

**Development of recombinant protein based
chemical conjugate malaria vaccines
targeting the pre-erythrocytic stage,
transmission blocking, or both**

David L. Narum, Nicholas MacDonald, David Jones,
Ruth Ellis, Yimin Wu, and Patrick E. Duffy

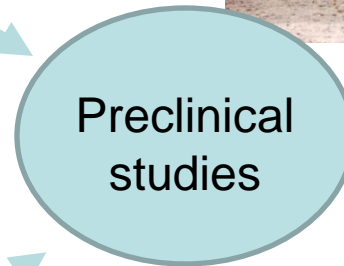
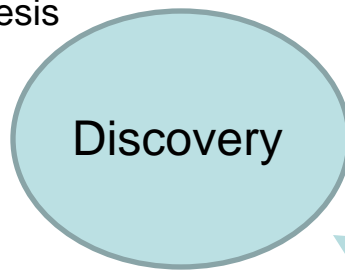
Laboratory of Malaria Immunology and Vaccinology

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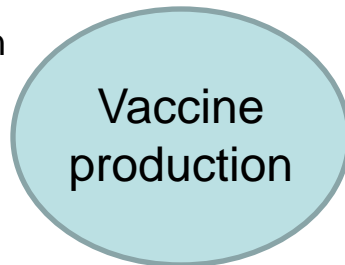
Vaccine Development



- Immunopathogenesis
- Ag & biomarker discovery



- Protein production
- Conjugation
- Formulation
- QA/QC



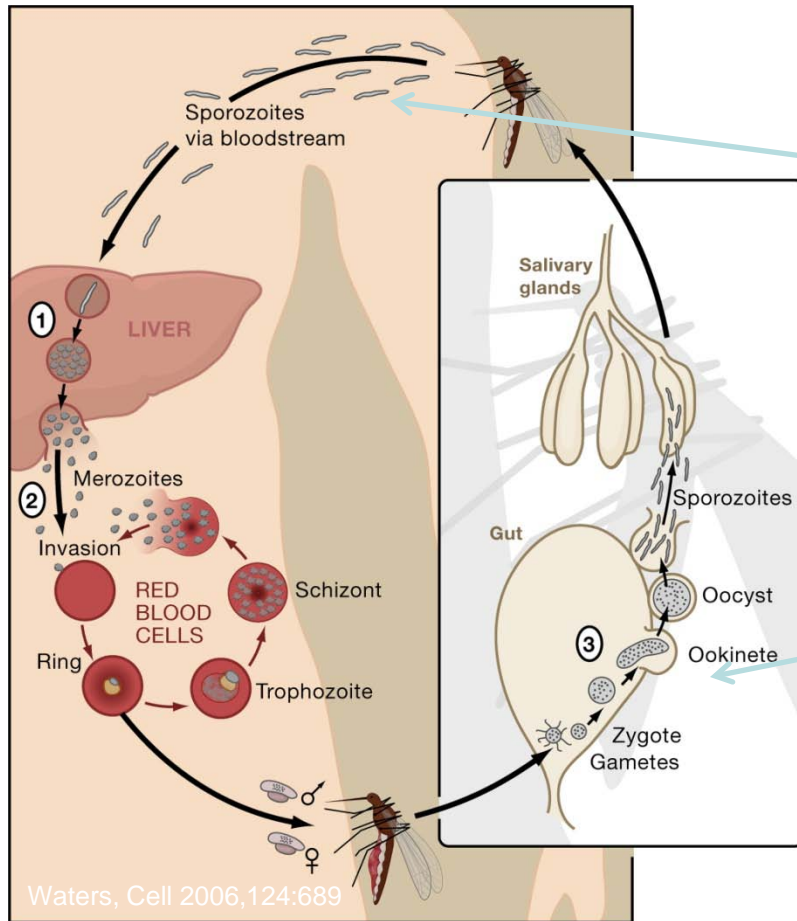
- Animal studies
- GLP tox

- Phase 1 & 2, US & Mali
- Proof of concept

LMIV Mission

- Pre-erythrocytic vaccine
- Transmission Blocking Vaccine
- Pregnancy malaria vaccine
- Merozoite vaccine

