PATH’s Work to Apply Adjuvants to Inactivated Poliovirus Vaccine

11th WHO/UNICEF Consultation with OPV/IPV Manufacturers and National Regulatory Authorities

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October 25, 2012
Summary

- The IPV adjuvant project:
  - Inactivated poliovirus vaccine (IPV) formulation with poly[di(carboxylatophenoxy)-phosphazene] (PCPP)

- IPV potency reagents and assays
The IPV Adjuvant Project
IPV Adjuvant Project

• **Goal:**
  — Develop low-cost IPV through antigen dose reduction using adjuvants.
  — Achieve 5-10 fold dose reduction.

• **Strategy:**
  — Evaluate and compare several advanced adjuvants.

• **Project start:**
  — November 2011

• **PATH’s role:**
  — Develop PCPP-IPV formulation.
  — Coordinate head-to-head comparison of different adjuvants and formulations under development by other grantees.
Project Activities and Timeline

Year 1

- Several formulation groups develop and optimize IPV formulations using:
  - PCPP (PATH)
  - Aluminum salts (University of Lausanne)
  - dmLT (Tulane University)
  - CAF01 (Statens Serum Institut)

Year 2

- Conduct head-to-head comparisons using lead adjuvanted-IPV formulations in rats.
- Analyze cost and regulatory requirements.
- Advance down-selection and technology transfer of the selected IPV-adjuvant formulation.
Introduction to PCPP

• **Existing data:**
  — Forms non-covalent complexes with antigen.
  — In vivo performance assessed using 23 antigens in 11 animal models.
  — Phase II human studies previously advanced with influenza vaccine.
  — Enhanced immune responses (mostly Th2) achieved.
  — Multimeric presentation to antigen-presenting cells.
  — No alarming safety issues in preclinical or clinical studies.

• **PCPP compared to aluminum salts:**
  — Typically more potent than aluminum salt.
  — No association with depot formation.
Antigen-PCPP Complex: Possible Mechanism of Action
Formulation Strategy with PCPP and Animal Models

• **Formulation:**
  — *Dose sparing target:* 5-10 fold dose reduction.
  — **Vaccine:** Trivalent IPV from Statens Serum Institut.
  — **PCPP:** Parallel Solutions, Inc.
  — **Strategy:** Mixing trivalent IPV (at reduced dose but at the same ratio of the three components as in adjuvant-free trivalent IPV) with an selected PCPP dose.

• **Animal models:**
  • Mice and rats
  • **Serology:**
    — *Primary:* Serum neutralization titers.
    — *Secondary:* Serum and mucosal antibodies measured using ELISA.
Aluminum Formulation Control: Which Aluminum?

**Aluminum salts:**
- Aluminum hydroxide (Alhydrogel®, Brenntag Biosector, IEP: 11.4).
- Aluminum phosphate (Adju-Phos®, Brenntag Biosector, IEP: 5).

**Buffer:**
- Tris buffered saline pH 7.4.

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**Figure 1:** DU ELISA for IPV3 in supernatant to determine percent of IPV absorbed.
Aluminum Formulation Control: Which Buffer?

Aluminum salt:
- Aluminum hydroxide (Alhydrogel®, Brenntag Biosector, IEP: 11.4).

Buffers:
- Dulbecco’s phosphate buffered saline, pH 7.4.
- Tris buffered saline, pH 7.4.
- Medium 199.

Figure 2: DU ELISA of supernatant for quantifying absorbed IPV.
# Adjuvant Effect: Intramuscular Immunization in Mice

<table>
<thead>
<tr>
<th></th>
<th>IPV 1</th>
<th>IPV 2</th>
<th>IPV 3</th>
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<tbody>
<tr>
<td><strong>Fold increase in neutralization titers</strong></td>
<td></td>
<td></td>
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<tr>
<td>IPV alone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IPV with alum</td>
<td>1.5</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>IPV with PCPP</td>
<td>1.5</td>
<td>1.7</td>
<td>4.2</td>
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<tr>
<td><strong>Fold increase in binding antibody titers (ELISA)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPV alone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IPV with alum</td>
<td>8.0</td>
<td>8.0</td>
<td>4.0</td>
</tr>
<tr>
<td>IPV with PCPP</td>
<td>5.3</td>
<td>6.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>

**Notes:**

1. Antigen: 1/30 of a human dose.
2. Alum: 100 µg.
3. PCPP: 100 µg.
4. Data are titers on day 56 after 3 immunizations on days 0, 21, and 42.
### Adjuvant Effect: Intradermal Immunization in Mice

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<td>IPV alone</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IPV with alum</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>IPV with PCPP</td>
<td>2.9</td>
<td>5.6</td>
<td>3.9</td>
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**Notes:**
1. Antigen: 1/30 of a human dose.
2. Alum: 100 µg.
3. PCPP: 100 µg.
4. Day 56 antibody titers following three doses on days 0, 21, and 42.
Preliminary Observations and Next Steps

Preliminary observations:

- Both PCPP and alum have a modest adjuvant effect on IPV.
- Intradermal delivery of IPV or IPV with PCPP does not appear to offer an advantage over intramuscular injection.
- Both PCPP and alum caused some degree of histopathology at the injection sites.

