Update on Status of Investigation of Reduced LAIV Effectiveness

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ACIP Meeting: Feb 22, 2017
Presentation Overview

- Review of 2015-2016 vaccine effectiveness data including recent data on effectiveness of LAIV against influenza hospitalization
- Progress on non-clinical investigation
- Update on A/H1N1 strain selection for 2017-2018 season
- Ongoing studies and timelines for data availability
LAIV and IIV effectiveness estimates for all strains: 2015-2016 influenza season

Lower bound of CIs was truncated at –30.

1. Ambrose C. Presented at Advisory Committee on Immunization Practices Meeting; June 22, 2016; Atlanta, GA.
2. Flannery B. Presented at Advisory Committee on Immunization Practices Meeting; June 22, 2016; Atlanta, GA.
3. Caspard H et al. Presented at International Society for Influenza and Other Respiratory Virus Diseases (ISIRV) Options IX for the Control of Influenza Conference; August 25, 2016; Chicago, IL.
6. Caspard H. Abstract Accepted for Publication PAS, May 6-9, 2017; San Francisco, CA.
LAIV and IIV effectiveness estimates for B Strains: 2015-2016 influenza season

Lower bound of CIs was truncated at –30.

LAIV and IIV effectiveness estimates for A/H1N1pdm09 strains: 2015-2016 influenza season¹,²

Lower bound of CIs was truncated at –30.* Effectiveness estimate against any A strain.

1. Caspard H et al. Abstract accepted for presentation at: Pediatric Academic Societies Meeting; May 6-9, 2017; San Francisco, CA.
## LAIV effectiveness against influenza hospitalization in England and Scotland: 2015-2016 Influenza Season

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Public Health England(^1)</th>
<th>Health Protection Scotland(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-confirmed influenza due to any strain</td>
<td>54.5% (32, 68)</td>
<td>63% (50, 72)</td>
</tr>
<tr>
<td>Lab-confirmed influenza due to H1N1 pdm09 strains</td>
<td>48.3% (17, 68)</td>
<td>NA</td>
</tr>
<tr>
<td>Lab-confirmed influenza due to B strains</td>
<td>70.7% (33, 87)</td>
<td>NA</td>
</tr>
<tr>
<td>Clinical diagnosis of influenza</td>
<td>NA</td>
<td>68% (42, 83)</td>
</tr>
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</table>

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• **Progress on non-clinical investigation**

• Update on A/H1N1 strain selection for 2017-2018 season
• Ongoing studies and timelines for data availability
Vaccine effectiveness investigation

• Investigation currently focused on two potential hypotheses for root cause
  o Reduced replicative fitness of H1N1pdm09 LAIV strains in human cells
  o Vaccine virus interference from quadrivalent formulation

• Investigation approach
  o Biological characterisation of recent H1N1 strains vs historical effective LAIV strains
  o Focus on differences between pdm09 H1N1 CA09 & BOL13 vs pre-2009 H1N1 strains NC09 & SD07

<table>
<thead>
<tr>
<th>Pre-pandemic strains</th>
<th>Post-pandemic strains</th>
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<tbody>
<tr>
<td>New Caledonia 1999 (NC99)</td>
<td>California 2009 (CA09)</td>
</tr>
<tr>
<td>South Dakota 2007 (SD07)</td>
<td>Bolivia 2013 (BOL13)</td>
</tr>
<tr>
<td></td>
<td>Slovenia 2015 (SOLV15)</td>
</tr>
<tr>
<td></td>
<td>Pandemic (pdm)</td>
</tr>
</tbody>
</table>
Initiation of life-cycle focused investigation

Viral entry
- HA Stability
- HA Receptor binding

Replication & assembly
- HA abundance
- UTR mismatch

Biophysical
- Size, shape
- Defective particles

Budding / spread
- Multi-cycle replication
- Neuraminidase

Primary human cells
- Growth curves
- Competition

Ferret model
- Infectivity
- Immunogenicity
- Competition

Biological profiling of effective vaccine strains

Performance in translatable models
LAIV strains differ only in their external surface glycoproteins HA and NA

2 external HA & NA genes from WT virus
Confer antigenic match

6 internal genes from Master Virus
Attenuated phenotype

Hemagglutinin (HA) - responsible for the initial phase of virus replication
  – cell binding and cell fusion
Neuraminidase (NA) - responsible for late phase of virus replication
  – virus release and spread
The hemagglutinin (HA) protein is responsible for cell binding and cell fusion.

HA stability

- pH 7.0
- pH 5.5

Importance in Early phase of viral replication

- pH 7.0
- pH 5.5

Lee KK - EMBO J. (2010)

The hemagglutinin (HA) proteins of post-pandemic H1N1 viruses have properties that differ from pre-pandemic H1N1 viruses.

A/California differs as it is less thermostable

Potential susceptibility to heat

The hemagglutinin (HA) proteins of post-pandemic H1N1 viruses have properties that differ from pre-pandemic H1N1 viruses.