Current aims



Pre-clinical development,
pre-erythrocytic vaccine

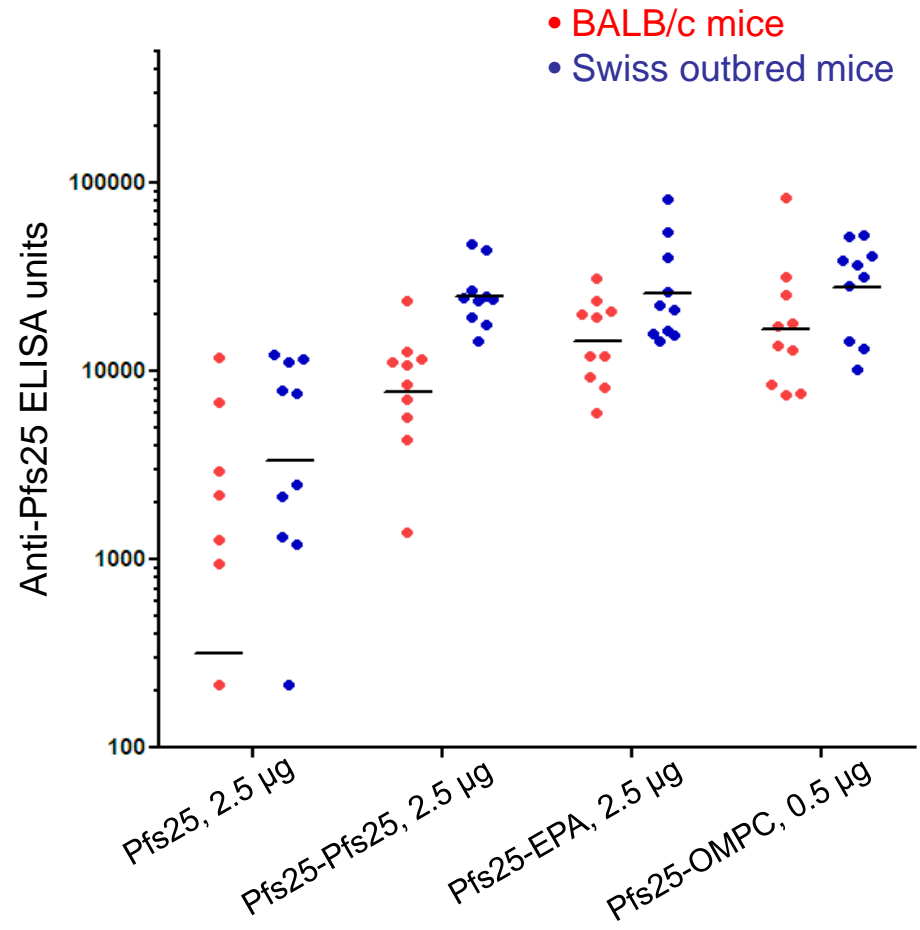
- CSP
 - Sporozoite surface protein
 - Produced in *Pichia pastoris* and in *E. coli*

Clinical development, TBV

- Pfs25
 - Ookinete surface protein
 - Produced in *P. pastoris*
 - ± HIS₆ fusion tag
- Pvs25
- Bio-assay: Membrane feeding

TBV: Pfs25 is a Lead Candidate

- Recombinant Pfs25 (& Pvs25) consistently induces functional antisera assessed by membrane feeding assay
- TB activity observed using purified IgG, with or without complement
- Human serum antibodies against Pfs25 demonstrate TB activity
- Development goal: enhance immunogenicity and longevity:
 - Conjugation
 - Adjuvants



- Immunized protein on Alum IM on D0 & D28
- Abs measured on D42

Transmission Blocking Activity in Mouse Sera

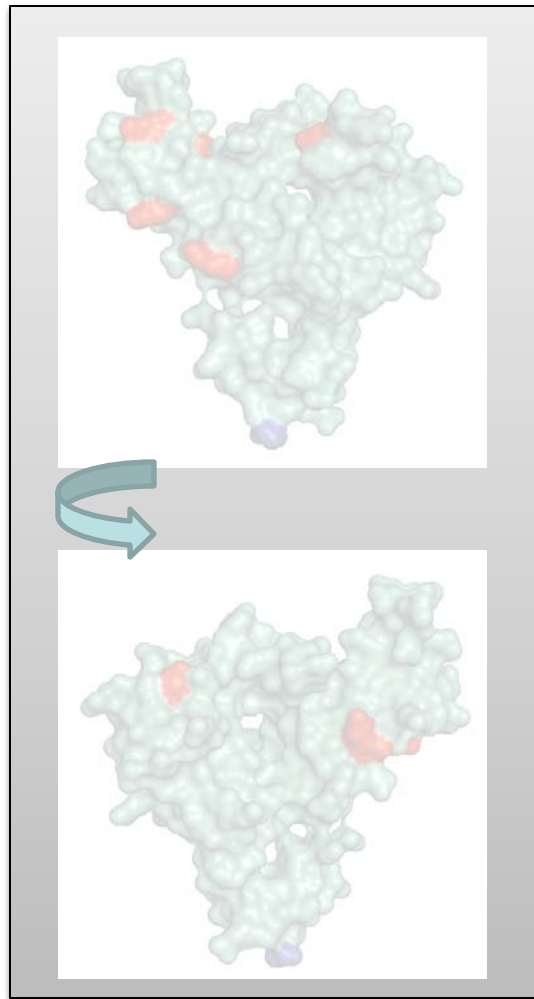
Membrane feeding assay

	Pfs25	Pfs25-EPA
% Inhibition of oocyst count	55.7	99.3
% Inhibition of prevalence	12%	90%

