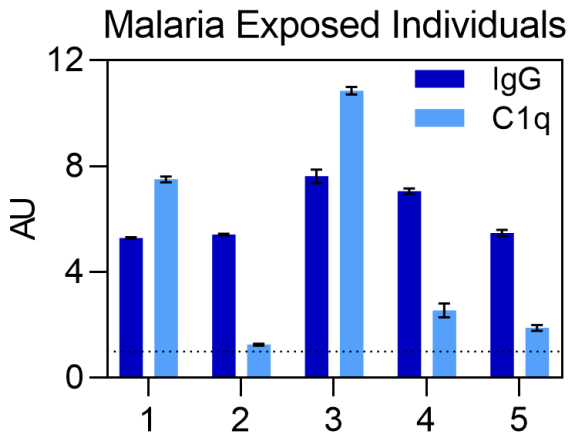


Figure created with BioRender.com; Kurtovic et al., BMC Med 2018

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?

P. vivax proteins tested

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:

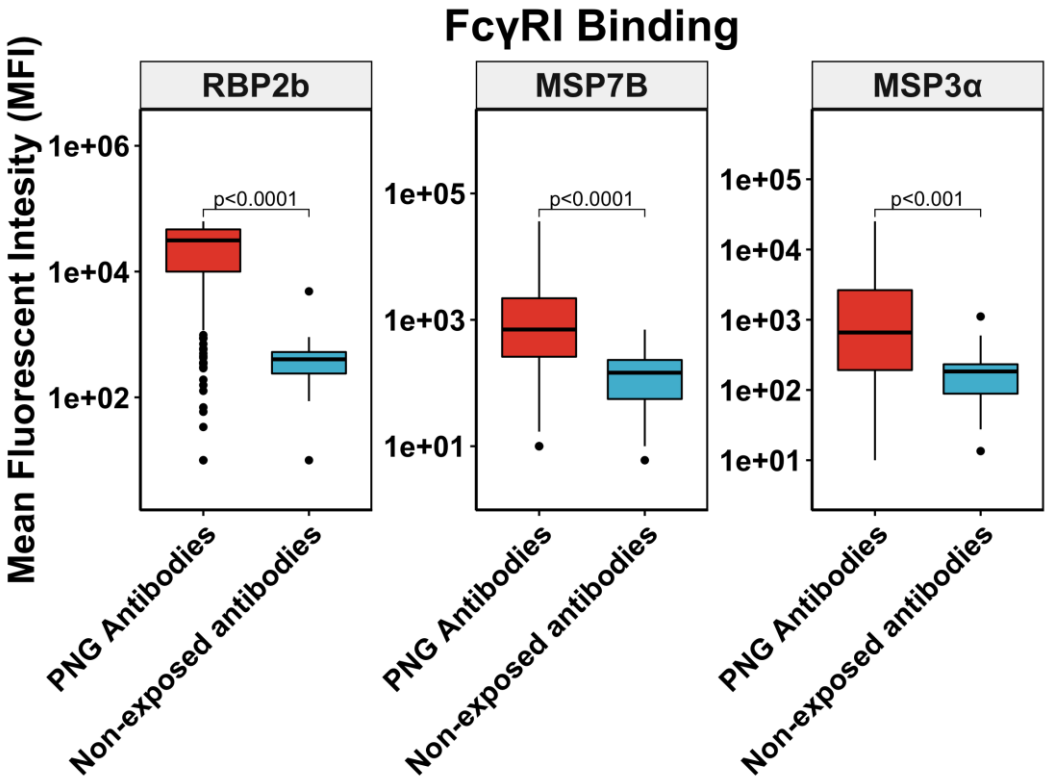
- CSP247, CSP210, TRAP, SIAP2

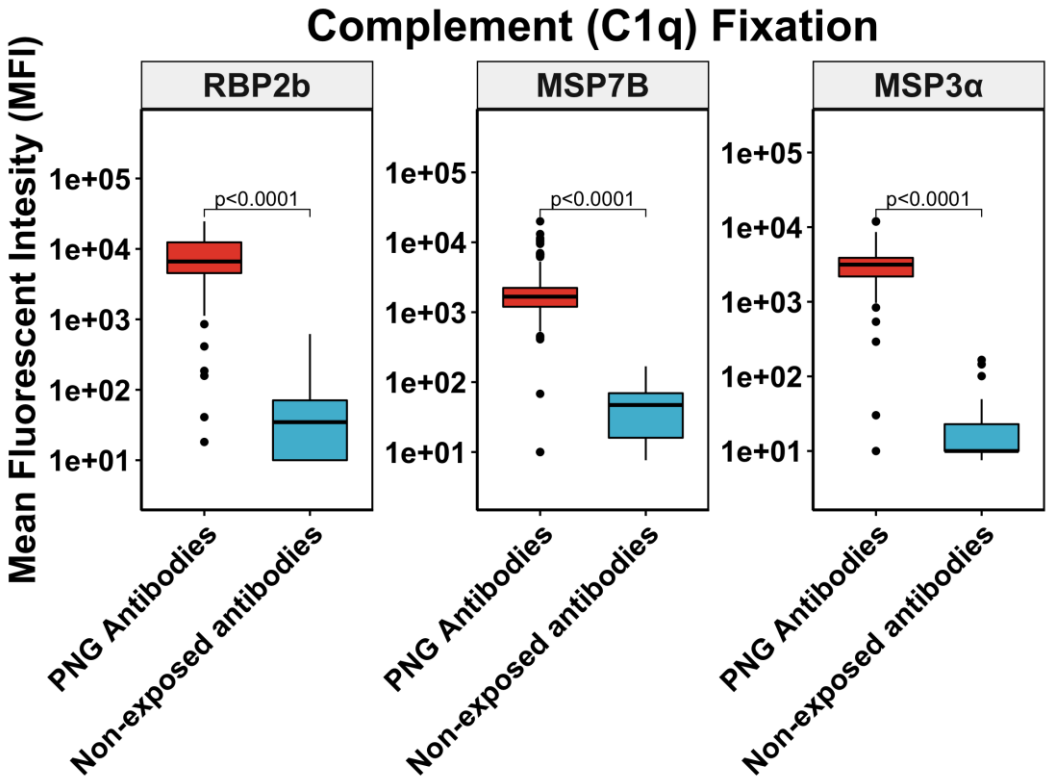
Other:

