Development of Recombinant Pertussis Vaccines

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DCVMN Workshop: Global Registration and Vaccine Shortage
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Bordetella pertussis
Pathogenesis

- **PT**
  - Principal toxin secreted by Bp, 5 subunits, A-B structure
  - Many pathologic effects mediated by ADP ribosylation of G protein effectors

- **FHA**
  - Filamentous adhesion factor

- **PRN ("69K")**
  - Impurity present in Japanese T-type vaccines
  - RGD sequences promoting adhesion to cells

- **Agg 2+3**
  - or Fimbriae

Source: http://www.my-pharm.ac.jp/~yishibas/research/Pertussis1.jpg

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Pertussis Vaccines
Three Types of Vaccines

• Whole-cell Pertussis vaccines (wP)

• Acellular Pertussis vaccines using chemically detoxified Pertussis Toxin (cPT)
  • Co-purified antigens (Asia)
  • Individually purified antigens (Western countries)

• Recombinant Pertussis vaccines
  • Live-attenuated (nasal route)
  • Inactivated
    • Genetically-detoxified Pertussis Toxin (rPT)
    • Recombinant antigens such as PT, ACT, PRN...
Resurgence of Pertussis
An Increasing Concern Worldwide

• Waning immunity
• Genetic shifts of circulating Bp strains

Sources:
1. WHO (2013)

Table 1. Possible Vaccination Strategies to Control the Resurgence of Pertussis

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Return to the use of wcP</td>
<td>Probably unacceptable</td>
</tr>
<tr>
<td>Develop less-reactogenic wcP</td>
<td>Not yet done</td>
</tr>
<tr>
<td>Maternal vaccination to provide transplacental antibody to protect newborn</td>
<td>Now generally recommended</td>
</tr>
<tr>
<td>Vaccination of newborn contacts (cocoon strategy)</td>
<td>Difficult to obtain complete coverage</td>
</tr>
<tr>
<td>More frequent boosters with acP</td>
<td>Costly and difficult to put in place</td>
</tr>
<tr>
<td>Change antigens in acP to those from currently circulating strains</td>
<td>Uncertain effect</td>
</tr>
<tr>
<td>Increase quantities of current antigens</td>
<td>Would require large trials</td>
</tr>
<tr>
<td>Inactivate PT by genetic mutation or milder chemical</td>
<td>Probably advisable to increase immunogenicity</td>
</tr>
<tr>
<td>Add new virulence factors</td>
<td>Would require large trials</td>
</tr>
<tr>
<td>Use stronger adjuvants</td>
<td>May require large trials</td>
</tr>
<tr>
<td>Administer live attenuated <em>Bordetella pertussis</em> intranasally</td>
<td>Early development Probably best as a boost strategy</td>
</tr>
</tbody>
</table>

Abbreviations: acP, acellular pertussis vaccine; PT, pertussis toxin; wcP, whole-cell pertussis vaccine.
Call for New Pertussis Vaccines
Genetically-Inactivated PT, the Solution?

The Diphtheria and Pertussis Components of Diphtheria-Tetanus Toxoids—Pertussis Vaccine Should Be Genetically Inactivated Mutant Toxins

John B. Robbins,1 Rachael Schneerson,2 Birger Trollors,1 Hiroko Sato,1 Yoji Sato,1 Nina Rappuoli,1 and Jerry M. Keith1
1National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; 2Department of Pediatrics, University of Sitsa, Sweden; 3Chiron Vaccines, Serra, Italy

Relative Contribution of Th1 and Th17 Cells in Adaptive Immunity to *Bordetella pertussis*: Towards the Rational Design of an Improved Acellular Pertussis Vaccine

Pádraig J. Ross1, Caroline E. Sutton2,*, Sarah Higgins3,*, Ailéen C. Allen1, Kevin Walsh1, Alicja Misiał1, Ed C. Leveille4, Rachel M. McLoughlin5, Kingston H. G. Mills1,2
1Immunology Research Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; 2Adjunct Research Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; 3Host Pathogen Interactions Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

The rise in pertussis cases urges replacement of chemically-inactivated with genetically-inactivated toxoid for DTP

John B. Robbins1,*, Rachael Schneerson3, Jerry M. Keith1, Joseph Shiloach2, Mark Miller3, Birger Trollors4