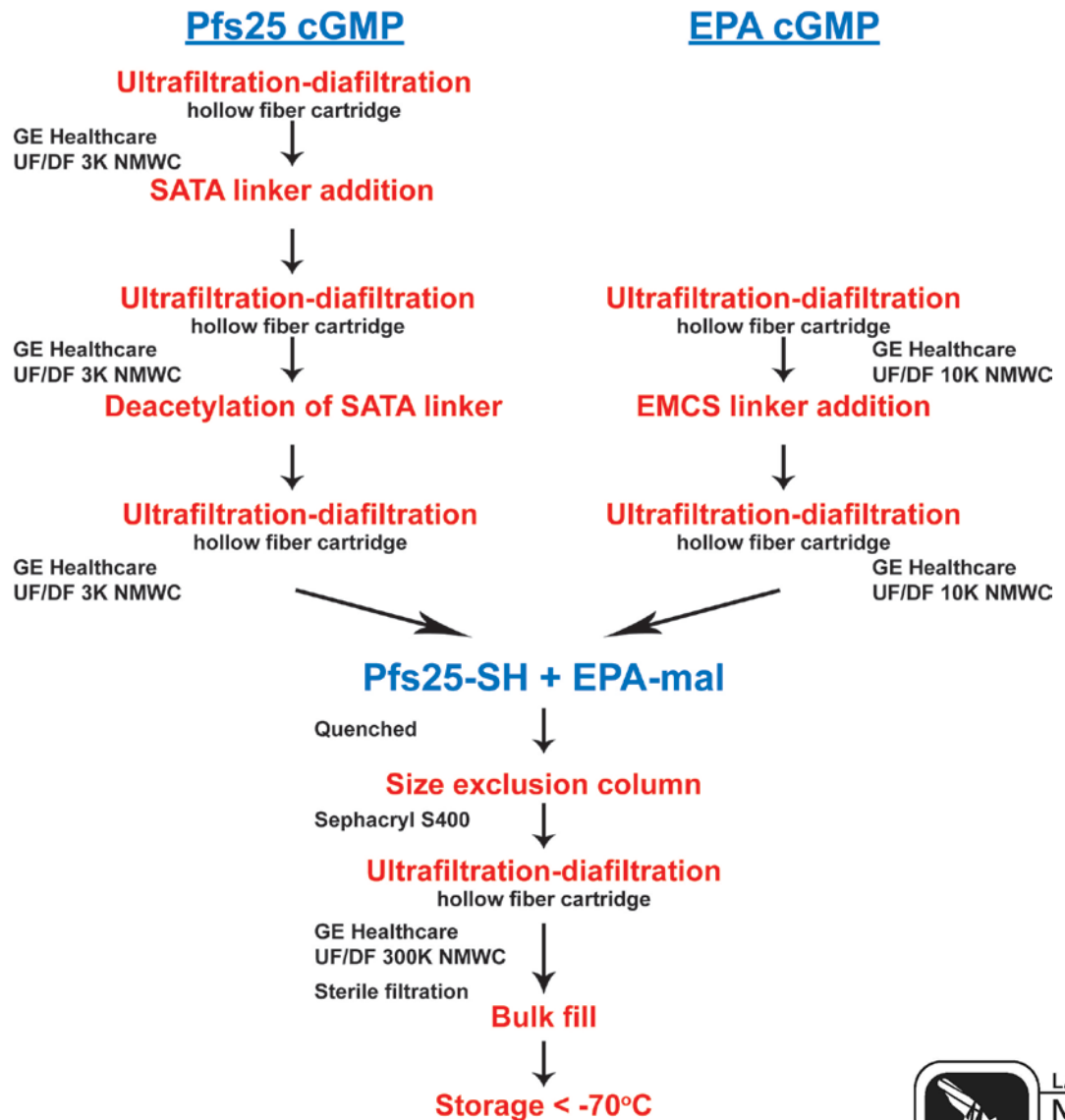
➔ Goal is zero oocysts; percent inhibition is assay correlate

