Vaccines based on Recombinant Proteins and Adjuvant Systems: GSK's malaria vaccine candidate as a case study.

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Vaccine workshop

M.-C. Uwamwezi, Senior scientist Regulatory Affairs, GSK Biologicals
Outline

- Case-study: GSK Malaria vaccine candidate : RTS,S/AS01
  - Rationale for RTS,S vaccine antigen design
  - Selection and justification of Adjuvant system
  - Rationale for vaccine presentation

- Characterization of vaccine antigen

- Characterization of Adjuvant System

- Compatibility of vaccine antigen and Adjuvant system

- Quality control of clinical lots

- Quality control of commercial lots

- Conclusion
GSK’s Malaria Vaccine Candidate

1) The RTS,S antigen

- **Sporozoites**
- **Liver-Stage Parasites**

**CircumSporozoite Protein (CSP)**
- Major surface protein of the sporozoite
- Involved in binding of sporozoite to liver cells
- Shown to be protective in animal models

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**Co-Expression of RT-S (Fusion Protein) and HBs Protein in S. cerevisiae that assemble into mixed particles**

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(2) Hoffman S.L. ASM Press, 1996; pages 15-76
(3) Cerami C et al. Cell, 70; pages 1021-1033

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*The images used are graphical representations. They are not the molecular or the exact shape of the elements described. The amounts of each element do not represent the exact composition of the vaccine.*
GSK’s Malaria Vaccine Candidate
2) A specific Adjuvant System to induce protection against *P. falciparum* infection

Human challenge model at the Walter Reed Army Institute of Research

<table>
<thead>
<tr>
<th></th>
<th>Protected</th>
<th>Infected</th>
<th>VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTS,S / AS02</td>
<td>14</td>
<td>30</td>
<td>32% (95% CI: 20; 47)</td>
</tr>
<tr>
<td>RTS,S / AS01</td>
<td>18</td>
<td>18</td>
<td>50% (95% CI: 35; 66)</td>
</tr>
</tbody>
</table>

VE AS01 vs VE AS02: p = 0.11

*K. Kester et al, JID, 2009*

**RTS,S/AS01 protects African children against malaria disease:**

53% VE for 8 M in 5-17 M old children in Kenya & Tanzania

*Bejon et al 2008 NEJM 359; 24: 2521-32*

The most promising formulation is the one that consistently induced superior humoral and CMI responses in preclinical and clinical testing, it is now evaluated in Phase III.

**AS02:** MPL, QS21, o/w emulsion based Adjuvant System

**AS01:** MPL, QS21, liposomes based Adjuvant System
RTS,S Antigen and AS01 have to be stored in separate Final Containers and mixed extemporaneously.

Stability evaluation of RTS,S+ AS01

2 M at 2-8

Aggregation

Degradation

2 vials approach
The RTS,S antigen is a well-characterized protein (*ICH guideline Q6B*).

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Test</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural Characterization and Confirmation</strong></td>
<td>Terminal amino acid sequence</td>
<td>Edman degradation</td>
</tr>
<tr>
<td></td>
<td>Peptide map</td>
<td>LC-MS</td>
</tr>
<tr>
<td></td>
<td>Sulfhydryl group(s)</td>
<td>MS</td>
</tr>
<tr>
<td><strong>Physicochemical Properties</strong></td>
<td>Molecular weight</td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td>Electrophoretic patterns</td>
<td>SDS-PAGE reducing and non-reducing conditions</td>
</tr>
<tr>
<td></td>
<td>Spectroscopic profiles</td>
<td>Fourier-Transformed IR spectroscopy</td>
</tr>
<tr>
<td><strong>Immunological Properties</strong></td>
<td>Ab reactivity vs. specific epitopes</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Western Blot analysis (reducing and non-reducing conditions)</td>
</tr>
<tr>
<td><strong>Particle characterization</strong></td>
<td>Microscopy</td>
<td>Electron microscopy</td>
</tr>
<tr>
<td></td>
<td>Liquid chromatographic patterns</td>
<td>SEC-HPLC</td>
</tr>
<tr>
<td></td>
<td>Particle size</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td></td>
<td>Lipid content</td>
<td>spectrophotometry</td>
</tr>
</tbody>
</table>

