Optimization of a Surrogate Assay for Efficacy of a Malaria Transmission Blocking Vaccine

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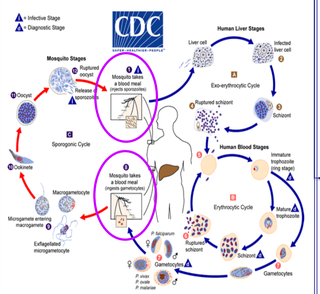
Abstract

Efforts to develop a higher efficacy malaria vaccine are a global priority, but parasites pose a complex biological puzzle for vaccine development. Thus, innovative vaccine strategies are necessary to better combat the complexity of the parasite. Among these, transmission blocking vaccines (TBV) target the mosquito stages of parasite development to inhibit parasite transmission and thereby pursue regional elimination and ultimately eradication of malaria across the globe. The current leading TBV called Pfs230D1-EPA targets Pfs230, a Plasmodium falciparum gamete surface protein that mediates binding of microgametes to red blood cells.

The conventional functional assay of TBV activity is the standard membrane feeding assay (SMFA) , but this biological assay using cultured parasites and laboratory mosquitoes is low throughput and has not been correlated to vaccine efficacy. Here, we are exploiting a panel of human monoclonal antibodies (hmAbs) generated from human volunteers who received Pfs230D1-EPA formulated in Alhydrogel or AS01 adjuvants, to develop a competitive ELISA platform. This assay will quantify epitope-specific serum antibodies along with their features and effector functions. In future, the assay will be assessed as a serum measurement that correlates with vaccine efficacy.

Background



TBV candidate **Pfs230D1-EPA** is in trials in Bamako, Doneguebougou, Bancoumana.

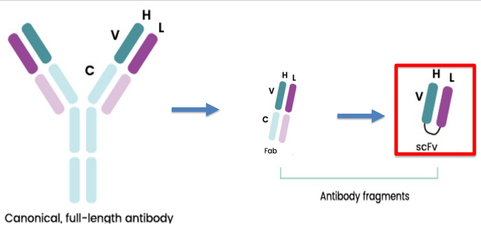
A surrogate assay for Pfs230D1-EPA efficacy is needed to monitor late-phase trials

- SMFA results have not been shown to correlate with vaccine efficacy
- Vaccine trials have been a source to generate vaccine-induced hmAbs.
- hmAb LMIV230-01 binds and lyses gametes in a complement-dependent manner to block parasite transmission through mosquitoes
- The broadly neutralizing epitope on Pfs230D1 defined by LMIV230-01 could be utilized to quantify functional antibody made in response to vaccination.
- An assay measuring functional antibody targeting specific epitopes of Pfs230D1 may be an improved surrogate measure of efficacy for our transmission blocking vaccine

TERMS

- **SMFA** is a labor intensive, low throughput biological assay of serum activity
- **Pfs230**: *P falciparum* gamete 14-domain surface protein required to bind red cells
- **Pfs230D1:** Pfs230 domain 1 targeted by leading TBV Pfs230D1-EPA
- **LMIV230-01:** functional human monoclonal antibody made in response to vaccination with Pfs230D1 that binds highly conserved neutralizing epitope on Pfs230D1 (Coehlo, C. et. al. Nature Communications, 2021)

Strategy Rationale



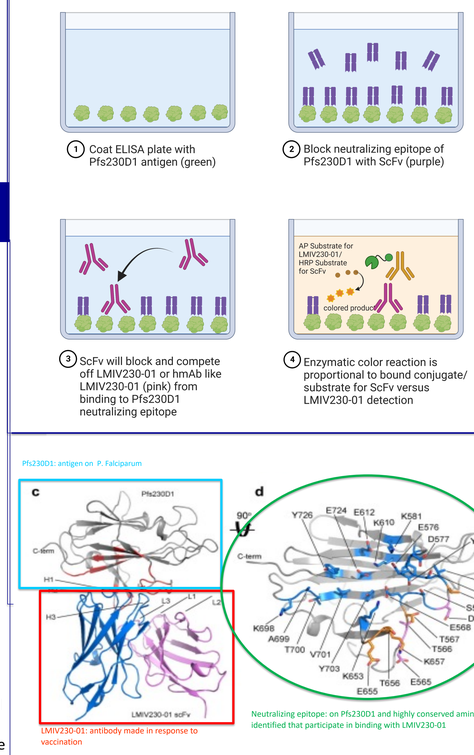
hmAb: LMIV230-01 antibody binds gamete surface Pfs230 and mediates complement dependent lysis to block parasite transmission to mosquitoes.