Genetically Detoxified Pertussis Toxin Induces Th1/Th17 Immune Response through MAPKs and IL-10-Dependent Mechanisms

Maria Nasso2,*, Giorgio Fedele,2* Fabiana Spensieri,3* Raffaella Palazzo,3 Paolo Costantini,7 Rino Rappuoli1, and Clara Maria Ausiello2,6
Genetically Detoxified Pertussis Toxin
A Non-Toxic and Superior Immunogen

**rPT** is a PT devoid of toxicity while maintaining the other properties of the native PT.

**cPT** introduces dramatic changes on the toxin surface.

- Chemical treatment can destroy up to 80% of surface epitopes
- The rPT preserves the epitopes for T-cell binding significantly better than cPT.

Source: Ibsen H (1996)
Pertussis (whooping cough) is an important cause of death in infants worldwide, and continues to be a public health concern despite high vaccination coverage. In 2013, according to WHO estimates, pertussis was still causing around 63,000 deaths in children aged <5 years. Two types of pertussis vaccines are available: wP vaccines and aP vaccines.

A switch from wP to aP vaccines for the primary schedule should only be considered if additional periodic booster or maternal immunization can be assured and sustained. National programmes currently administering wP vaccination should continue to use wP vaccines for primary vaccination series. National programmes currently using aP vaccine may continue using this vaccine but should consider the need for additional booster doses and additional strategies such as maternal immunization in case of resurgence of pertussis.
BioNet Patented Pertussis Technology
Translational Research: From Concept to Clinical Proof

- BioNet Genetically detoxified PT (PTgen)
  - Mutation in two positions
    - Two amino-acids replaced
    - ARG9 to LYS9 and GLU129 to GLY129
  - Resulting in the loss of catalytic and toxic effects

Source: Buasri et al. (2012) BMC Microbiology 12:61

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Clinical Phase I/II Study

Procedure Overview

• Randomized, Observer-blind and controlled study (TCTR20140703001)

• **Objective:** To assess safety and immunogenicity of a single injection of BNA’s aP or BNA’s TdaP or licensed TdaP (Adacel®, Sanofi Pasteur) vaccines

• **Study Population:** Healthy adult volunteers (Male & Female), 18-35 years of age

• **Number of Subjects:** 60 (20 per group)
  • Group 1 – BNA’s aP
  • Group 2 – BNA’s TdaP
  • Group 3 – Licensed TdaP (Adacel®, Sanofi Pasteur) as comparator

• **Study site:** Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Source: Sirivichayakul C. et al. (2017) Human Vaccin Immunother
### Study Vaccines

#### Vaccine Composition

- Presented in pre-filled syringe for intramuscular injection

<table>
<thead>
<tr>
<th>Active ingredients per 0.5-mL dose</th>
<th>BNA’s aP</th>
<th>BNA’s TdaP</th>
<th>Adacel®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus toxoid (TT)</td>
<td>-</td>
<td>7.5 Lf</td>
<td>5 Lf</td>
</tr>
<tr>
<td>Diphtheria toxoid (DT)</td>
<td>-</td>
<td>2.0 Lf</td>
<td>2 Lf</td>
</tr>
<tr>
<td>Pertussis toxoid (PT)</td>
<td>5 µg*</td>
<td>5 µg*</td>
<td>2.5 µg</td>
</tr>
<tr>
<td>Filamentous hemagglutinin (FHA)</td>
<td>5 µg</td>
<td>5 µg</td>
<td>5 µg</td>
</tr>
<tr>
<td>Pertactin (PRN)</td>
<td>2.5 µg</td>
<td>2.5 µg</td>
<td>3 µg</td>
</tr>
<tr>
<td>Fimbriae type 2/3</td>
<td>-</td>
<td>-</td>
<td>5 µg</td>
</tr>
</tbody>
</table>

* Recombinant Pertussis Toxin (PTgen)  

Lf = Limit of flocculation  

Source: Sirivichayakul C et al. (2017) *Human Vaccin Immunother*

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The study was conducted according to ICH-GCP guidelines.
EC approval of the study and individual consent were obtained.
Safety and Immunogenicity assessment were conducted according to GLP or relevant guidelines using standardized methods.

