### Protocol Amendment

**Title:** Assessment of the Safety and Immunogenicity of three Formulations of the Recombinant *Pichia pastoris* Apical Membrane Antigen 1 (PIAMA-I-FVO[25-45]), Blood-stage Malaria Vaccine in Healthy Dutch Adult Volunteers: a Phase 1, Single-Blind, Randomised, Dose-escalating, Unicentre trial.

**Trial code:** AMA-I_1_03  
**Version No.:** 1  
**Effective Date:** 16/05/05

## Signatures

I have read the amendment and agree that the trial will be conducted according to the procedures described.

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<th>Function</th>
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<tr>
<td>Principal Investigator</td>
<td>Prof Robert Sauerwein</td>
<td>24/6/05</td>
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<tr>
<td>Investigator</td>
<td>Dr Meta Roestenberg</td>
<td>24/6/05</td>
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<tr>
<td>E.M.V.I. Project manager</td>
<td>Dr Hildur E. Blythman</td>
<td>24/6/05</td>
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<td>E.M.V.I. Director of Clinical and Regulatory Affairs</td>
<td>Dr Odile Leroy</td>
<td>22/6/05</td>
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_A2_PAMA1_050516_
2. Justification of the amendment

The paragraphs modified by this amendment are described below. Other paragraphs remain unchanged.

7.2.2. Cellular Immune response:

2. Parameters to be measured, Method and Timing of Measurement

Cytokine production will be assayed by ELISPOT for the cytokines IL-4 and IFNγ

Change to:

Cytokine production will be assayed by ELISPOT for the cytokines IL-5 and IFNγ

Justification for the change:

- The IL-4 production of Polymorphic Blood Mononuclear Cells (PBMC's) measured in the ELISPOT is low as compared to the IL-5 production. Therefore, due to the low numbers of spots, the read-out will be less reproducible and the intra-assay variability will be higher as compared to IL-5.
- ELISPOT results from this study can easily be compared with the IFNγ/IL-5 data published by researchers of GSK[1], [2], one of the co-investigators in this study.


3. Appendixes
   1. Amendment List

<table>
<thead>
<tr>
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<th>EC submission</th>
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2. Amended protocol