Pilot-scale conjugation process

Pfs25-SH



Pfs25-SH: ~3 linkers
EPA-EMCS: ~7 linkers



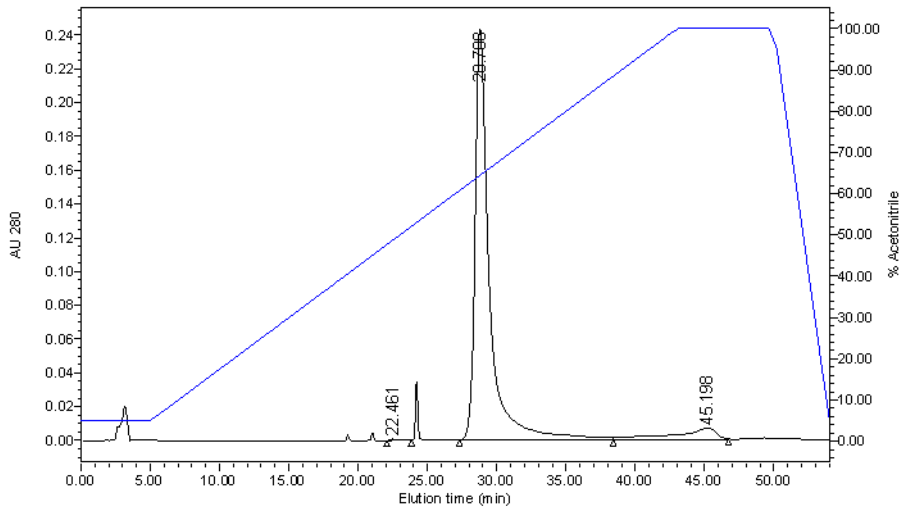
Pfs25-EPA characterization

In process and bulk

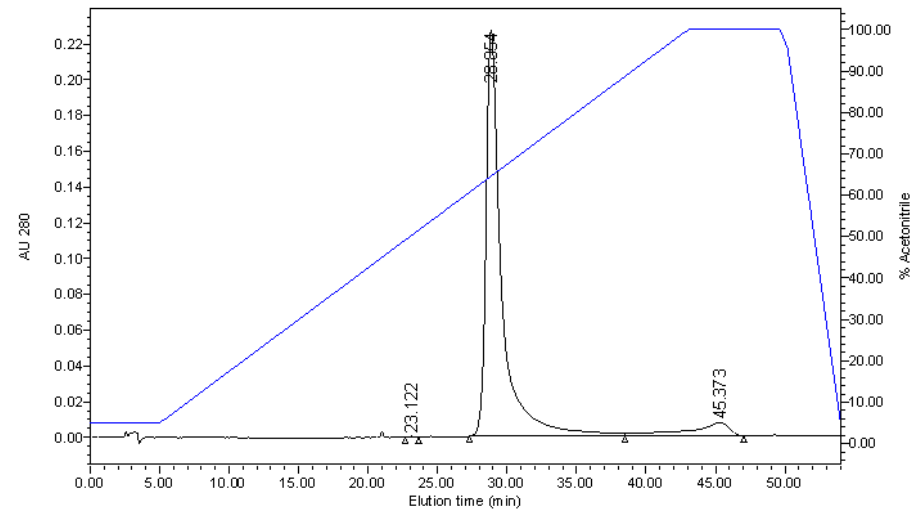
- Appearance
- AAA
- Absorbance 280 nm
- AFM
- BCA
- Coomassie blue stained
 - SDS-PAGE
 - SDS-agarose_GE
- Endotoxin
- Trp fluorescence
- General safety
- HCP
- Linker addition
- pH
- RP-HPLC
- SEC-MALS-HPLC
- Sterility
- Western blot