- Hypothetical proteins x3, 1x “unspecified protein”, 2x “exported proteins”, PTEX150, SSA s16, Pv-fam-a x5

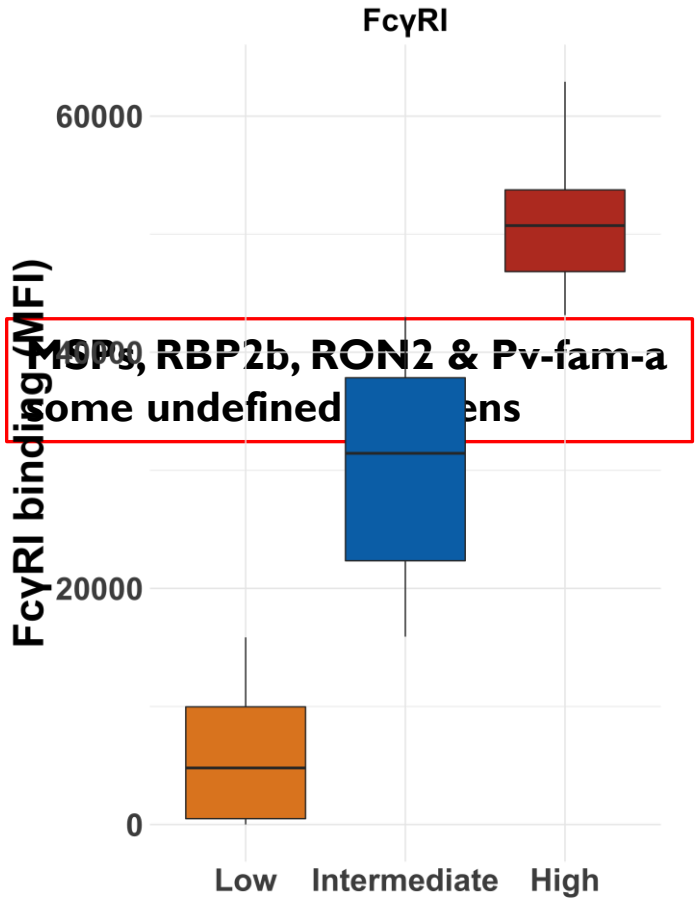
P. vivax antigens are targets of functional antibodies

Longitudinal cohort of ~200 children (1-3yrs old) in East Sepik Province in **PNG vs naïve controls**