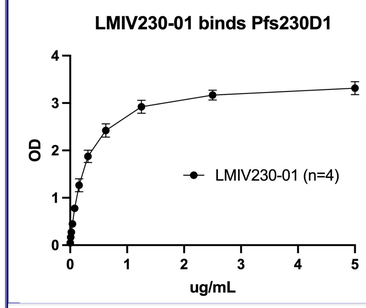
ScFv: Single Chain Variable Fragment specific for LMIV230-01 retains the variable region of the LMIV230-01 full antibody but no longer has Fc portion of antibody

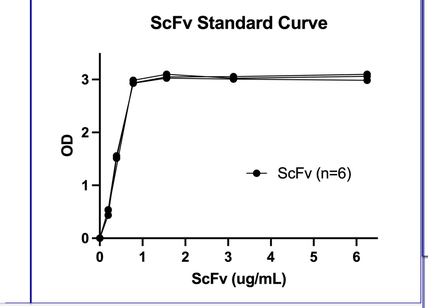
Competitive ELISA: single-chain variable fragment (scFv) against Pfs230 neutralizing epitopes will displace serum antibodies to quantify epitope-specific antibodies

Assay Optimization

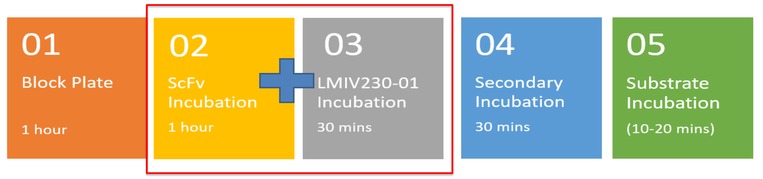
Figure 1: Optimization of competitive ELISA assay using ScFv to block Pfs230D1 neutralizing epitope





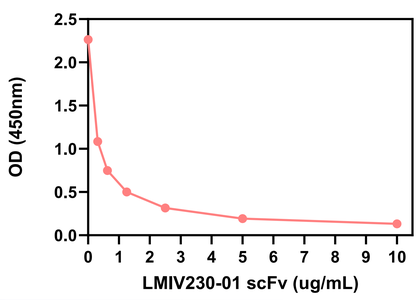


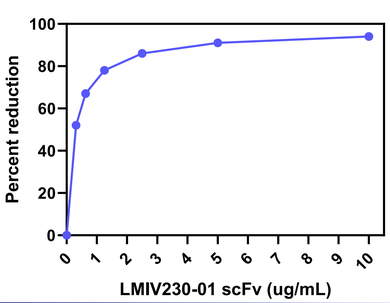
- ELISA plates were coated with Pfs230D1 and then incubated with LMIV230-01 epitope-specific ScFv to block functional epitope of LMIV230-01.
- The competitive ELISA was optimized by a 15-minute pre-incubation of ScFv and co-incubating the ScFv and LMIV230-01 together (combining steps 2 and 3). The main optimization variables of the assay included:
  a) Optimal **coating concentration** of Pfs230D1 antigen coating
  b) Optimal **concentrations** of ScFv and LMIV230-01 to detect competition
  c) Order of addition ScFv and LMIV230-01
  d) Co-incubation versus no wash between ScFv and hmAb incubations?
- Assay optimized through iterative process of changing protocol variables to achieve the greatest competition signal as defined by the reduction in signal when no scFv is present and signal then converted to amount of hmAbs competed by the ScFv.