Comparison of Bulk Pfs25-EPA Conjugates

Reverse-phase HPLC analysis



In-house reference: MV1351

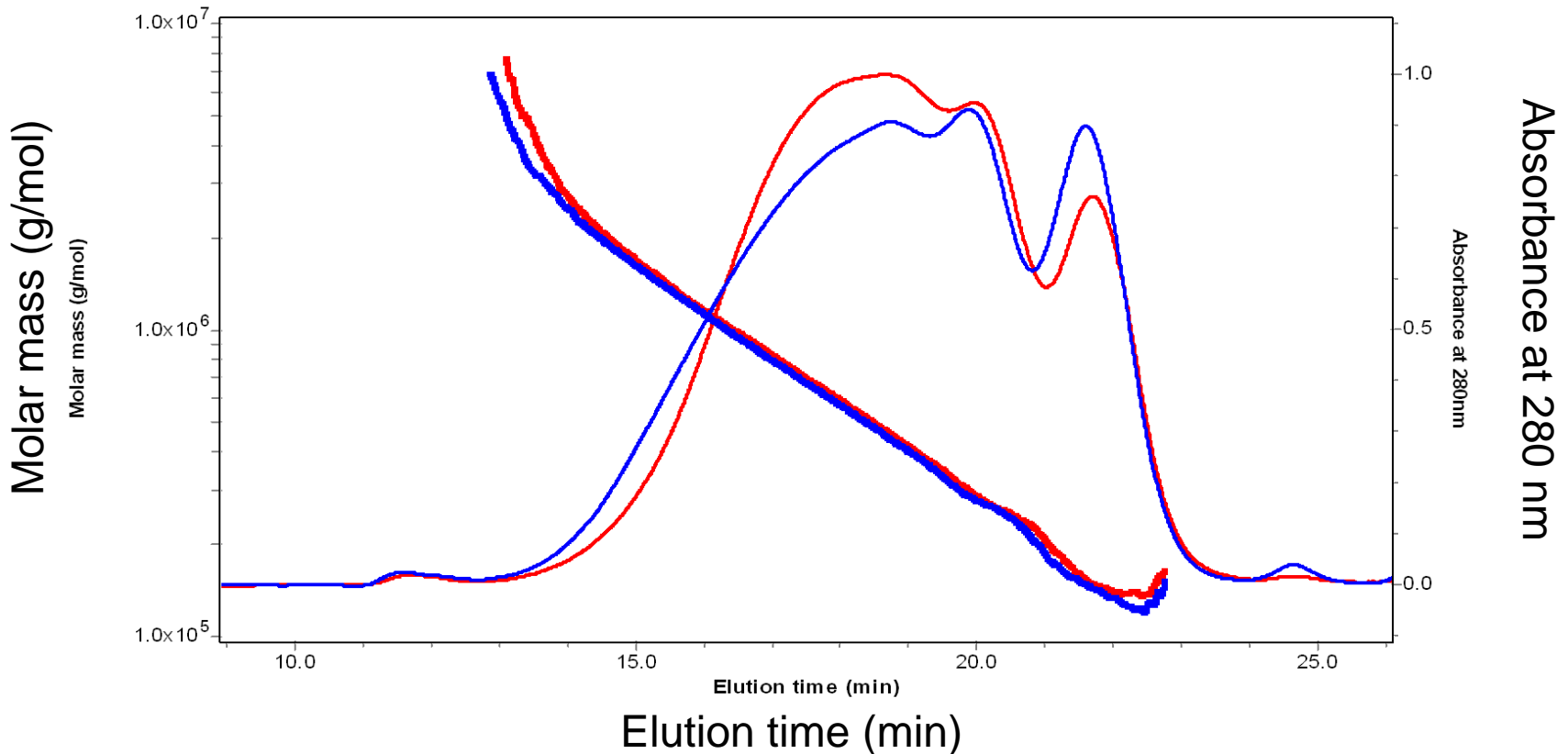


cGMP lot

Comparison of Pfs25-EPA conjugates by SEC-MALS-QELS-HPLC

molar mass vs. time

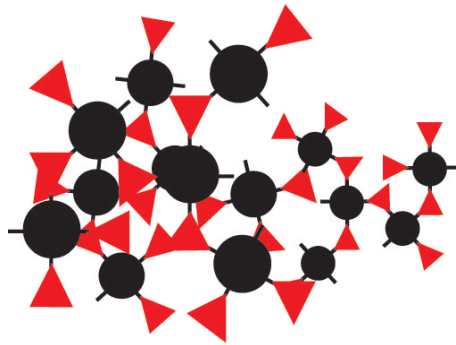
☑ Pfs25rEPA WRAIR 1634 Inj 2 12May10 Configured ☑ Pfs25rEPA MV1351 Inj 2 12May10 Configured



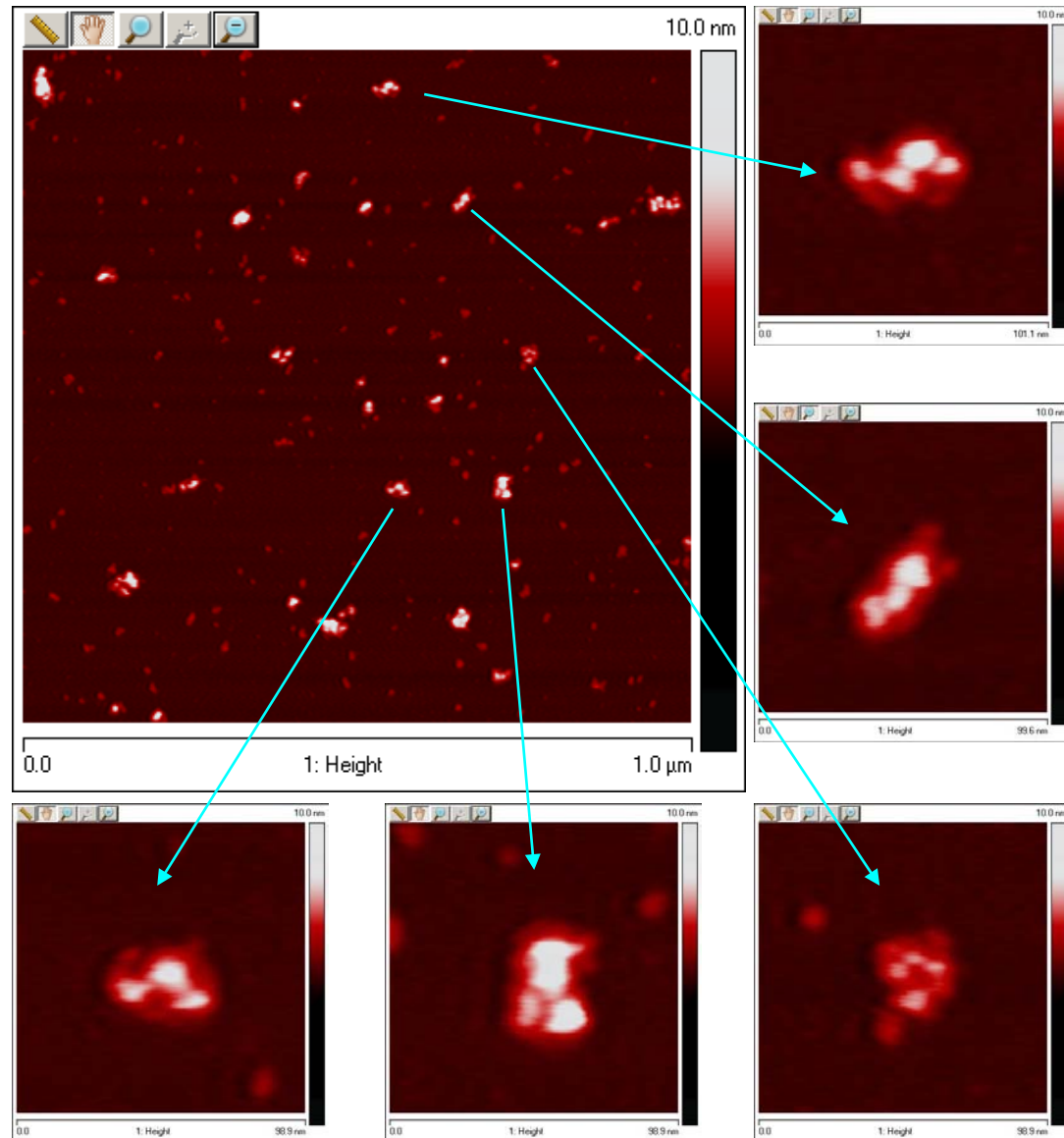
- In-house reference (MV1351), Mw = 558 kDa, Rh= 12.4 nm
- cGMP lot, Mw = 545 kDa, Rh= 12.3 nm