**HA stability**
- pH 7.0: Low temp
- pH 5.5: High temp

**HA thermostability**

**HA activation pH**
- Pre-pdm H1N1
- Pdm09 H1N1

**A/California differs as it is less thermostable**
Potential susceptibility to heat exposure during shipping/handling

**A/Bolivia differs as it is less pH sensitive**
Potential impact on viral replication life cycle

Viral Entry: Post-pandemic H1N1 viruses have reduced binding to human α2-6 cell receptors\textsuperscript{1,2}

- A/California and A/Bolivia strains have reduced binding to cell receptor

1. modified from Swiss Institute of Bioinformatics http://viralzone.expasy.org/
Replication: Post-pandemic H1N1 viruses less able to support multiple rounds of replication compared to pre-pdm H1N1 viruses

- Two assays used to measure infectivity
  - Fluorescent Focus Assay (FFA): one round of replication
  - Tissue Culture Median Infectious Dose (TCID$_{50}$): multiple rounds of replication

- For previous LAIV viruses these assays have given very similar results

- For A/California and A/Bolivia results differ

- Suggests that H1N1pdm09 viruses are less able to support multiple rounds of replication

Replication: Post-pandemic H1N1 LAIV strains have reduced replication in primary human nasal epithelial cells

![Graph showing virus titre (TCID₅₀/mL) over time (days post infection) for pre-pandemic and post-pandemic strains. Pre-pandemic strains include NC99, CA09, SDO7, and BOL13. Post-pandemic strains show reduced replication compared to pre-pandemic strains.]

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• **Update on A/H1N1 strain selection for 2017-2018 season**

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Assays previously used to select effective vaccine strains

**Virus Characteristics**

- Growth in Eggs
- Plaque Morphology
- Sequence Stability

**Immune-Response**

- Immunogenicity & Attenuation
- Antigenicity
- HAI & Neutralisation
New characterization assays introduced into strain selection process

**Virus Characteristics**

- Growth in Eggs
- Plaque Morphology
- Morphology
- HA Thermostability
- Sequence Stability
- Receptor Binding
- Acid stability and Fusion pH
- TCID\textsubscript{50} v FFA

**Immune-Response**

- Growth in Primary Human Nasal Epithelial Cells
- Immunogenicity & Attenuation
- Antigenicity HAI & Neutralisation
A/Slovenia strain has improved HA properties

- Higher activation pH compared to A/Bolivia strain

- Data suggest improved receptor binding vs Bolivia strain

A/Slovenia strain has significantly improved replication kinetics compared to A/Bolivia strain

- FFA and TCID$_{50}$ similar, unlike A/California and A/Bolivia

A/Slovenia strain has significantly improved replication kinetics compared to A/Bolivia strain

- FFA and TCID$_{50}$ similar, unlike A/California and A/Bolivia

• Improved replication in primary human cells compared to A/Bolivia

Summary of non-clinical data

• Initial findings of reduced replicative fitness with H1N1pdm09 viruses

• Underlying mechanism likely to be multi-factorial:
  - E.g. HA stability, HA activation pH, receptor binding, neuraminidase

• Current lead H1N1 candidate (A/Slovenia) identified for 2017-2018 LAIV:
  - No deficiency with multiple rounds of replication (FFA and TCID$_{50}$ match)
  - Higher HA activation pH vs. A/Bolivia
  - Higher replication in nasal epithelium vs. A/Bolivia

• Investigation ongoing:
  - Cell and ferret studies evaluating interference and formulation
  - Planned clinical study with 2017-2018 LAIV
A pediatric study is being planned to further compare the new A/Slovenia strain to the previous A/Bolivia strain.

Randomized, double-blind, study will enroll ~ 200 children 24 to <48 months of age.

Subjects will be randomized (~65 subjects per group) at 1:1:1 ratio to receive two doses of:

- LAIV4 2017-2018 (A/H1N1 Slovenia strain)
- LAIV4 2015-2016 (A/H1N1 Bolivia strain)
- LAIV3 2015-2016 (A/H1N1 Bolivia strain)

**Primary endpoint:**
- HAI antibody seroconversion rates after each dose

**Secondary endpoints:**
- Neutralizing antibody seroconversion rates after each dose
- Mucosal IgA increases after each dose
- Shedding after each dose
- Safety
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Timeline for data availability

**February 2017**
- 2015-16 VE meta-analysis
- 2015-16 VE hospitalized flu
- Preliminary 2017-18 H1N1pdm09 strain characterization

**June 2017**
- Final 2017-18 H1N1pdm09 (A/Slovenia) strain characterization
- 2016-17 VE (H3N2) data: UK, Finland, Canada

**October 2017**
- US pediatric shedding / immunogenicity data (new H1N1pdm09 strain)
- Japan 2016-17 pediatric efficacy study data (A/H3N2)
Conclusions

• LAIV demonstrated overall effectiveness in most studies conducted in 2015-16:
  o H1N1 effectiveness more variable and lower than IIV in all studies
  o Effectiveness against influenza hospitalization recently demonstrated

• Initial findings from investigation indicate that post-pandemic strains have reduced replicative fitness compared to pre-pandemic strains

• Based on investigation, new assays introduced into strain selection process:
  o Replacement A/H1N1 Slovenia strain selected for 2017-2018 has characteristics similar to pre-pandemic strains
  o Final nonclinical strain characterization data for the new A/Slovenia strain will be available in Q2 2017
Back up slides
Vaccine effectiveness investigation

- Following in-depth investigations, no support for the following:
  - H1N1 A/Bolivia development
    - Homology to circulating strains, antigenic match to A/California, growth in eggs, morphology, thermostability, ferret immunogenicity, MDCK cell infectivity, fusion pH
  - Manufacturing / Processing
  - QC Testing
  - Storage Stability
  - Distribution / Logistics
Pre-existing immunity among vaccinated children

- No statistically significant effect of prior season vaccination on LAIV VE was observed in either CDC or ICICLE studies in 2013-14 or 2015-16.

- In ICICLE and Finland studies, H1N1 VE estimates trended higher among previously vaccinated vs. not previously vaccinated.
  - ICICLE: 19% vs. 9% (2013-14); 60% vs. 35% (2015-16)
  - Finland: 74% vs. 25% (2015-16)

- Considered an unlikely root cause of the reduced VE

1. Ambrose C. Presented at Advisory Committee on Immunization Practices Meeting; June 22, 2016; Atlanta, GA