Source: Sirivichayakul C et al. (2017) Human Vaccin Immunother
# Safety

## Solicited Local & Systemic Reactions at Day 7

<table>
<thead>
<tr>
<th></th>
<th>BNA’s aP (n = 20)</th>
<th>BNA’s TdaP (n=20)</th>
<th>Adacel® (n=20)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LOCAL REACTIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>16 (80.00)</td>
<td>15 (75.00)</td>
<td>17 (85.00)</td>
<td>0.73</td>
</tr>
<tr>
<td>Redness</td>
<td>0 (0.00)</td>
<td>3 (15.00)</td>
<td>1 (5.00)</td>
<td>0.15</td>
</tr>
<tr>
<td>Induration</td>
<td>0 (0.00)</td>
<td>4 (20.00)</td>
<td>1 (5.00)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>SYSTEMIC REACTIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (5.00)</td>
<td>-</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (10.00)</td>
<td>1 (5.00)</td>
<td>3 (15.00)</td>
<td>0.86</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3 (15.00)</td>
<td>2 (10.00)</td>
<td>5 (25.00)</td>
<td>0.43</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (5.00)</td>
<td>2 (10.00)</td>
<td>3 (15.00)</td>
<td>0.86</td>
</tr>
<tr>
<td>Chills</td>
<td>1 (5.00)</td>
<td>0 (0.00)</td>
<td>1 (5.00)</td>
<td>-</td>
</tr>
<tr>
<td>Malaise</td>
<td>1 (5.00)</td>
<td>1 (5.00)</td>
<td>5 (25.00)</td>
<td>0.08</td>
</tr>
<tr>
<td>Myalgia</td>
<td>5 (25.00)</td>
<td>2 (10.00)</td>
<td>7 (35.00)</td>
<td>0.21</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>-</td>
</tr>
</tbody>
</table>

*; Fisher’s exact and P < 0.05 considering as statistically significant

Source: Sirivichayakul C et al. (2017) Human Vaccin Immunother
## Safety

### Solicited Adverse Events during 28 days Post-vaccination

<table>
<thead>
<tr>
<th>Summary</th>
<th>BNA’s aP (n = 20)</th>
<th>BNA’s TdaP (n = 20)</th>
<th>Adacel® (n = 20)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adverse events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with one or more AEs</td>
<td>5 (25.00)</td>
<td>5 (25.00)</td>
<td>4 (20.00)</td>
<td>14 (23.33)</td>
</tr>
<tr>
<td>vaccine-related AEs</td>
<td>1 (5.00)</td>
<td>1 (5.00)</td>
<td>1 (5.00)</td>
<td>3 (5.00)</td>
</tr>
<tr>
<td>with no AE</td>
<td>15 (75.00)</td>
<td>15 (75.00)</td>
<td>16 (80.00)</td>
<td>46 (76.67)</td>
</tr>
<tr>
<td>discontinued due to an AE</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td><strong>Serious adverse events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with SAE</td>
<td>1 (5.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (1.67)</td>
</tr>
<tr>
<td>vaccine-related SAE</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>discontinued due to a SAE</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

*Arm pain*  
*Vaccination site pain*

Source: Sirivichayakul C et al. (2017) *Human Vaccin Immunother*
Immunogenicity

ELISA Total IgG Anti-pertussis GMT at Day 28 (Post-vaccination)

**Day 28**

<table>
<thead>
<tr>
<th>Seroconversion rate</th>
<th>BNA's aP</th>
<th>BNA's TdaP</th>
<th>Adacel®</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) (95% CI)</td>
<td>19</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>PT Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>18 (94.44) (84.0–100.0)</td>
<td>16 (94.44) (84.0–100.0)</td>
<td>16 (94.44) (84.0–100.0)</td>
</tr>
<tr>
<td>Day 28</td>
<td>19 (94.44) (84.0–100.0)</td>
<td>16 (94.44) (84.0–100.0)</td>
<td>16 (94.44) (84.0–100.0)</td>
</tr>
<tr>
<td>MT (ELISA) IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNA's aP</td>
<td>76.0–100.0</td>
<td>84.0–100.0</td>
<td>84.0–100.0</td>
</tr>
<tr>
<td>BNA's TdaP</td>
<td>76.0–100.0</td>
<td>84.0–100.0</td>
<td>84.0–100.0</td>
</tr>
<tr>
<td>Adacel®</td>
<td>76.0–100.0</td>
<td>84.0–100.0</td>
<td>84.0–100.0</td>
</tr>
</tbody>
</table>