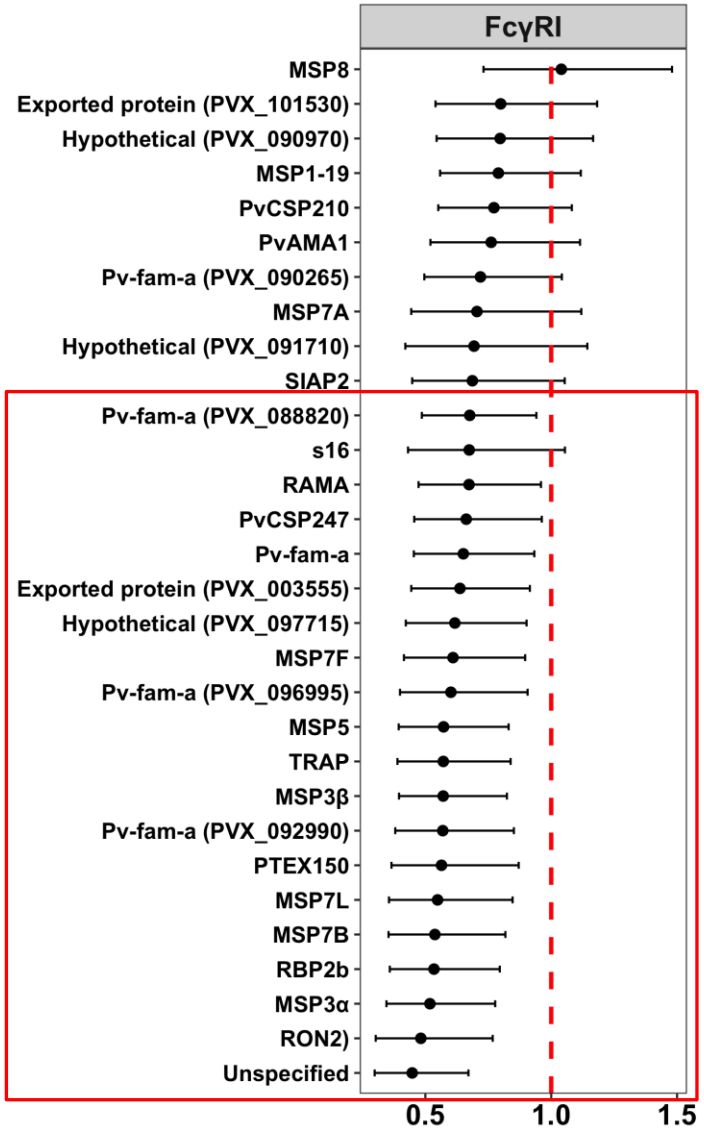


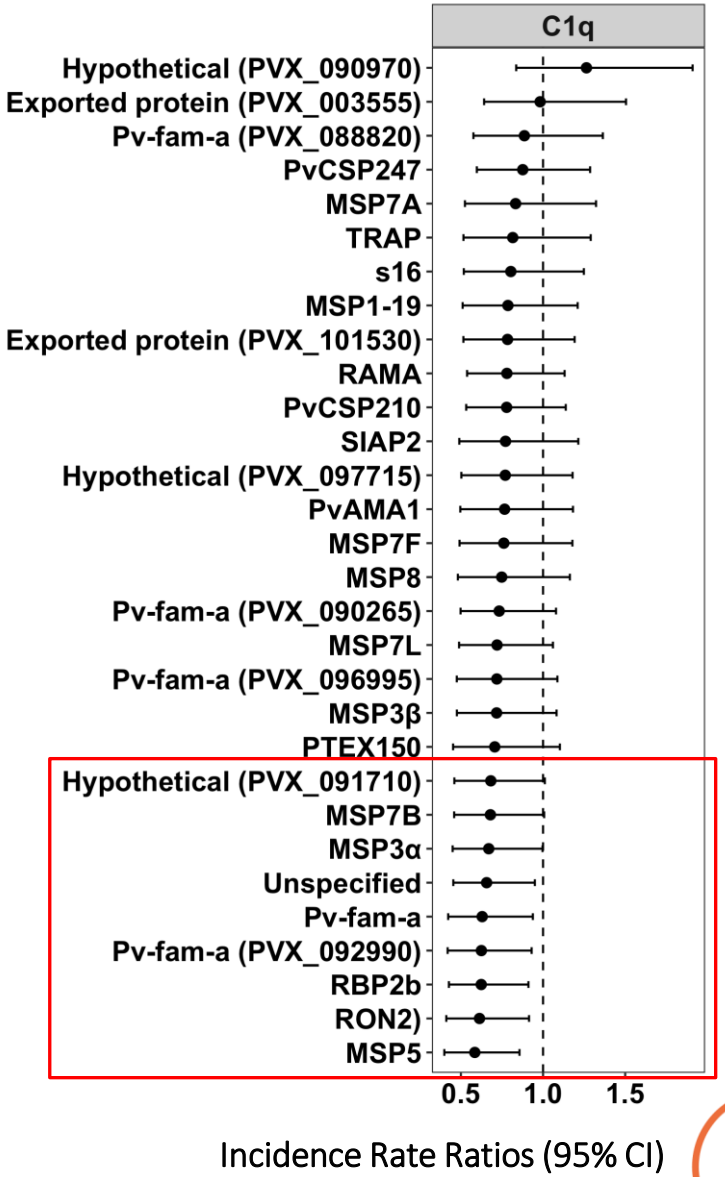


Functional antibodies targeting *P. vivax* associated with protection

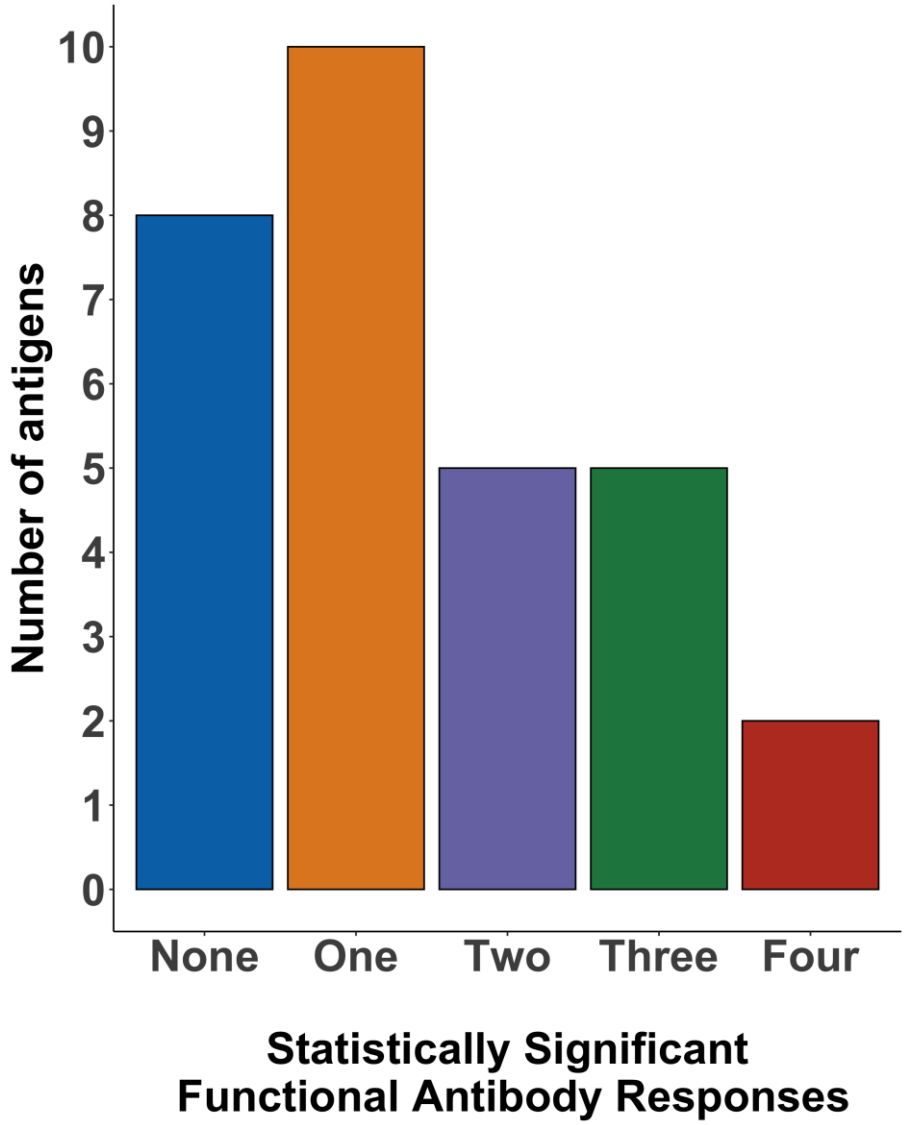


MSP3, RBP2b, RON2 & Pv-fam-a
some undefined ens

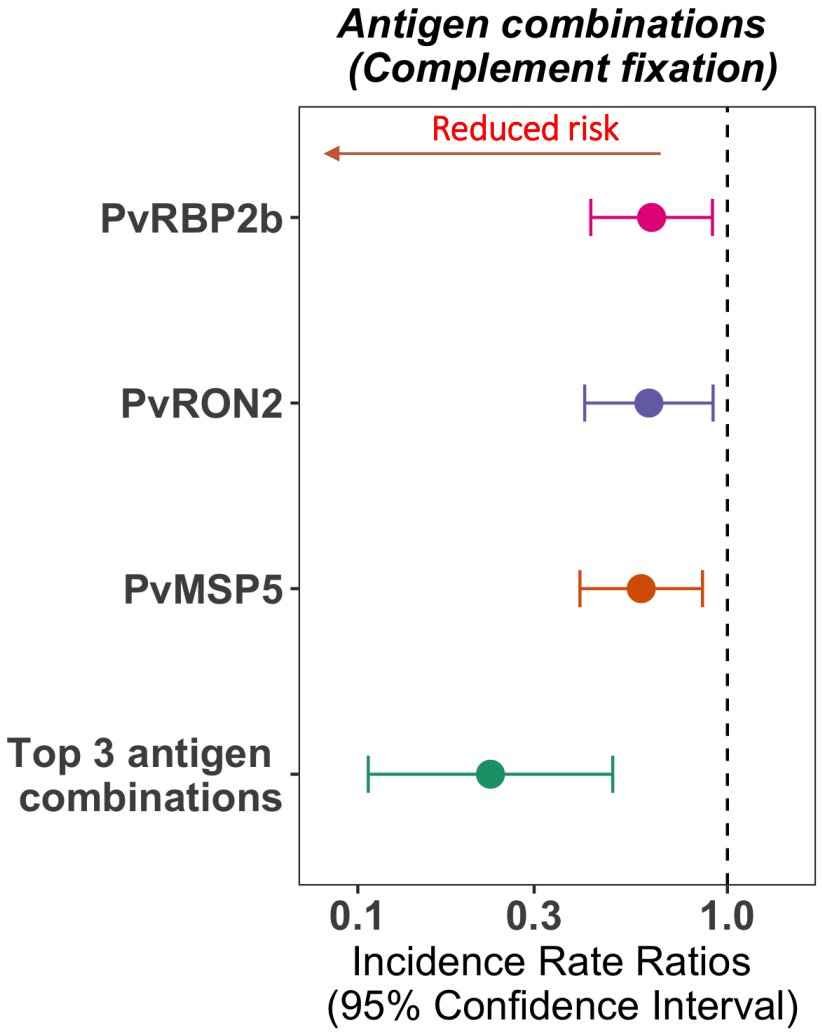