Analysis of Pfs25-EPA conjugate by AFM

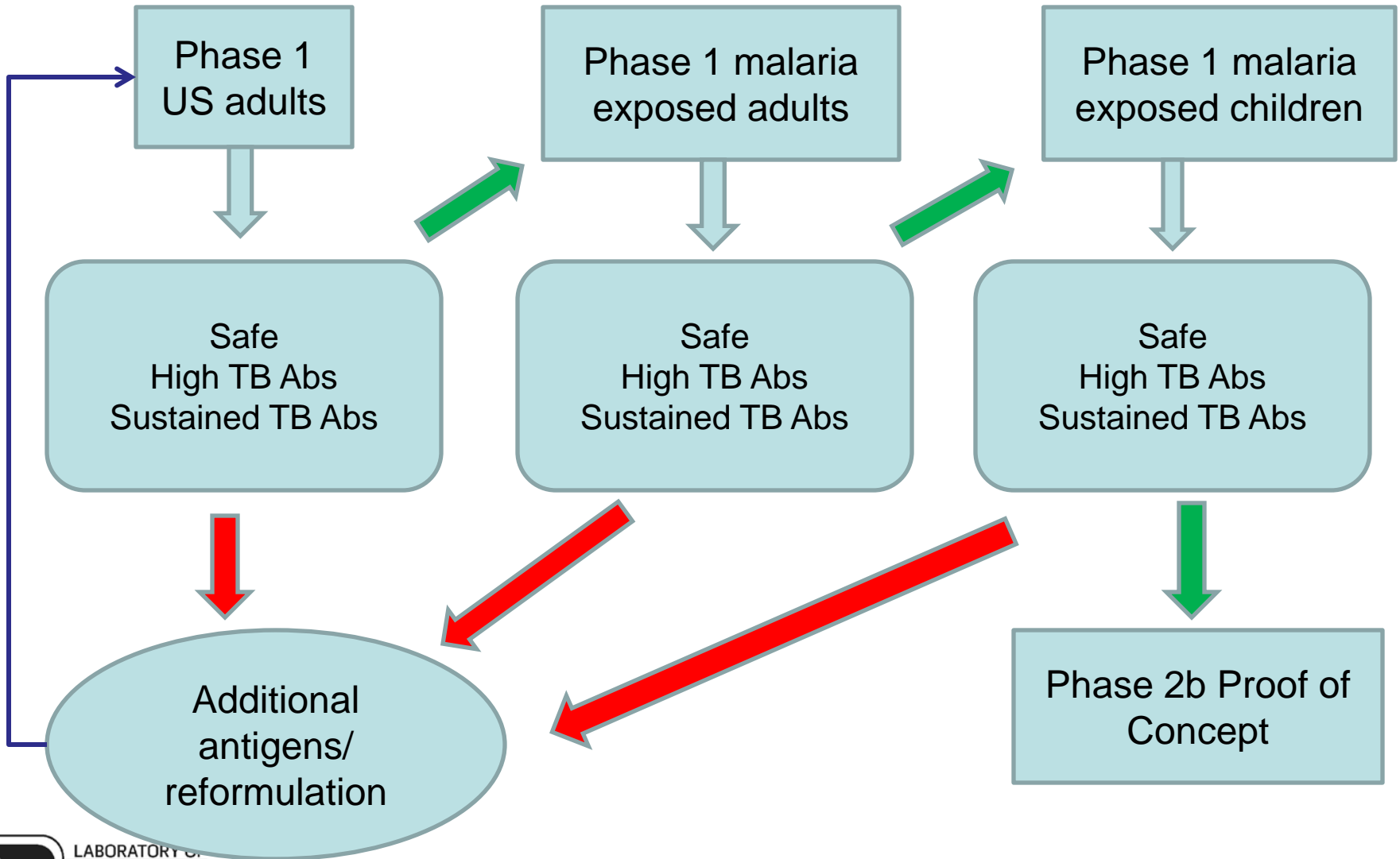
- Pfs25-EPA MV1351
- Conditions:
 - Deposition 10 min. on clean mica in PBS



