Plague vaccine pipeline: Overview of next generation vaccine approaches

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Virulence factors as source of vaccine antigens

- *Y.pestis* has many virulence factors e.g. genome–encoded adhesins and plasmid-encoded factors

- F1 capsular protein
  - MT
  - Phospholipase D:
  - Persistence in flea gut

- V antigen
  - Type III Secretion

- Pesticin
  - Coagulase
  - PLA
F1-antigen

- Surface located in *Y. pestis*, forms a polypeptide capsule
- Antiphagocytic effect
- Aggregates to >200kDa
- *Caf* operon encoded

- *Psa*+ *caf*+ *Y. pestis* has pH6 antigen fimbrial adhesin & F1 capsular protein

- *Δcaf Δpsa* mutant was more efficiently internalised by respiratory epithelial cells
  
  (Liu *et al* 2006)
V-antigen secreted through injectisome

- V antigen secreted unfolded through injectisome
- Re-folds at tip surface (enabled by C_{177} and C_{234} Ligtenberg et al 2013)
- Optimal pentameric conformation
- V is chaperone for Yop’s B and D
- Pore-forming in host cell
- Translocates Yop’s into host cell
- 37kDa protein
<table>
<thead>
<tr>
<th>Sub-unit</th>
<th>Function</th>
<th>Immunogenic</th>
<th>Protective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pla</td>
<td>Plasminogen activator protease</td>
<td>Y</td>
<td>Partially</td>
</tr>
<tr>
<td>pH6 antigen</td>
<td>Putative surface adhesin</td>
<td>Y</td>
<td>No</td>
</tr>
<tr>
<td>YopD/B</td>
<td>T3SS – translocation Yop</td>
<td>Y</td>
<td>TTSS factors; some partially protective</td>
</tr>
<tr>
<td>YopH</td>
<td>T3SS-PTPase effector Yop</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YopE</td>
<td>T3SS-cytotoxin effector Yop</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YopN</td>
<td>T3SS-regulates Yop release?</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YopK</td>
<td>T3SS-regulates Yop release?</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YopM</td>
<td>T3SS-effector Yop</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YpkA</td>
<td>T3SS-ser/Thr kinase effector yop</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>LcrG</td>
<td></td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>YscF</td>
<td>Forms injectisome</td>
<td>Y</td>
<td>Partially</td>
</tr>
<tr>
<td>LcrV</td>
<td>TT3S- tips the injectisome</td>
<td>Y</td>
<td>Bubonic &amp; pneumonic</td>
</tr>
<tr>
<td>F1 antigen</td>
<td>Surface capsular protein</td>
<td>Y</td>
<td>Bubonic</td>
</tr>
</tbody>
</table>

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Protective efficacy against aerosol challenge at d.68 in outbred (TO) mice after single immunisation (Williamson et al 1997, 2001)

![Graph showing percent survival against different doses of Y.pestis inhaled for different treatments.]

- F1+V (dose increased to 75mcg)
- Plague vacc USP
- Alhydrogel

MLD Y.pestis inhaled
Immunised mice showed no clinical signs

Williamson et al Biomed. Microdevices 2007, 9, 51-60
### F1/V combination protects against pneumonic plague

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>NHP</th>
<th>Immunogenic</th>
<th>Protective</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+V</td>
<td>Cynomolgus macaque</td>
<td>+</td>
<td>$10^4$ cfuY.p.CO92 i.n.</td>
<td>Cornelius et al 2008</td>
</tr>
<tr>
<td>Nicotiana LcrV-F1</td>
<td>Cynomolgus macaque</td>
<td>+</td>
<td>$10^5$ cfu aerosol Y.p. CO92</td>
<td>Chichester et al 2009</td>
</tr>
<tr>
<td>F1-V</td>
<td>Cynomolgus macaque</td>
<td>+</td>
<td>Serum passively protected mice</td>
<td>Fellows et al 2010</td>
</tr>
<tr>
<td>F1+V</td>
<td>Cynomolgus macaque</td>
<td>+</td>
<td>$10^4$ cfu (10 LD$_{50}$) Y.p, Co92 aerosol</td>
<td>Williamson et al 2011</td>
</tr>
</tbody>
</table>
## Cumulative evidence for safety of F1/V sub-units in CT’s

