Live attenuated pertussis vaccines
Are they the future of pertussis control?

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Sydney, August 25/26, 2011
Acute *Bordetella pertussis* infection in 2 months old infants

27 one to four months-old *B. pertussis* infected children

44 two months-old non-immune infants

**Antigen-specific IFN-γ secretion**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>IFN-γ Secretion (SC / 10^6 PBMC)</th>
<th>IFN-γ Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHA</td>
<td><img src="chart1.png" alt="Bar Graph" /></td>
<td><img src="chart2.png" alt="Bar Graph" /></td>
</tr>
<tr>
<td>PTX-R</td>
<td><img src="chart3.png" alt="Bar Graph" /></td>
<td><img src="chart4.png" alt="Bar Graph" /></td>
</tr>
</tbody>
</table>

- **Absence of antigen-specific IL-4 and IL-13**

Live attenuated *B. pertussis* for intranasal administration

- Mucosal administration
  - Induction of systemic and mucosal immune responses
- Ease of administration
- Persistence of the bacteria in the host
  - Long-lived immune responses
  - Reduced number of administrations to induce protection
- Potential as a multivalent vaccine
Safety - natural biology of pertussis

- *B. pertussis* = strictly upper-respiratory pathogen (no dissemination)
- No fever (although high fever after WCV)
- Pertussis = rare in AIDS patients (Cohn *et al.*, 1993)
  (in contrast to *B. bronchiseptica*)
- *B. pertussis* = extremely sensitive to erythromycin
- Very limited survival of *B. pertussis* in environment
  (Porter & Wardlaw, 1993)

Feasibility

- *B. bronchiseptica* in dogs and pigs (2-days old)
  (Bey *et al.*, 1981; De Jong, 1987)
Protection against *B. pertussis* challenge after i.n. vaccination of infant mice with BPZE1

In 1-week-old mice, BPZE1 provides better protection than two i.p. doses of aPV

Feunou *et al.*, in preparation
Passive transfer to SCID mice
(200 µl/50,106 WSC)

CFU Log$_{10}$

- WSC naives   WSC BPZE1   serum naives   serum BPZE1

Feunou et al., PLoS One, 2010
Longevity of BPZE1-induced immunity

3 weeks

Feunou et al., Vaccine, 2010
Early protection induced by BPZE1 vaccination

Naive
BPZE1

B. pertussis

B. pertussis

B. pertussis

B. pertussis

D0
D7
D14
D21
D28

CFUs in mouse lung (Log10)

Time post vaccination (weeks)

Total IgG titer

Debrie et al., in preparation
<table>
<thead>
<tr>
<th>Microbe</th>
<th>PAMP</th>
<th>PRR</th>
<th>Site of recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Glycoproteins</td>
<td>TLR2, TLR4</td>
<td>Cell surface</td>
</tr>
<tr>
<td></td>
<td>5’PPP RNA</td>
<td>RIG-I</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td></td>
<td>ss RNA</td>
<td>TLR7, TLR8</td>
<td>Endosomes</td>
</tr>
<tr>
<td></td>
<td>dsRNA</td>
<td>TLR3, MDA5, RIG-I</td>
<td>Endosomes/cytoplasm</td>
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<tr>
<td></td>
<td>Genomic DNA</td>
<td>TLR9, DAI</td>
<td>Endosomes/cytoplasm</td>
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<tr>
<td>Bacteria</td>
<td>Lipopeptides</td>
<td>TLR2</td>
<td>Cell surface</td>
</tr>
<tr>
<td></td>
<td>Lipoteichoid acid</td>
<td>TLR2</td>
<td>Cell surface</td>
</tr>
<tr>
<td></td>
<td>Peptidoglycan</td>
<td>TLR2</td>
<td>Cell surface</td>
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<tr>
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<td>Flagellin</td>
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<td>Cell surface</td>
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<td>CpG DNA</td>
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<td>B-form DNA</td>
<td>DAI</td>
<td>Cytoplasm</td>
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<tr>
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<td>Diaminopimelic acid</td>
<td>NOD1</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td></td>
<td>Muramyl dipeptide</td>
<td>NOD2</td>
<td>Cytoplasm</td>
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<td>GPI anchors</td>
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<td>Endosomes</td>
</tr>
<tr>
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<td>CPG DNA</td>
<td>TLR9</td>
<td>Endosomes</td>
</tr>
<tr>
<td></td>
<td>Haemozoin</td>
<td>TLR9</td>
<td>Endosomes</td>
</tr>
<tr>
<td></td>
<td>Profilin-like protein</td>
<td>TLR11</td>
<td>Cell surface</td>
</tr>
</tbody>
</table>
Role of TL4 in early protection

Debrie et al., in preparation
Protection against Influenza A virus-induced mortality

Li et al., J. Virol., 2010
QuickTime™ et un décompresseur TIFF (non compressé) sont requis pour visionner cette image.
Manufacturing Process of BPZE1 DP