N=3
The RTS,S antigen is a well-characterized protein (*ICH guideline Q6B*).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity &amp; Product-Related Impurities</td>
<td>Electrophoretic pattern</td>
<td>SDS PAGE analysis in reducing conditions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SDS PAGE analysis non-reducing</td>
</tr>
<tr>
<td>Process-Related Impurities and Contaminants</td>
<td>Cell substrate-derived impurities</td>
<td>Residual DNA content by Threshold method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCP by ELISA</td>
</tr>
<tr>
<td></td>
<td>Upstream and Downstream-derived impurities</td>
<td>Colorimetry, GC-MS, HPLC</td>
</tr>
</tbody>
</table>

N=8
## Characterization of the Adjuvant System (EMA Guideline on adjuvants for vaccines for human use)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical composition</strong></td>
<td>Chromatographic methods (HPLC, UPLC,..)</td>
</tr>
<tr>
<td>(qualitative and quantitative)</td>
<td></td>
</tr>
<tr>
<td><strong>Physical characteristics</strong></td>
<td>Visual appearance</td>
</tr>
<tr>
<td></td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>Zeta-potential</td>
</tr>
<tr>
<td></td>
<td>Size and size distribution (Dynamic)</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Manufacturing residuals (HPLC)</td>
</tr>
<tr>
<td></td>
<td>Endotoxin (pyrogens)</td>
</tr>
<tr>
<td></td>
<td>Bioburden</td>
</tr>
<tr>
<td></td>
<td>Sterility</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td>Electron microscopy</td>
</tr>
</tbody>
</table>

![Dynamic light scattering Volume distribution profile](image)

N=10
Compatibility and Absence of Interaction between Antigen and AS are demonstrated

- Compatibility of Ag and AS:
  - Integrity of Ag in presence of AS
  - Integrity of AS in presence of Ag
  - Visual appearance

- Absence of Interaction:
  - Isothermal Titration Calorimetry
**Approach followed for Clinical lots QC Release**

- **Pairs** of final containers: one lot of Antigen + one lot of AS

- **QC tests** on:
  - Ag Final Container: Standard release tests (Identity, content, pH, sterility, residual moisture etc..;)
  - AS Final Container: Standard release tests (Identity, contents, pH, sterility, etc…)
  - **Ag reconstituted in AS**: Abnormal Toxicity Test, appearance, *in vivo* Potency: Humoral response in mice
Proposed approach for **Commercial** lots QC Release

**Individual** Final containers: Antigen and AS

**QC tests** on:
- Ag Final Container: Standard release tests (Identity, content, pH, sterility, residual moisture etc…)
- AS Final Container: Standard release tests (Identity, contents, pH, sterility, volume, etc…)
- No tests on Ag reconstituted in AS
Progression from *in-vivo* to *in-vitro* potency

3Rs principles for animal use: “Reduce, Refine, Replace”

**Immunogenicity**
- Extensive *in vivo* exploration of immune response

**Potency**
- *In vivo* measurement of specific humoral responses

**Antigenicity**
- *In vitro* Evaluation of antigenicity

Correlation with human response?

Knowledge of vaccine including manufacturing process

Consistency tools
Conclusion

- Vaccine presentation justified by stability profile

- Extensive characterization data package of the recombinant protein and of the Adjuvant System during development
  - assessment of key quality attributes
  - establishing consistency of quality profile

- Compatibility of these 2 moieties was established

- Routine release of commercial lots proposal:
  - Testing of individual antigen and Adjuvant system containers
  - *In-vitro* potency (3 R’s)
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