▼ Pfs25 ● EPA



Clinical development path for TBVs



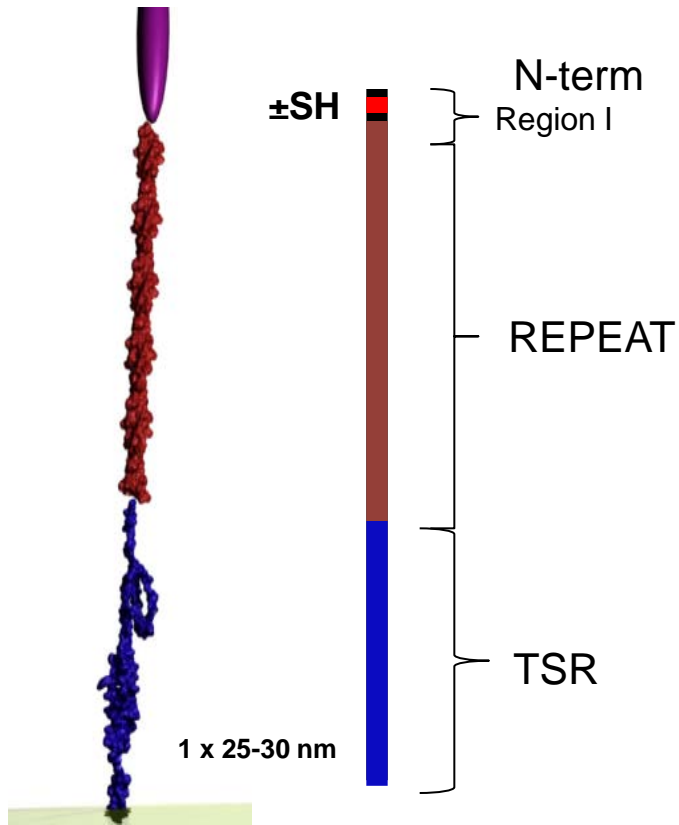
Approaches to Improve TBV Efficacy

- Combine with other target antigen(s)
 - CSP (pre-erythrocytic)
 - Pfs230 (TBV)
 - Novel antigens (pre-erythrocytic and/or TBV)
- Other carriers
 - CRM197
 - Qbeta
 - OMPC

CSP Pre-erythrocytic Vaccine

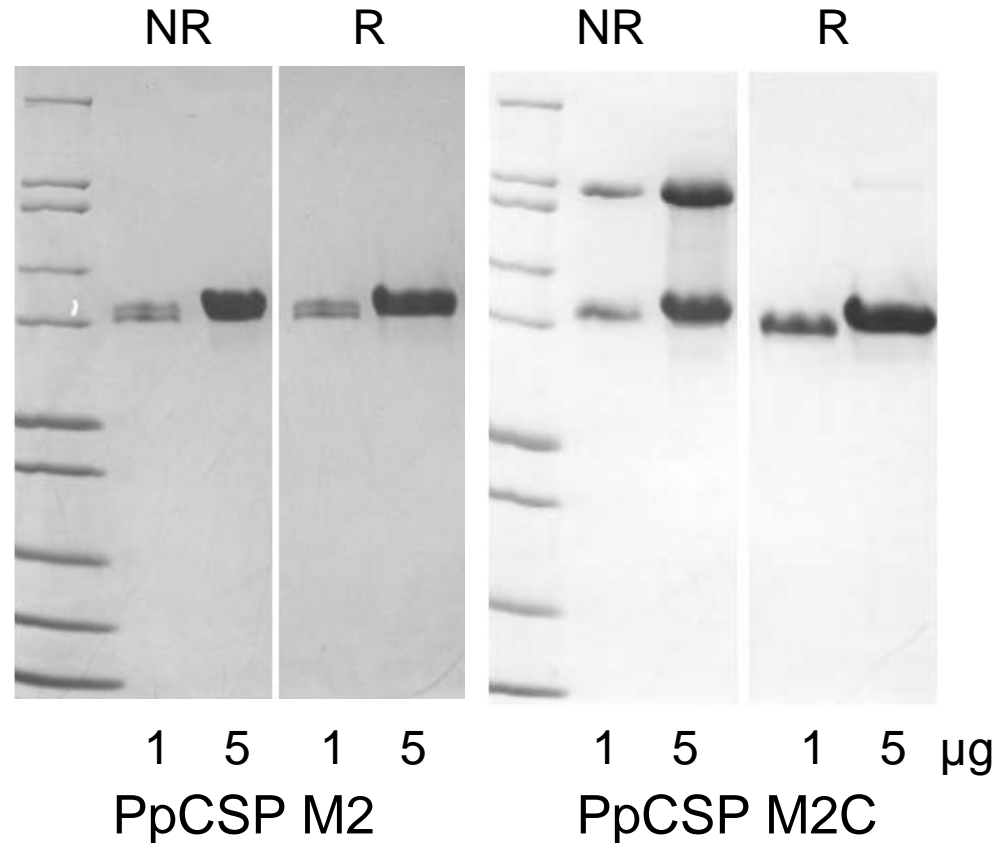
- Identify suitable CS protein construct
 - No HIS tag
 - Scalable process
 - Compatible with conjugation strategies
- Two forms of recombinant CSP produced
 - AN87606 (India Strain)
 - *E. coli* full length without signal sequence and GPI anchor plus HIS₆ tag
 - *P. pastoris* near full-length with and without free thiol
 - NP473175 (3D7)
 - *P. pastoris* near full-length with and without free thiol