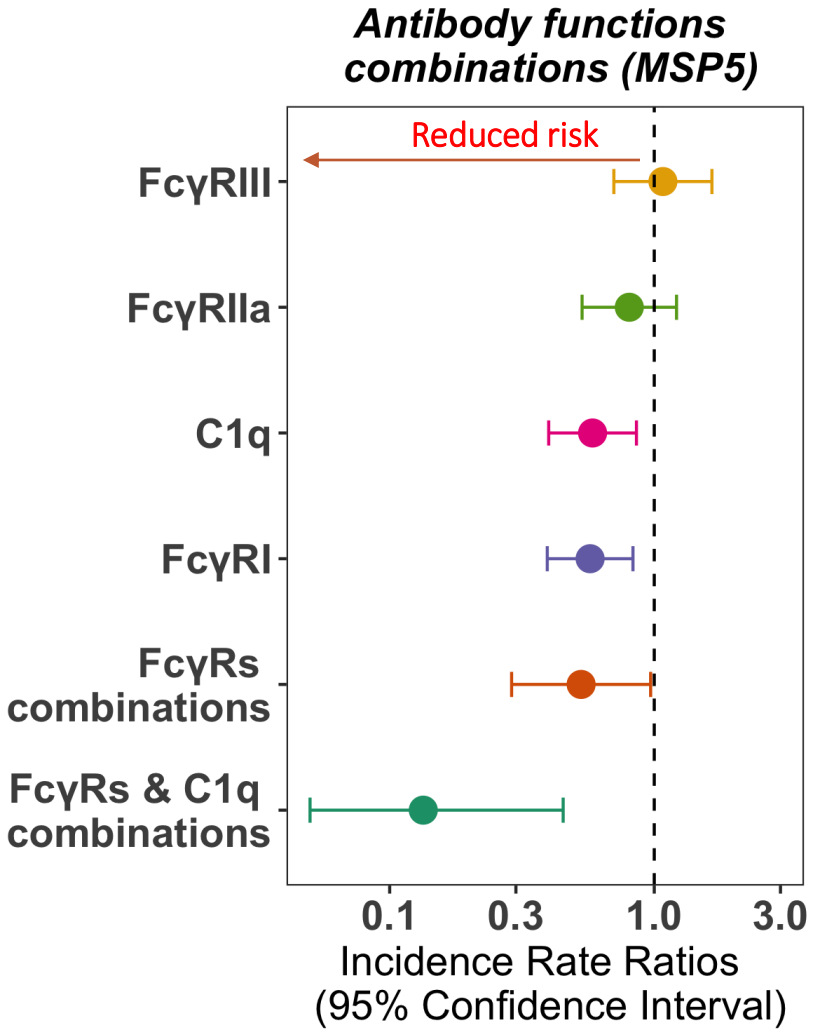


Few antigens elicit poly-functional protective responses

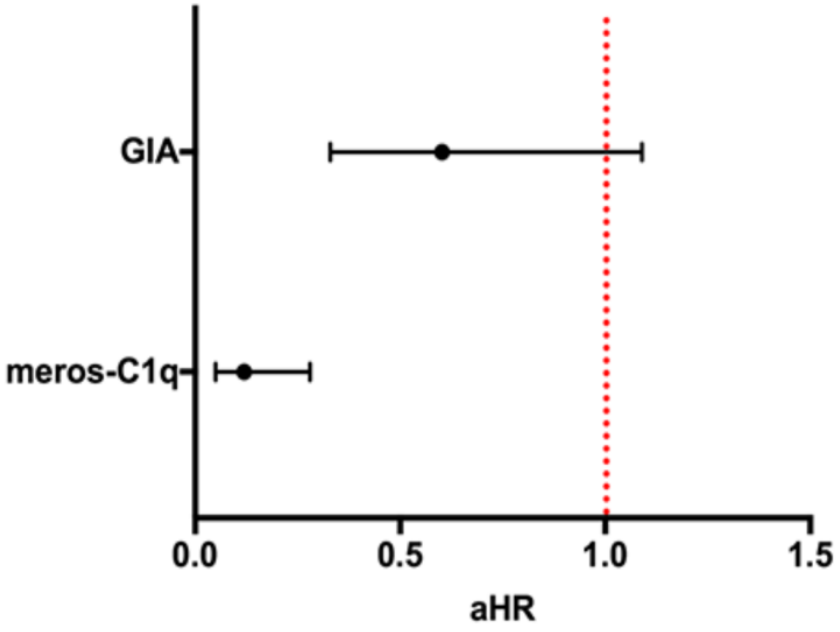


Increased protection with multiple antigens and antibody functions

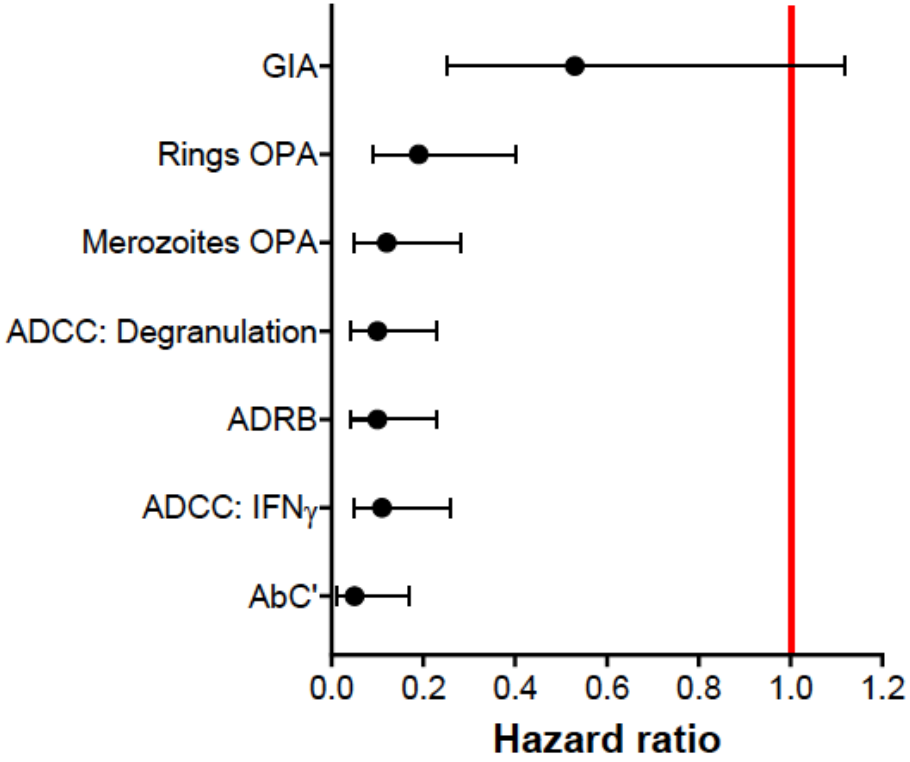




Functional antibodies more strongly associated with protection than GIA (*P. falciparum*)