1. MCB
2. WCB
3. BPZE1 cell culture
4. Harvest: cell pellet
   - Wash and resuspend in formulation buffer
5. DS: Cell suspension in formulation buffer
6. Dilution of DS to final concentration and filling
7. Drug Product
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Assay</th>
<th>MCB</th>
<th>WCB</th>
<th>DS</th>
<th>DP</th>
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<tbody>
<tr>
<td>Description</td>
<td>Visual aspect</td>
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<tr>
<td></td>
<td>pH</td>
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<tr>
<td></td>
<td>Osmolarity</td>
<td></td>
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</tbody>
</table>
|                               | cfu – assay                    | #   | #   | #  | Δ/+
|                               | OD-measurement                 | #   | #   | #  |    |
|                               | Delivered dose uniformity      |     |     |    |    |
|                               | (EP2.9.40)                     |     |     |    |    |
|                               | Endotoxin                      |     |     |    |    |
| Quantity                      | Colony morphology             | +   | +   | +  | +  |
|                               | Genetic tests (PT, DNT, ampG)  | +   |     | +  |    |
| Identity (attenuated BPZE1)   |                               |     |     |    |    |
|                               | Microbial purity               | +   | +   | +  | +  |
| Safety*                       | Absence of revertant *B. pertussis* | +   | +   | +  |    |
|                               | Absence of active PTX: in vitro |     |     |    |    |
|                               | CHO clustering test            |     |     |    | #  |
| Stability plan                | cfu/ml in function of storage time | +   | +   |    | +  |

Δ: must have for Tox; +: must have for Phase I; # In Process Control, internal test for information only
Study Objectives

✓ First-in-man, dose-escalating, placebo-controlled, double blind, safety trial

✓ Primary Objective

Assess general safety and local tolerability in the respiratory tract after single ascending dose of BPZE1

✓ Secondary Objectives

- Evaluate colonization of the human respiratory tract after a single ascending dose of BPZE1

- Evaluate T and B cell immune responses to *B. pertussis* after a single ascending dose of BPZE1
Inclusion Criteria

- Healthy male volunteer born between 1979 and 1991, who has not experienced pertussis during the last 10 years and has not been vaccinated with any pertussis vaccine.

- Informed consent form signed by subject.

- Subject able to attend all scheduled visits and to understand and comply with the study procedures (able to read and write Swedish).
Exclusion Criteria

1. Pertussis toxin serum IgG $\geq$ 20 units/mL
2. Blood pressure after resting $\geq$ 150/90 mmHg
3. Heart rate after resting $\geq$ 80 bpm
4. Respiratory rate after resting $\geq$ 20/min
5. Unwillingness to refrain from nicotine from screening to day 28
6. Donated blood or blood loss ($\geq$ 450 ml) within 60 days prior to screening
7. Receipt of Ig/blood products/immunosuppressive drugs within the previous 3 ms
8. Receipt of corticosteroids in respiratory tract 30 days prior to day 0
9. Use of herbal medications or dietary suppl. 7 days prior to day 0 and for 30 days
10. Any vaccine within the last 30 days and 30 days after day 0
11. Evolving encephalopathy within 7 days of a previous vaccination
12. Known hypersensitivity to any component of the study vaccine
13. Participation in other trials in the previous 3 months
14. Inability to adhere to the protocol
15. Family history of congenital or hereditary immunodeficiency
16. Infection with HIV, HCV, HBV
17. Any medical condition which, in the opinion of the investigator, might interfere with evaluation of the study objectives
18. Clinically significant abnormal lab. Values
19. Frequent contact with children $< 1$ year of age
Phase I Trial Primary Objective

- Local, systemic, haematologic
- Classified in diary
- Any cough (Start, duration, severity, outcome)
## Phase I Trial Secondary Objective

<table>
<thead>
<tr>
<th></th>
<th>d0</th>
<th>d4</th>
<th>d7</th>
<th>d11</th>
<th>d14</th>
<th>d28</th>
<th>6 months</th>
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<tbody>
<tr>
<td><strong>Culture</strong></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><strong>Serology</strong></td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>(FHA, PT, Prn, Fim, WCL)</td>
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<tr>
<td><strong>Saliva</strong></td>
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<td>X</td>
<td>X</td>
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<tr>
<td>(FHA, PT, Prn, Fim, WCL)</td>
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<td><strong>B/T Elispot</strong></td>
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</tr>
</tbody>
</table>
Phase I Trial Study Design (I)

Thursday, September 9, 2010  1 V
Friday, September 10, 2010   1 V

Monday, September 12, 2010  2 V/P
Tuesday, September 13, 2010  3 V/P
Wednesday, September 14, 2010  4 V/P
Thursday, September 15, 2010  5 V/P

TOTAL  12 V + 4 P
Phase I Trial Design (II)

- **Low dose $10^3$**
  - 2010: Aug, Sept, Oct
  - 2011: Jan, Feb
  - IDMC

- **Medium dose $10^5$**
  - 2010: Nov, Dec
  - 2011: March, Apr
  - IDMC

- **High dose $10^7$**
  - 2011: May, June, July
  - IDMC
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