Pre-erythrocytic vaccine development recombinant *P. pastoris* CSP

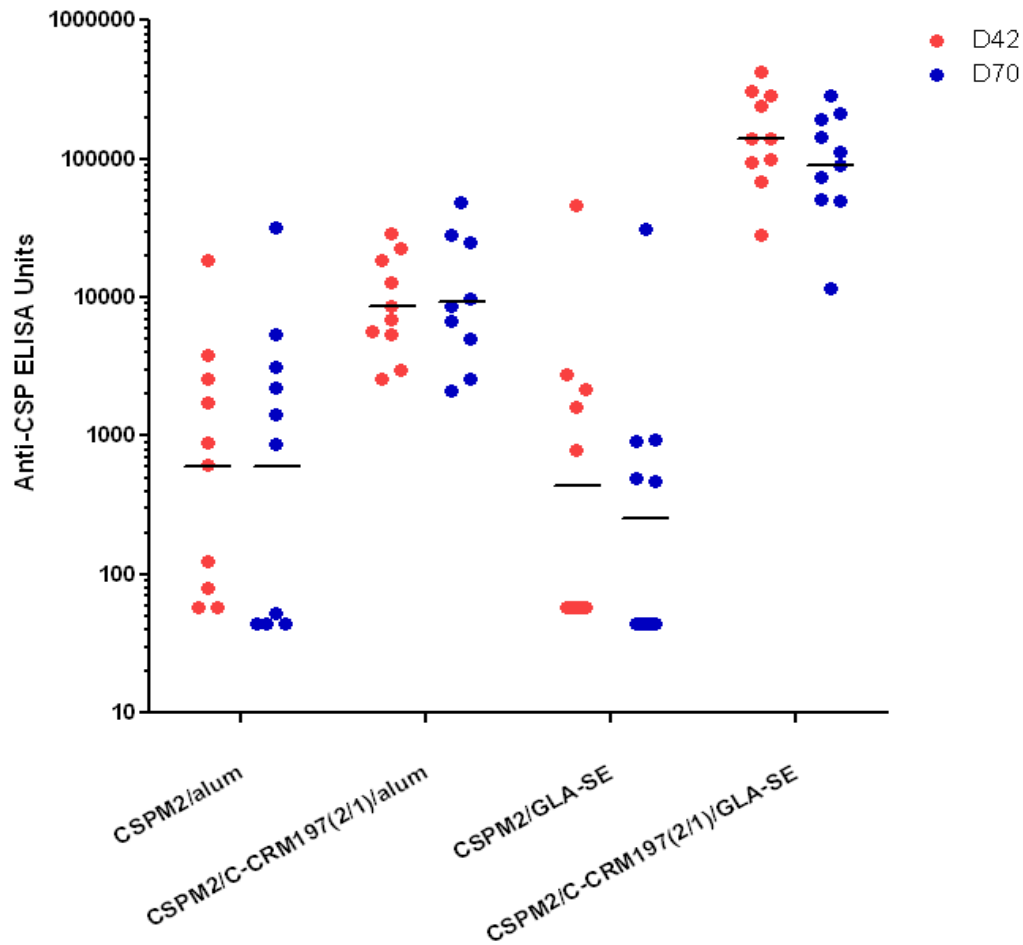


Recombinant PpCSP

Coomassie blue stained SDS-PAGE gels



Enhanced CSP immunogenicity: CRM¹⁹⁷ conjugate, GLA/SE adjuvant



Mice immunized on D0 and D28

Summary

- cGMP pilot-scale production of Pfs25-EPA successful
- Analytical parameters qualified for testing of bulk substance
- Formulation development for Pfs25-EPA ongoing
- Human phase 1 trial planned for 1qtr 2011
- Pre-clinical studies ongoing for enhancing TBV efficacy

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Karine Reiter

Rich Shimp

Yanling Zhang

Immunology Unit

Joan Aebig

Olga Muratova

Bhanumati Ramenini

Formulation

Kelly Rausch

Animal Science Unit

Lynn Lambert

Quality Control Unit

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