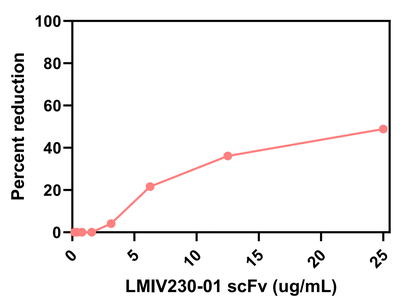


Results Monoclonal Experiments

Figure 2: Demonstration of optimized competitive ELISA with single ScFv



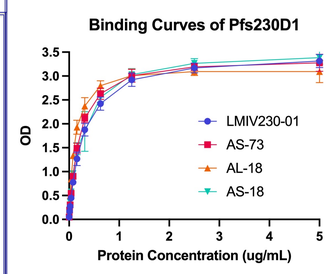




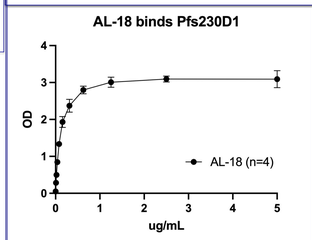
As the goal of this assay is to interrogate epitope-specific antibodies that are present in serum of vaccine trial subjects who received Pfs230D1-EPA formulated in Alhydrogel or AS01 adjuvants, vaccine induced LMIV230-01 functional antibody and its corresponding scFv were used to model the competition ELISA. ELISA format relies on single-chain variable fragments (scFv) against Pfs230D1 neutralizing epitopes to displace serum antibodies. This effect was demonstrated above in the full reduction of LMIV230-01 OD signal in the presence of 10 ug/mL ScFv concentration. This competition assay allows for quantification of epitope in serum through surrogate measure of reduction in signal when no scFv is present. These pilot experiments above with purified antibody (LMIV230-01), LMIV230-01 scFv displaces full-antibody (red, left) with percent reduction in the signal (blue) and LMIV230-01 scFv displaces half of the signal when a mixture of 50% LMIV230-01 and a second hmAb (LMIV230-02) is used (red, right).

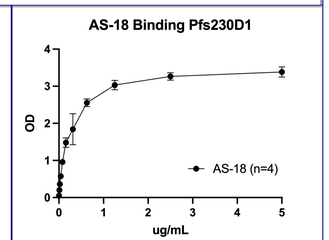
Results Polyclonal Experiments

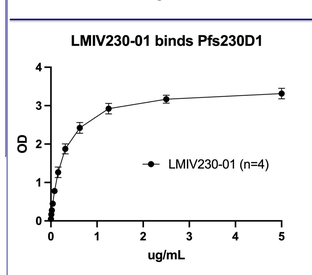
Figure 3: Multiple ScFv platform for competitive ELISA assays on polyclonal sera

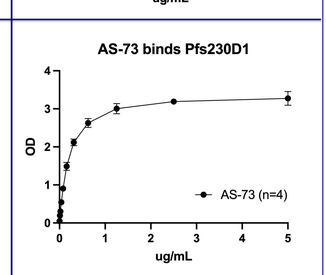


Binding curves run for Pfs230D1-specific functional hmAbs with unique binding epitopes. LMIV230-01 AS-18 and AL-18 bind fully unique epitopes on Pfs230D1 and AS-73 binds overlapping epitope regions. Binding capacity to Pfs230D1 clearly demonstrated for all functional antibodies of interest for the multiple ScFv platform that will next be run with each antibody-specific ScFv.









Future Directions

Successful optimization of the competitive assay demonstrates concentration-dependent epitope-specific competition between ScFv and hmAbs. Efforts now focus on expanding this competition assay to a multiple ScFv platform with various functional hmAbs that bind unique epitopes of Pfs230D1. This polyclonal platform will then be expanded to test with trial serum to compare efficacy measures to SMFA. The assay will then be adapted to measure complement fixing and isotype specific antibodies. These efforts will lay the foundations for standardized assays that can assess correlates of efficacy and durability of Pfs230D1-EPA vaccine.

References and Funding

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