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

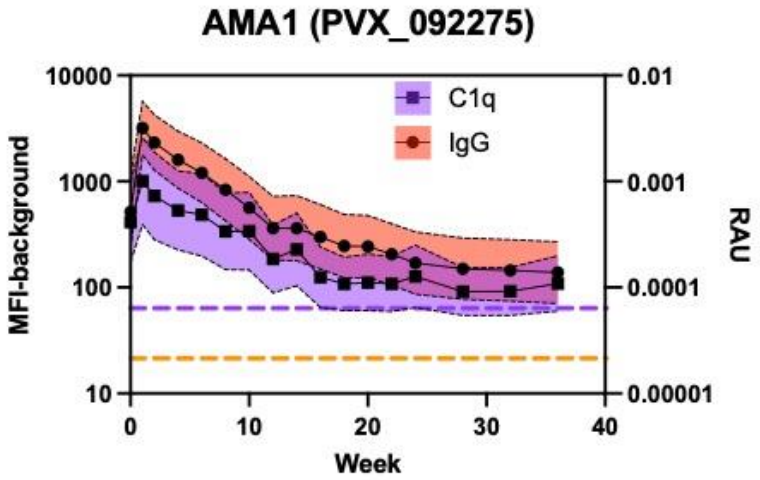


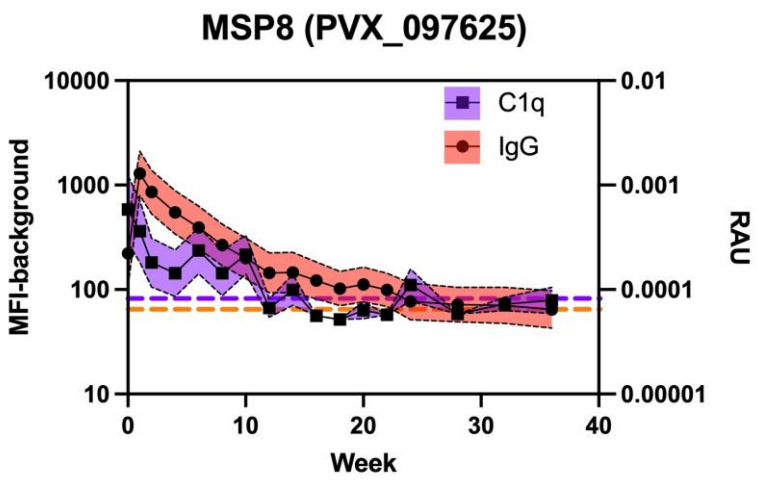
Nkumama, I.N. *et al*. bioRxiv, 2022

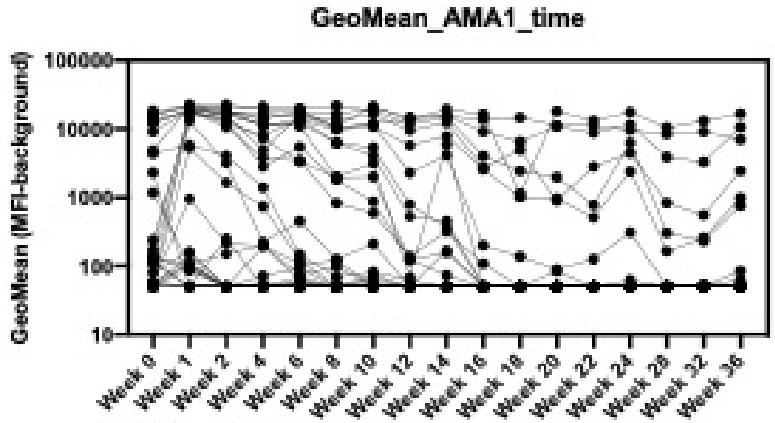
Studies on antibody decay to achieve long-lived immunity