Next steps:

- Investigation and optimization of PCPP’s adjuvant activity and safety since the material used in this study was made 10 years ago.
- The dose-sparing effect will be determined in the next experiment.
- Comparison of the dose-sparing effect of PCPP with other adjuvants—including alum, oil-in-water emulsion, dmLT, and CAF01.
IPV Potency Reagents and Assays
Goals:

- To generate and qualify IPV potency reagents, develop potency assay protocol, and make the reagents and protocol available to all project partners.
- Ideally, to make the reagents and assays applicable to both Sabin IPV and Salk IPV.
- Ideally, to make the reagents and assays applicable to IPV containing different strains of Type 1, Type 2, or Type 3 poliovirus.
Information Gathered from Expert Consultation

• No standardized potency assay or reagents exist for Sabin IPV.
• For Salk IPV:
  — All assays are based on capture ELISA to quantify the D antigen.
  — Manufacturer, EU, and US use different reagents, reference standards, and assay protocols.
  — Some use a pair of polyclonal Ab, others use polyclonal - monoclonal Ab pair.
  — For those we talked to, the existing stock of the reagents is limited.
• Potency reagents for Salk IPV might be appropriate for Sabin IPV but might need optimization (titration of the reagents and optimization of the protocol).
• Strong suggestion to use polyclonal antibodies (from different rabbits) as coating and detection antibodies because the project may involve Sabin IPV, Salk IPV, and different strains of poliovirus.
Potency Assay Reference Standard

- **Salk IPV references:**
  - A European reference standard.
  - USFIDA reference standard calibrated against European reference.
  - There may be an international standard?

- **Sabin IPV references:**
  - ??

- **Reactivity of references to different strains of IPV1:**
  - Mahoney poliovirus type 1 vs Brunhilde Virus)?
Project Workflow and Estimated Timeline

Rabbit serum generation
Spring Valley Labs
Completed

Reagent preparation and testing
CBER/USFDA
2–3 months

Reagent lyophilization
PATH
1–2 weeks

Reagent validation
CBER/USFDA
1–2 months

Current
Rabbit* Immunization Schedule

<table>
<thead>
<tr>
<th>Group #</th>
<th>Prime</th>
<th>Boost 1</th>
<th>Boost 2</th>
<th>Boost 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPV1</td>
<td>OPV1</td>
<td>OPV1</td>
<td>IPV1 + adjuvant</td>
</tr>
<tr>
<td>2</td>
<td>OPV2</td>
<td>OPV2</td>
<td>OPV2</td>
<td>IPV2 + adjuvant</td>
</tr>
<tr>
<td>3</td>
<td>OPV3</td>
<td>OPV3</td>
<td>OPV3</td>
<td>IPV3 + adjuvant</td>
</tr>
<tr>
<td>4</td>
<td>IPV1**</td>
<td>IPV1</td>
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<tr>
<td>5</td>
<td>IPV2</td>
<td>IPV2</td>
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<tr>
<td>6</td>
<td>IPV3</td>
<td>IPV3</td>
<td>IPV3</td>
<td>IPV3</td>
</tr>
</tbody>
</table>

Notes:
*New Zealand white female rabbits (n=5).
**Monovalent Salk IPV.
Characterization of Rabbit Sera

- **Binding antibodies (ELISA):**
  - High titer, low or absence to other IPV types.

- **Neutralization titers:**
  - All >1448.

- **Block ELISA titer:**
  - Low to moderate titers.
Binding Antibody Titers in IPV1 (or OPV1+IPV1) Immunized Rabbits

Group 1
(OPV1+IPV1 w/adjuvant)

Group 4
(IPV1)

Reactivity to:

- IPV1
- IPV2
- IPV3
Binding Antibody Titers in IPV2 (or OPV2+IPV2) Immunized Rabbits

**Group 2**
(OPV2+IPV2 w/adjuvant)

**Group 5**
(IPV2)

Reactivity to:

- IPV1
- IPV2
- IPV3
Binding Antibody Titers in IPV3 (or OPV3+IPV3) Immunized Rabbits

Group 3
(OPV3+IPV3 w/adjuvant)

Group 6
(IPV3)

Reactivity to:
Preparation of Reagents and Assay Protocol

- **Pilot experiments of preparing assay reagents completed.**
  - Specificity (differential reactivity to D and H antigens) is acceptable for type 1 and type 2 IPV but seems low for type 3 IPV.
    - *Note:* a monoclonal antibody will be used as the detection antibody if the specificity is unacceptable.

- **The next step is to prepare reagent stock and develop the assay protocol.**

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**Improved ELISA test for determination of potency of Inactivated Poliovirus Vaccine (IPV)**

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*Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, HFM 470, Rockville, MD 20852, USA

Biologicals 33 (2005) 17–27
Acknowledgements

• PATH’s adjuvant project team.

• Adjuvant developer partners:
  – University of Lausanne.
  – Statens Serum Institut.
  – Tulane University.
  – Parallel Solutions, Inc.

• Preclinical study partners:
  – University of Washington.
  – University of Lausanne.
  – United States Centers for Disease Control and Prevention.

• Potency assay reagent development partners:
  – USFDA.
  – Spring Valley Labs.