PRIMALVAC: Pregnancy Associated Malaria Anti-Disease Vaccine

Dr Benoît Gamain
- 5 plasmodial species are commonly infected humans

  *Plasmodium falciparum*

  *Plasmodium vivax*

  *Plasmodium malariae*

  *Plasmodium ovale*

  *Plasmodium knowlesi*

- *Plasmodium falciparum* is responsible for the most severe clinical cases and deaths

- In 2011, WHO reported an estimated 216,000,000 cases of Malaria worldwide and 655,000 subsequent deaths from the disease
Facts about Malaria and Pregnancy

• 50 million women become pregnant in Malaria endemic areas yearly
  (Half in Sub-Saharan Africa) where *P. falciparum* is endemic
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• In malaria-endemic areas, malaria during pregnancy may account for:
  – Up to 15% of maternal anemia
  – 5–14% of all low birth weights in children worldwide
  – 35% of all preventable low birth weights in children worldwide
  – Low birth weights can lead to premature births and intrauterine growth retardation
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• 200,000 – 363,000 neonates deaths worldwide each year attributed to malarial infection during pregnancy
Malaria Prevention and Treatment during Pregnancy

WHO recommends a package of interventions for the prevention and control of malaria during pregnancy:

- The use of insecticide treated nets (ITNs) to prevent infection
- Effective case management for malaria illness and anemia
- Intermittent Preventive Treatment (IPT) to prevent asymptomatic infections among pregnant women living in areas of moderate or high *P. falciparum* transmission
- Current recommended drug: sulfadoxine-pyrimethamine administered at least two times during pregnancy

(new WHO recommendations: providing at least 3 doses during pregnancy at each antenatal care visit in the 2nd and 3rd trimester)
• Within the infected erythrocyte, the parasite successively:
  expresses, exports and presents parasite-derived proteins at the surface of the cell.

• Alteration of the morphological and physical properties of the red blood cell

• Represent an Achille heel allowing immune system recognition

• Evasion strategy → antigenic variation
Antigenic variation of PfEMP1

- PfEMP1 is a major parasite virulence factor
- Display extensive antigenic variation
- Encoded by a highly diverse family of \( \text{var} \) genes
- Only one \( \text{var} \) gene expressed at a time
- Switching to another \( \text{var} \) gene allow immune escape

The parasite is concurrently changing host receptor recognition and tissue tropism

→ The properties of the exposed PfEMP1 variant may determine the outcomes of the disease
Antigenic variation

- 100 to 300 Kda proteins
- 2-7 DBL (Duffy Binding Like) domains
- 0-2 CIDR (Cysteine-rich interdomain region)
- Sequence homologies (and functions):
  - 6 types of DBL domains
  - 3 types of CIDR domains

CD36 ICAM-1 PECAM

Var A
Var B
Var C
Var D

PfEMP-1

• 100 to 300 Kda proteins
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• 0-2 CIDR (Cysteine-rich interdomain region)
• Sequence homologies (and functions):
  - 6 types of DBL domains
  - 3 types of CIDR domains
• Cytoadhesion to the endothelium to avoid transit and clearance in the spleen

• Sequestration and the clogging the microvasculatures

Hypoxic capillary vessels creates a favorable environment for parasite growth

→ rupture of the microvasculature wall and to the damage/necrosis of surrounding tissues
**P. falciparum** sequestration and severe malaria

**BRAIN (children)**

Silamut et al Am. J. Pathol 1999

**PLACENTA**

Smith et col. Nat. med. 1999

- Microvascular occlusion and anoxia
- Preterm delivery
  - Coma
  - Fœtal hypotrophy
  - Increase newborn mortality
  - Anemia and maternal mortality
- Death
**P. falciparum Pregnancy Associated Malaria**

**Microvasculature**
- Endothelium
- Specific antibody
- CD36-binding PEMP1
- Sequestered infected erythrocyte
- Microvasculature