<table>
<thead>
<tr>
<th>Vaccine &amp; regimen</th>
<th>CT Phase</th>
<th>Outcome</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>nF1+rV/alum</td>
<td>2a; n=240</td>
<td>Vaccine safe; 30µg &gt;15µg (p&lt;0.05)</td>
<td>Chu et al, HuVacclImmunother 2016, 12, 2334</td>
</tr>
<tr>
<td>15µg / 30µg of each Day 0,28 i.m.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>rF1-V /alum</td>
<td>2a; n=400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80µg/160µg Day 0,28,182 or d.0,56,182</td>
<td></td>
<td></td>
<td>NCT01122784 DVC,2008</td>
</tr>
<tr>
<td>rF1+rV/alhydrogel</td>
<td>1; n=32</td>
<td>Vaccine safe; IgG to F1+V at d.21 Correlated with PT protection (p&lt;0.001)</td>
<td>Williamson et al. Infect.Immun 2005, 73, 3598</td>
</tr>
<tr>
<td>5µg-40µg of each Day 0,21 i.m.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rF1+rV/alhydrogel</td>
<td>1a/1b; n=164</td>
<td>Vaccine safe; 6 month follow-up; 100% immune response; 80µg optimal</td>
<td>Avecia 2006</td>
</tr>
<tr>
<td>40µg-120µg of each Day 0,21 i.m.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n reported</td>
<td>N=836</td>
<td>No vaccine-related SAE</td>
<td></td>
</tr>
</tbody>
</table>
Presentation & immunogenicity

- Need both sub-units
  - rV added to original KWCV restored efficacy (Jones et al, 2003)
  - Provide protection against F1-negative Y.pestis (e.g. C12, Anderson et al, 1996)
  - F1 polymerises, V dimerises (Tito et al, 2001a, 2001b)
  - conformational effect on immunogenicity (Derewenda et al, 2004)
  - variants of V exist, but Y.pestis strains predominantly V03 sequence
- B-cell epitopes identified in N terminal region of V (Mab29.3) and central domain (Mab7.3) (Hill et al, 2009)
- F1 and V expressed as a recombinant fusion/single protein (Heath et al, 1998) or in free association.
F1 and V can induce memory responses


HLA-DR restricted epitope in F1(C-terminus) at 141-160 (Musson et al, 2010)
T-cell epitope map for rV

Murine T-cell epitope map for V
(Parent et al 2005, Shim et al, 2006)

HLA-DR1 restricted T cell epitopes (unpublished)
Appropriate formulation to meet requirement

- Conventional formulation: F1/V in liquid alhydrogel/alum ± excipients with injected delivery (expect ~ 2y shelf-life at 5°C)
- Requirement to protect against all forms of plague
  - Need to induce systemic & mucosal immunity
  - Need to induce rapid response (control outbreak)
  - Need to induce durable immunity
- Sub-unit proteins are amenable to other formulations & non-invasive delivery
- Alternative formulation may enhance stability, be dose-sparing/ reduce dosing frequency or number of doses required.
Non-invasive delivery

• Oral delivery ideal; immunostimulants may be incorporated to maximise immunogenicity

• Combine with another route e.g. parenteral prime plus oral boost to maximise systemic & mucosal immunity

• Nasal delivery: good induction of both systemic & mucosal immunity-need to demonstrate safety

• Transcutaneous delivery a possibility but patient compliance/environment may be problematic
Point- of- use convenience

• Consider context of use-may be resource poor setting
  – Ambient temperature/humidity /poor infrastructure

• Physical presentation-liquid or dry powder
  – If liquid, then may need incorporated stabiliser
  – If dry powder, then minimal manipulation preferred-use dry/wet barrel syringe for rehydration of lyophilised vaccine

• Single use presentation (vial or dual-barrel syringe) may be preferable to avoid dosing errors (cost implication)
Stability: the last mile test

- Vaccine distribution without need for cold chain highly desirable
- What presentation maximises stability?
- Solid /liquid/aerosol presentations possible
- Need stringent stability testing under accelerated conditions of temperature/RH etc.
Summary

• Next generation plague vaccine-F1 and V in combination
• Preferably fully recombinant
• Able to induce systemic and mucosal immunity
• Needs to induce rapid immunity (to curtail outbreak situations)
• Need to induce durable immunity/memory response
• Can formulate to give significant advantages
  – point-of-use convenience
  – rapid onset of immunity
  – non-invasive dosing
  – enhanced stability/shelf-life
  – adequate duration of immunity

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