**Source:** Sirivichayakul C et al. (2017) Human Vaccin Immunother
Immunogenicity

Anti-PT Neutralizing Titers at Day 28 (Post-vaccination)

Source: Sirivichayakul C et al. (2017) Human Vaccin Immunother
Immunogenicity

Proportion of Subjects at Various Cut-off Titers

Source: Sirivichayakul C et al. (2017) Human Vaccin Immunother

DCVMN Workshop Taiwan 2017
Clinical Phase II/III Study
A Pivotal Study in Thailand in 12-17 Years of Age

• Objectives:
  • Primary: To demonstrate non-inferior immunogenicity of one dose of BioNet’s combined Tetanus, reduced dose of Diphtheria and acellular Pertussis vaccine (Boostagen™) as compared to Adacel® vaccine.
  • Secondary:
    • To assess safety of Boostagen™ and Pertagen™ vaccines and to demonstrate non-inferior immunogenicity of Pertagen™ vaccine as compared to Adacel®.
    • To assess immunopersistence at 1 year.

• Primary endpoint:
  • Non-inferior immunogenicity – antibody response in all subjects
  • Seroconversion rates as defined by proportion of subjects with ≥ 4-fold increase with respect to baseline of ELISA antibodies to PT and FHA in Boostagen™ and Adacel® vaccine groups.
Study Design

Single Injection with 28 Days and 1 Year Follow-up

- Day 0: Screening & Vaccination
- Day 7: Follow-up Visit
- Day 28: Follow-up Visit
- Day 336: Follow-up Visit

Safety

- Day 0: Green
- Day 7: Green
- Day 28: Green
- Day 336: Green

Immunogenicity

- GMT Ab (ELISA)
  - Day 0: Blue
  - Day 7: Blue
  - Day 28: Blue
  - Day 336: Orange
- PT NAb (CHO)
  - Day 0: Yellow
  - Day 7: Yellow
  - Day 28: Yellow
  - Day 336: Yellow
- CMI
  - Day 0: Red
  - Day 7: Red
  - Day 28: Red
  - Day 336: Red

A subset of 50 subjects/group
A subset of 20 subjects/group

DCVMN Workshop Taiwan 2017
BioNet Clinical Study

Summary

Results
Local and systemic post-immunization reactions at seven days after vaccination and incidence of Adverse Events one month after vaccination were similar in the three vaccine groups. One unrelated Serious Adverse Event was reported in one subject in the Pertagen® group. ELISA anti-PT, ELISA anti-FHA, and anti-PT neutralizing anti-body GMTs as well as serconversion rate (≥ 4 fold increase) were statistically significant higher in Pertagen® and Boostagen® vaccine groups than in Adacel® group (p ≤ 0.05). Non-inferiority of Pertagen® and Boostagen® vaccines vs Adacel® vaccine was demonstrated (difference level 10%).

Conclusions
The newly developed aP vaccines either standing alone (Pertagen®) or in formulation as TdaP (Boostagen®) showed to be as tolerated and safe as Adacel®. The higher ELISA and neutralizing anti-PT titres observed in Pertagen® and Boostagen® vaccine groups vs Adacel® vaccine group are consistent with epitopes conservation of the genetically detoxified PT.
BioNet PTgen delivered via Innovative Patch
Initiating Phase I Study in Europe in 2016 after Pre-Clinical Proof of Concept

DBV Technologies, BioNet-Asia and Geneva University Hospitals Complete Dosing in First Cohort of Phase I Study of Viaskin rPT for Booster Vaccination Against Pertussis

DSMB expressed no safety concerns with Viaskin rPT 25 μg

Following positive DSMB review, dosing with Viaskin rPT 50 μg has been initiated

PARIS, BANGKOK and GENEVA November 17, 2016 - DBV Technologies (Euronext: DBV – ISIN: FR0010417345 - Nasdaq Stock Market: DBVT), the Geneva University Hospitals (HUG) and BioNet-Asia Co. Ltd today announced
BioNet Pertagen Vaccine Evaluated in Europe
Phase II Study in Adolescents
“A world free of any preventable disease is our dream. To make this dream come true is our mission.”