**Placenta**
- Maternal Circulation
- CSA-binding infected erythrocyte
- Other PEMP1 proteins?
- Antibodies block placenta adhesion and/or trigger removal by monocytes

**Primigravidas: No Immunity to PAM**
- Placental isolates bind to CSA.
  - Fried and Duffy, Science, 1996
- Increased newborn mortality (200,000)
- Increased maternal morbidity and mortality (10,000)

**Multigravidas: Immunity to PAM**
- Protection correlated to inhibitory and cross-reactive antibodies that block adhesion of placental parasites to CSA.
  - Fried et al., Nature, 1998
- Develop vaccine and therapeutics

**Identify and characterize ligand(s)**
A single var gene is involved in CSA adhesion

- Var2CSA is specifically expressed in placental and CSA-binding parasites


![Image of gel electrophoresis](image1)

![Image of real-time PCR analysis](image2)

Real-time PCR analysis across 50 IT/FCR3 var genes (Smith et al)
A single var gene is involved in CSA adhesion

- Var2CSA disruption impairs parasite binding to CSA and placental cells


⇒ Var2csa is essential for CSA specific cytoadhesion of FCR3 infected erythrocytes.
A single var gene is involved in CSA adhesion

- Var2CSA disruption impairs parasite binding to CSA and placental cells


⇒ Var2csa is essential for CSA specific cytoadhesion of FCR3 infected erythrocytes.
Var2CSA orthologs are conserved among parasite isolates and strains.  

14 different full length Var2CSA sequences

<table>
<thead>
<tr>
<th>Var2CSA</th>
<th>DBL-1X</th>
<th>DBL-2X</th>
<th>DBL-3X</th>
<th>DBL-4ε</th>
<th>DBL-5ε</th>
<th>DBL-6ε</th>
<th>ATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIN</td>
<td>69.5</td>
<td>67.3</td>
<td>82.3</td>
<td>84.7</td>
<td>79.7</td>
<td>53.1</td>
<td></td>
</tr>
<tr>
<td>MAX</td>
<td>89.2</td>
<td>89.7</td>
<td>91.6</td>
<td>95.4</td>
<td>91.3</td>
<td>82.2</td>
<td></td>
</tr>
</tbody>
</table>

(percentage identity)

Full length var2CSA binds with High affinity and specificity to CSA


EMBL Grenoble

SAXS reveals that full length var2CSA has a compact organization
N-terminal multi-domain constructs bind placental CSPG with high affinity

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_D$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D7-DBL2X</td>
<td>5.3</td>
</tr>
<tr>
<td>FCR3-DBL3X</td>
<td>344</td>
</tr>
<tr>
<td>CYK39-DBL5ε</td>
<td>150</td>
</tr>
<tr>
<td>3D7-DBL6ε</td>
<td>92</td>
</tr>
<tr>
<td>3D7-DBL1X-2X</td>
<td>0.77</td>
</tr>
<tr>
<td>3D7-DBL1X-CIDR</td>
<td>1.58</td>
</tr>
<tr>
<td>3D7-DBL1X-3X</td>
<td>0.58</td>
</tr>
<tr>
<td>3D7-DBL1X-5ε</td>
<td>0.115</td>
</tr>
<tr>
<td>3D7-DBL1X-6ε</td>
<td>0.127</td>
</tr>
</tbody>
</table>

3D7-DBL1X-2X is the shortest var2CSA recombinant protein with an affinity and a specificity for CSPG comparable to the full length var2CSA

Antibodies against var2CSA, cross-react and block the adhesion to CSA


- **Dose dependent inhibition with anti DBL1-6 rabbit 2 plasma / purified IgG**
- **No or low cross-inhibition to other CSA binding strains**

