Developing a Trivalent Live Attenuated Influenza Vaccine

Workshop on Business Modeling for Sustainable Influenza Vaccine Manufacturing
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During the 2009-2010 H1N1 pandemic SIIL developed a monovalent live attenuated influenza vaccine (LAIV) against the pandemic 2009 H1N1 influenza virus strain.

This vaccine was licensed in July 2010 and >2.5 million doses have been marketed in India.

Based on this experience SIIL chose to develop a trivalent seasonal LAIV.
Seasonal trivalent LAIV

Individual monovalent pools of H1N1, H3N2 and Type B are prepared using master seed strains supplied by WHO.

- Manufacturing processes follow WHO guidelines and are identical for the three strains and follow the same steps used to prepare the H1N1 pandemic LAIV.
Influenza Vaccine (Human, Live attenuated)
Seasonal trivalent freeze dried

Lyophilized vaccine with the appearance of a white friable mass that is reconstituted with Sterile Water for Inhalation, USP

The final vaccine formulation consists of

- $H1N1 \geq 10^{7.0} \text{ EID}_{50}$
- $H3N2 \geq 10^{7.0} \text{ EID}_{50}$
- Type B $\geq 10^{6.5} \text{ EID}_{50}$

Antigenically identical to WHO recommended strains for that season
<table>
<thead>
<tr>
<th>Quality control test on bulk</th>
<th>Quality control tests on final vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Description</td>
<td>✓ Description</td>
</tr>
<tr>
<td>✓ Sterility</td>
<td>✓ Sterility</td>
</tr>
<tr>
<td>✓ Infectivity (Potency)</td>
<td>✓ Infectivity (Potency)</td>
</tr>
<tr>
<td>✓ Identity</td>
<td>✓ Identity</td>
</tr>
<tr>
<td>✓ Attenuation confirmation</td>
<td>✓ General Safety</td>
</tr>
<tr>
<td>- Genotypic &amp; Phenotypic</td>
<td>✓ Endotoxin</td>
</tr>
<tr>
<td>✓ Test for - Mycoplasma,</td>
<td>✓ Residual Moisture</td>
</tr>
<tr>
<td>- Mycobacteria</td>
<td></td>
</tr>
<tr>
<td>- Avian Leukosis Virus</td>
<td></td>
</tr>
<tr>
<td>✓ Test for Adventitious Agents</td>
<td></td>
</tr>
<tr>
<td>- In tissue culture and</td>
<td>✓ Inspection of Final container</td>
</tr>
<tr>
<td>- In embryonated eggs</td>
<td></td>
</tr>
</tbody>
</table>
Attenuation confirmation by genotypic and phenotypic testing

By RT-PCR followed by sequencing, the hemagglutinin (HA) and neuraminidase (NA) genes are matched to the reported sequences of the recommended strains. Presence of specific mutations on internal genes attributing to the temperature sensitive, cold adapted, attenuated phenotype are verified.

Temperature sensitivity, ts marker (RCT\textsubscript{40}) is determined by titration in chicken eggs at 40\textdegree C (Non-permissive temperature) and 32\textdegree C (Permissive temperature; confirmation of cold adaptation was done by verifying growth at 25\textdegree C).
## Virus Challenge studies in ferrets

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine</th>
<th>Challenge virus</th>
<th>Group description as used in text</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trivalent LAIV</td>
<td>H1N1 A/N’lands/602/2009</td>
<td>LAIV/pH1N1</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td></td>
<td>Placebo/H1N1</td>
</tr>
<tr>
<td>3</td>
<td>Trivalent LAIV</td>
<td>H3N2 A/N’lands/063/2011</td>
<td>LAIV/H3N2</td>
</tr>
<tr>
<td>4</td>
<td>Placebo</td>
<td></td>
<td>Placebo/H3N2</td>
</tr>
<tr>
<td>5</td>
<td>Trivalent LAIV</td>
<td>B/Brisbane/60/2008</td>
<td>LAIV/B</td>
</tr>
<tr>
<td>6</td>
<td>Placebo</td>
<td></td>
<td>Placebo/B</td>
</tr>
</tbody>
</table>
Haemagglutination inhibition titres

A

Trivalent

pH1N1 | H3N2 | B

Placebo

pH1N1 | H3N2 | B

Day after immunization

HI antibody titer

det limit
Results from ferret challenges

Single intranasal immunization with a trivalent live attenuated influenza vaccine induced:

- High HI antibody responses and to a lesser extent VN antibody responses,
- Reduced viral load,
- Reduced viral shedding and
- Reduced pathology after challenge
Present status

✓ Phase I, II/III clinical trials of Influenza Vaccine (Human, Live attenuated) Seasonal trivalent have been completed.

✓ A single dose of the both formulations of the Seasonal LAIV was found safe and immunogenic in adults, elderly and children above 2 years.

✓ Data have been submitted for licensure.
Manufacturing hurdles

• Requirement of strain specific license for the importation of new strains
• Availability of eggs due to lack of prior order commitment
• Final product testing at SIIL and the national laboratory not done in parallel delaying batch release
• Absence of clear guidelines for formulation using new strains
Scientific hurdles

• Clarification of IPR issues relating to the use of vaccine strains generated using reverse genetics technology

• Efficacy standards for LAIV: The IgG response to LAIV has been low in spite of high efficacy. Importance of assessing animal (ferret) data when evaluating LAIV.

Market uncertainties

Delays in licensure and pre-qualification plus the lack of vaccine orders from the Government of India and international agencies.