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







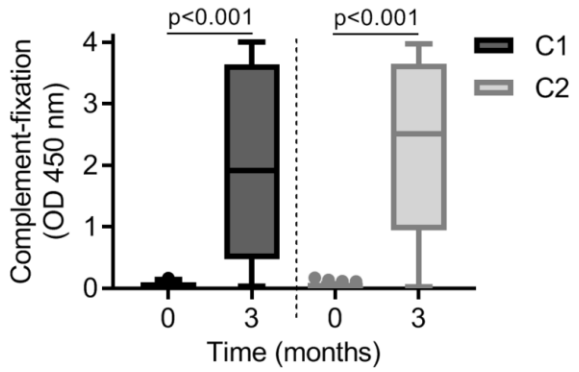
***P. vivax* functional antibodies wane rapidly**

Some antigens and individuals have better longevity

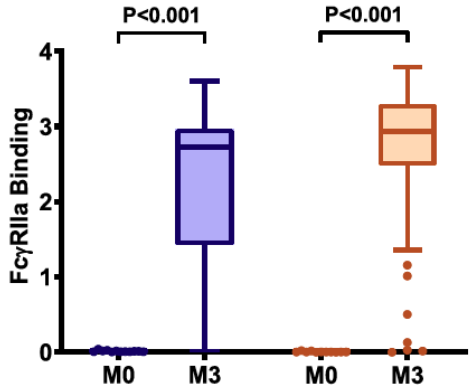
Studies on antibody decay to achieve long-lived immunity

