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

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8 June 2010
Vaccine Development

Discovery
- Immunopathogenesis
- Ag & biomarker discovery

Preclinical studies
- Animal studies
- GLP tox

Vaccine production
- Protein production
- Conjugation
- Formulation
- QA/QC

Clinical development
- Phase 1 & 2, US & Mali
- Proof of concept
LMIV Mission

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

Pfs25
- Ookinete surface protein
- Produced in P. pastoris
- ± HIS\textsubscript{6} fusion tag

Pvs25
- Bio-assay: Membrane feeding

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

Pre-clinical development, pre-erythrocytic vaccine

Clinical development, TBV
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

- BALB/c mice
- Swiss outbred mice

- Immunized protein on Alum IM on D0 & D28
- Abs measured on D42
Transmission Blocking Activity in Mouse Sera

Membrane feeding assay

<table>
<thead>
<tr>
<th></th>
<th>Pfs25</th>
<th>Pfs25-EPA</th>
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<tbody>
<tr>
<td>% Inhibition of oocyst count</td>
<td>55.7</td>
<td>99.3</td>
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<tr>
<td>% Inhibition of prevalence</td>
<td>12%</td>
<td>90%</td>
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Goal is zero oocysts; percent inhibition is assay correlate
Pilot-scale conjugation process

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

- 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
Clinical development path for TBVs

- Phase 1 US adults
  - Safe
  - High TB Abs
  - Sustained TB Abs

- Phase 1 malaria exposed adults
  - Safe
  - High TB Abs
  - Sustained TB Abs

- Phase 1 malaria exposed children
  - Safe
  - High TB Abs
  - Sustained TB Abs

- Additional antigens/reformulation

- Phase 2b Proof of Concept
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 HIS\textsubscript{6} 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

Coomassie blue stained SDS-PAGE gels

Recombinant PpCSP

1 x 25-30 nm

N-term Region I

REPEAT

TSR

Plassmeyer et al., 2009 JBC 284:26951
Enhanced CSP immunogenicity: CRM\textsuperscript{197} 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
Acknowledgements

Molecular Biology Unit
Merrit Hickman

Conjugation Unit
Beth Chen
Christopher Rowe

Process Development Unit
Martin Burkhardt
Jacqueline Glen
Raul Herrera
Dominique Jones
Vu Nguyen
Harold Obiakor
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
Daming Zhu

Former MVDB Chief
Louis Miller

NIH/NIBIB
Svetlana Kotova
Albert Jin

Infectious Disease Research Institute
Steve Reed

WRAIR BioProcess Facility
Jay Woods