### Table

<table>
<thead>
<tr>
<th>CSA-binding lines</th>
<th>Control lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR3</td>
<td>NF54</td>
</tr>
<tr>
<td>MFI</td>
<td>MFI</td>
</tr>
<tr>
<td>FB Mouse1 anti-DBL1-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Mouse2 anti-DBL1-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Mouse3 anti-DBL1-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Rabbit1 anti-DBL1-6</td>
<td>1/50</td>
</tr>
<tr>
<td>FB IgG Rabbit1 anti-DBL1-6</td>
<td>0.11mg/ml</td>
</tr>
<tr>
<td>FB Mouse1 anti-DBL4-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Mouse2 anti-DBL4-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Mouse3 anti-DBL4-6</td>
<td>1/25</td>
</tr>
<tr>
<td>FB Rabbit1 anti-DBL4-6</td>
<td>1/50</td>
</tr>
<tr>
<td>FB IgG Rabbit1 anti-DBL4-6</td>
<td>0.15mg/ml</td>
</tr>
</tbody>
</table>

### Graph

- Adjusted MFI = MFI Final bleed - MFI Preimmune

### Notes

- No or low cross-inhibition to other CSA binding strains
- Dose dependent inhibition with anti DBL1-6 rabbit 2 plasma / purified IgG
Criteria for Placental Adhesion Ligand(s) Vaccine Candidates

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Gene should be specifically transcribed in CSA binding parasite isolates or infected placentas</td>
<td>Yes</td>
</tr>
<tr>
<td>2- The protein should bind CSA (or other placental adherence receptors)</td>
<td>Yes</td>
</tr>
<tr>
<td>3- Genetic disruption should validate the importance of that gene in CSA adhesion</td>
<td>Yes</td>
</tr>
<tr>
<td>4- The protein, or epitopes within it, should be conserved among parasite isolates from different parts of the world</td>
<td>Yes/No</td>
</tr>
<tr>
<td>5- Gender and gravidity specific recognition by pregnant mother sera</td>
<td>Yes</td>
</tr>
<tr>
<td>6- The protein should be at the surface of CSA binding-infected erythrocytes from laboratory and infected placentas</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Var2CSA : leading candidate for vaccine and therapeutic strategies
Develop a vaccine to prevent PAM

- **PRIMALVAC**: Develop a var2CSA based vaccine inducing long lasting or rapidly boosted cross reactive and inhibitory antibodies. Budget: €6,864,050 (incl. 50% co-funding by EVI/Irish Aid, Inserm and INTS).

- Recombinant forms of var2CSA will be generated in different expression systems, and their activity as immunogens that elicit functional and cross-reactive antibodies against placental parasite will be assessed.
PRIMALVAC - Development / Next steps

• Expression screening by Pfenex in Pseudomonas fluorescens

• Expression screening by GTP Technology of 4-5 protein variants in *P. pastoris, L. lactis, E. coli* and CHO cells

• Characterisation of the proteins in CSA binding assays

• Selection of the best expression system and best protein variant, according to best yield, best immunogenicity in small animal models and best protein characteristics in in vitro assays. Testing and selection of the adjuvant

• Transfer of the selected variant/system to a CMO for process development and GMP production

• Selection of sponsor for clinical trial and of clinical trial sites for phase Ia/b in Europe and Africa
Acknowledgments

ATIP-Avenir team « Severe malaria pathogenesis »

Anand Srivastava (Post-Doc; FRM)
Sébastien Dechavanne (Technician, Primalvac)
Stéphane Gangnard (Post-Doc; Primalvac)
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Nathalie Imbault (QA Director)
Sten Larsen (Finance Director)

Former members: Nicola Viebig, Pablo Fernandez

- Funding:

EVI / BMBF through KfW PRIMALVAC
ATIP-Avenir / Sanofi
FP7 EU funded project « PreMalStruct » (Scientific Director)
DIM île de France maladie infectieuse
Appel d’offre actions structurantes paris diderot
Labex GR-Ex is funded by the program “Investissements d’avenir”
THANK YOU

PRIMALVAC - Project Team