RTS,S for *P. falciparum* in children

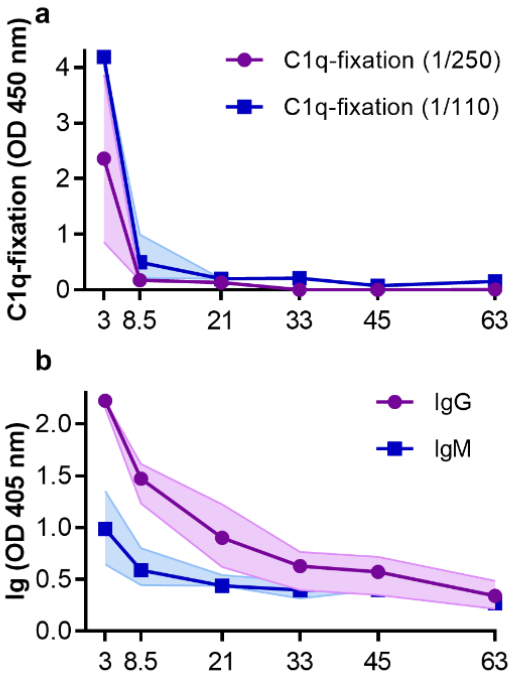
Induction of functional antibodies

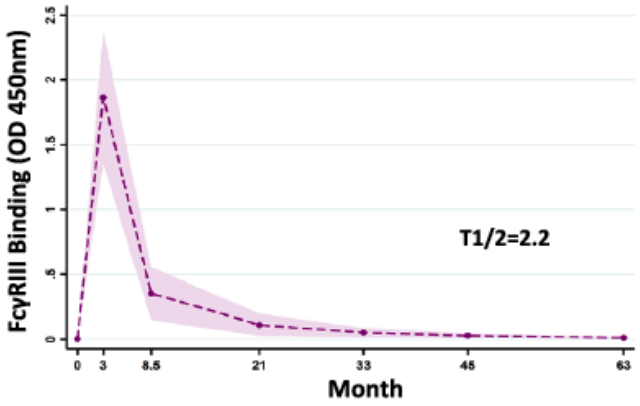


Two vaccine cohorts studied: C1 and C2



Rapid decay of functional antibodies during follow-up

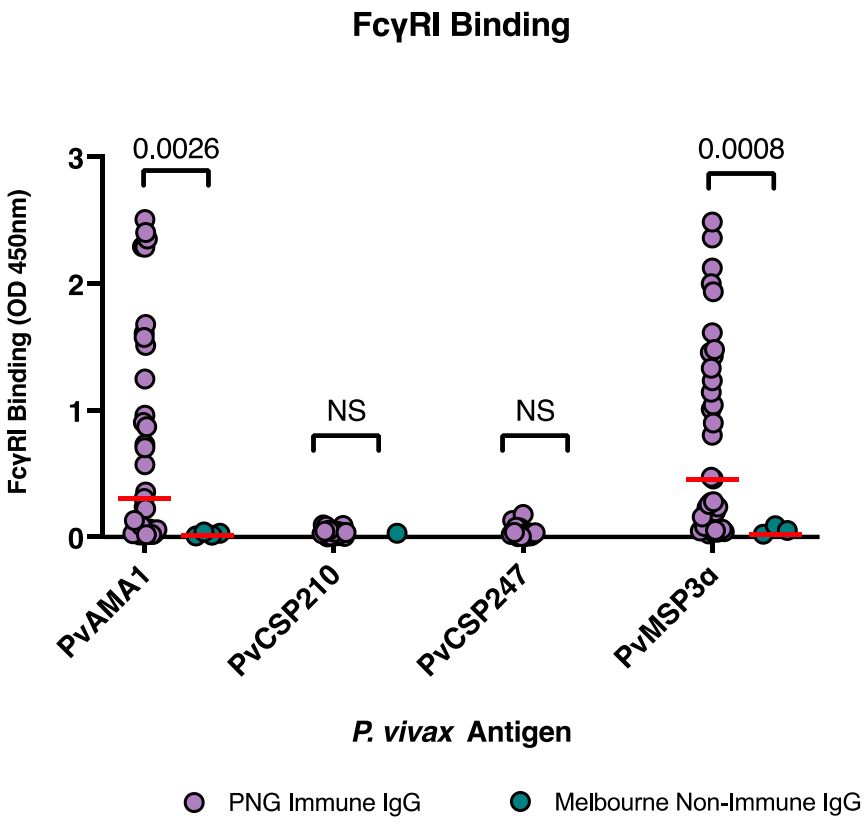


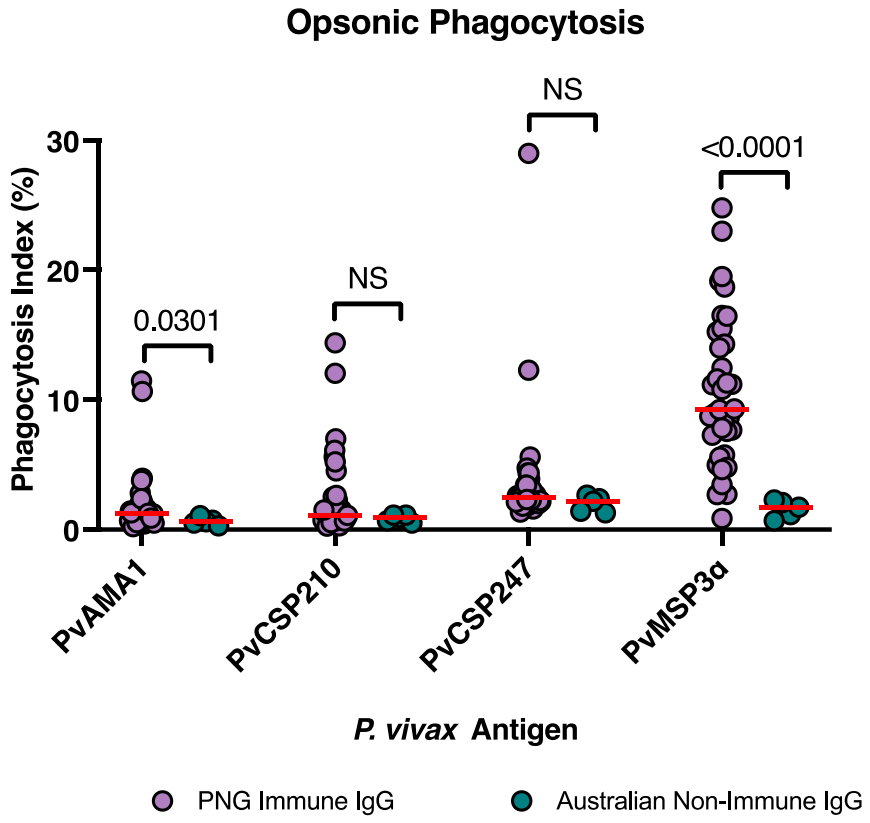


Corresponds with loss of vaccine efficacy

Kurtovic L, et al, BMC Med, 2019, Feng G, et al, BMC Med 2022

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

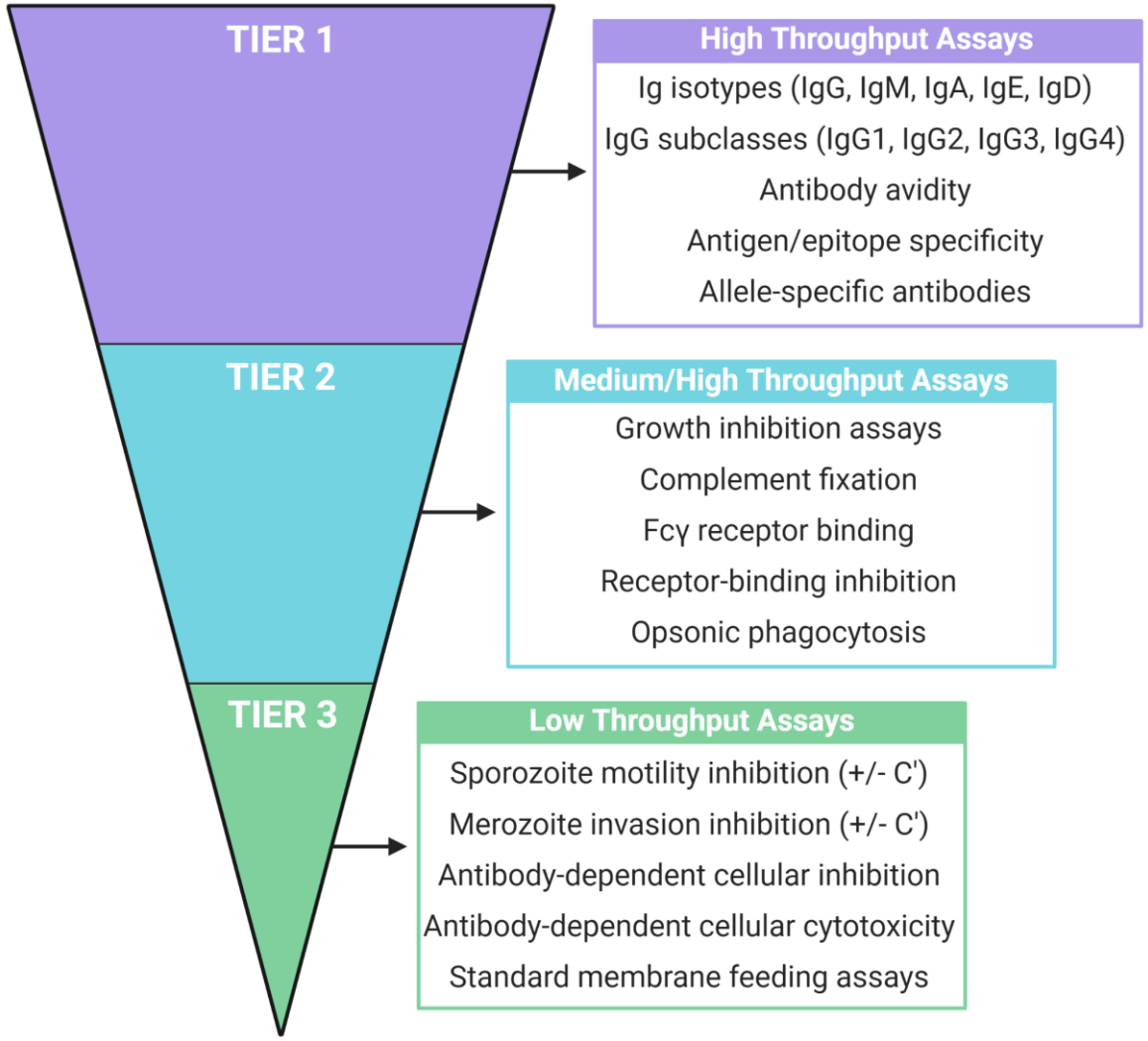




***Opsonic phagocytosis by THP-1 monocyte line of antigen-coated beads**

Multifunctional antibody profiling in vaccine evaluation and development

A complex parasite requiring
a complex approach



Opi, D.H. *et al.* Expert Review Vaccines, 2021

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

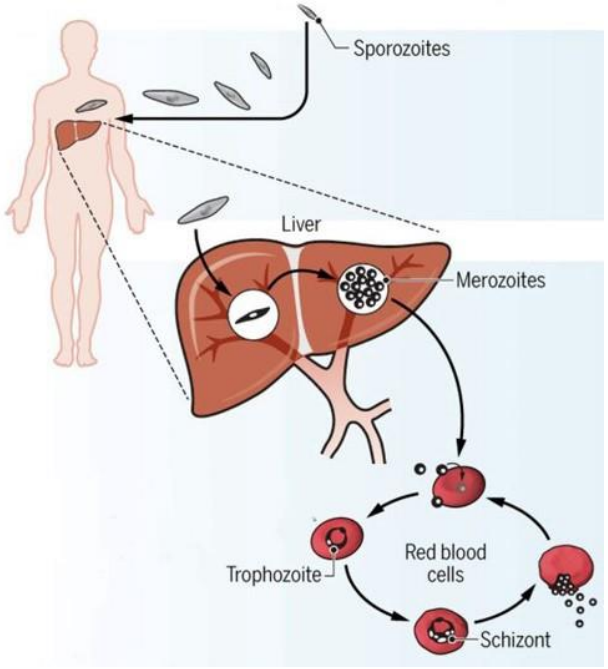
Our Approach:

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

MONASH University
Institute of Pharmaceutical Sciences

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

Chaudhary *et al* 2021 *Nat. Rev. Drug Discov.;* Beeson *et al* 2019 *Sci. Transl. Med.;* Beeson *et al* 2016 *FEMS Micro. Rev.;* Purcell *et al* 2007 *Nat. Rev. Drug Discov.*

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:

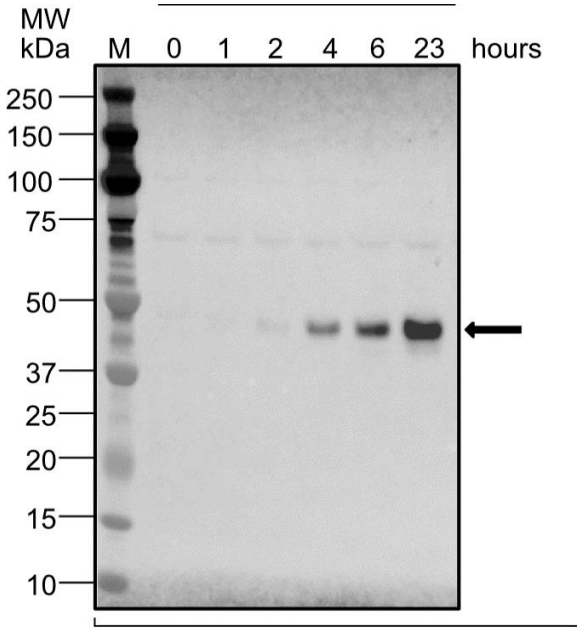
- Vaccine schedule and immunogenicity
- Longevity of immunity

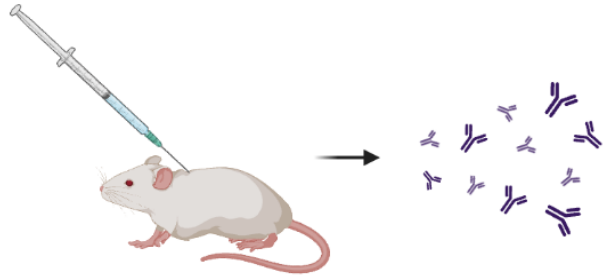
Ongoing work:

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

Future work

- Human trials



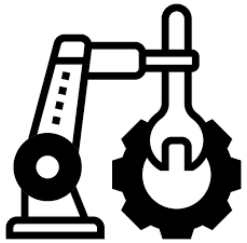


Industry Best Practices – Quality Management System

Incoming goods Quality Control and inventory

Documentation of laboratory processes

Record keeping- Electronic laboratory notebooks



Equipment, validation, calibration & maintenance

Internal Audits and traceability

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.

Capabilities

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

Vaccine implementation

- Health system strengthening
- Optimization of vaccination strategies

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

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

Acknowledgements

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+ team

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PNGIMR Team
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Enmoore Lin
Francesca Baiwog

Griffith University
Danielle Stanisic

Ehime University
Takafumi Tsuboi
Eizo Takashima

Cell Free Sciences
Mathias Harbers

Australian Government
National Health and Medical Research Council

CASS
The CASS Foundation

ACREME
AUSTRALIAN CENTRE OF RESEARCH EXCELLENCE IN MALARIA ELIMINATION

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Burnet
reach for the many

Thank you

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