Influenza Vaccines

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Associate Professor of Medicine
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Overview

1) Biology & Epidemiology of Influenza (and other Respiratory Viruses)
2) Currently Approved Influenza Vaccines
3) Manufacture Process
4) Next-Generation Influenza Vaccines
5) ACIP recommendations
Known Respiratory Viruses

- Influenza
- Parainfluenza
- RSV (Respiratory Syncytial Virus)
- Adenovirus
- Rhinovirus
- Coxsackieviruses/Enteroviruses/Echoviruses
- Human Parechovirus (1999)
- Human metapneumovirus (2001)
- Human Bocavirus (2005)

Influenza Virus Biology

Orthomyxovirus
- Enveloped
- Segmented, negative sense RNA

Types:

A  most virulent, subtype based on HA and NA
B  milder but still causes outbreaks
C  mild illness, no epidemic potential

“H”
17 subtypes
Attachment to host cells

“N”
10 subtypes
Release from host cells
Epidemiology, Seasonal Flu

Seasonal epidemic curve
• Winter time
• Attack rates highest at extremes of age
• Mortality primarily in elderly (90%)

Treanor, 6th ed PPID
Glezen & Couch NEJM 1978;298:587–593
Therefore, there is a “Northern Hemisphere” and “Southern Hemisphere” influenza vaccine formulation every year.
Drift vs. Shift

- **SEASONAL** Influenza Viruses experience antigenic **drift** over time
- Result – need to update seasonal vaccine **annually**
Drift vs. Shift

Reassortment $\rightarrow$ Phenotypic Change

- **PANDEMIC** Viruses are a result of antigenic **shift**
- Result – completely “new” virus
WHO’s Global Influenza Surveillance Network (GISN)

1. Assist regulatory authorities to recommend strain selection for vaccines
2. Early detection of emergence and spread of new variants
3. Expanded East & South-East Asian circulation network

*Schematic of the dominant seeding hierarchy of seasonal influenza A (H3N2) viruses – 2002-2007*

C A Russell et al. Science 2008;320:340-346
Influenza Virus Strain nomenclature

A/duck/Alberta/35/76 (H1N1)
Unpredictable

Lab-confirmed Influenza: 2012-2017
Co-circulation with other Respiratory Viruses

Respiratory Viruses: 2012-2017

Number of Patients Positive (EIA, DFA, PCR or culture)

Week Beginning on Sunday
Problem 1: Influenza Virus Drift

- Twice each year (Feb & Sep), WHO Vaccine Composition Meeting selects 3-4 representative viruses
- Need to make best guess for predicting the circulating strains
"I hate it when we’re not sure we’re inoculating against the right strain of flu virus."
Strain Match/Mis-match

2014-2015
- H1N1, A/California/7/2009
- H3N2, A/Texas/50/2012
- B/Massachusetts/2/2012 (Yamagata)

2016-2017
- H1N1, A/California/7/2009
- H3N2, A/HongKong/4801/2014
- B/Brisbane/60/2008 (Victoria)

Vaccines: H1N1, A/California/7/2009
H3N2, A/Switzerland/9715293/2013
B/Massachusetts/2/2012 (Yamagata) 70% Yamagata-like, 30% Victoria-like
B/Brisbane/60/2008 (Victoria) 50% Victoria, 50% Yamagata
Problem 2: Dependence on Eggs

Requires massive egg supply
What about people with severe allergies to eggs?

Requires healthy poultry population
Conventional Manufacture Process (inactivated vaccine)

- Certified (quality control), temp-controlled **embryonated chicken eggs** are cleansed and sanitized
- Eggs inoculated with *working seed* virus
- Incubate 2-3 days, then **harvest** allantoic fluid
- **Clarify**: centrifuge to remove cellular debris
- **Purify**: zonal centrifugation (with density gradient) or column chromatography
- **Inactivate**: formaldehyde or betapropiolactone
- **Disrupt**: surfactant
- **Subunit**: separate subviral core from surface proteins, by different sedimentation rates
- **Split virus**: surfactant to disassemble subviral core
- Final **Sterile Filtration** step [result **monovalent bulk solution**]
Live Attenuated (LAIV)

Master Virus Seed (MVS) development uses patented Reverse Genetics Technology
- Cold-adapted virus backbone
- Insertion of HA and NA

- Eggs inoculated with *working seed* virus
- Incubate 2-3 days, then harvest *allantoic fluid*
- Clarify: centrifuge to remove cellular debris
- Stabilized: buffer (sucrose, potassium phosphate, monosodium glutamate)
- Blending and Dilutions for Final Trivalent Bulk vaccine
Conventional Vaccine Manufacture Timeline

<table>
<thead>
<tr>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order birds</td>
<td>House birds</td>
<td>Receive candidate seed viruses</td>
<td>Prepare high-growth reassortants</td>
<td>Start 1&lt;sup&gt;st&lt;/sup&gt; strain</td>
<td>Start 2&lt;sup&gt;nd&lt;/sup&gt; strain</td>
<td>Start 3&lt;sup&gt;rd&lt;/sup&gt; strain</td>
<td>Monovalent concentrate production</td>
<td>Potency test reagent preparation</td>
<td>Vaccine formulation</td>
<td>Vaccine filling</td>
<td>CBER/CDC</td>
</tr>
</tbody>
</table>

- CBER/CDC
- Manufacturing and Distribution
U.S. Approved Influenza Vaccines

In 2004:
- Fluzone (Aventis Pasteur)
- Fluvirin (Chiron)
- Flumist (MedImmune, 2003)

2017:
- Fluzone (SP)
  - High-Dose (2009)
  - Intradermal (2011)
  - Quadrivalent (2013)
- Fluvirin (Seqirus)
- Flumist (MedImmune)
  - Quadrivalent (2012)
- Fluarix (GSK, 2005)
  - Quadrivalent (2012)
- FluLaval (ID Biomed, 2006)
  - Quadrivalent (2013)
- Afluria (CSL, 2007)
  - Quadrivalent (2016)
- Agriflu (Novartis, 2009)
- Flucelvax (Novartis, 2012)
  - Quadrivalent (2016)
- Flublok (Protein Sciences, 2013)
  - Quadrivalent (2016)
- Fluad (Seqirus, 2015)
Problem 3: Yield of viruses

If the virus grows well in chicken eggs: (high growth reassortants)

- Inactivated: ~1-5 doses per egg
- LAIV: ~90 doses per egg
- ? Growth in various cell cultures?
Cell Culture-based Vaccines

MDCK cell line – Novartis & Solvay
Vero cell line - Baxter
PER.C6 cell line – Sanofi Pasteur
EB66 cell line - GSK
Cell Culture Vaccine, Efficacy

Phase III trial of Novartis MDCK based seasonal influenza vaccine (2008-09 season), 18-49 year old subjects

**Vaccine Efficacy of 84%**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th># with influenza</th>
<th>Attack Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucelvax</td>
<td>3776</td>
<td>7</td>
<td>0.19%</td>
</tr>
<tr>
<td>Placebo</td>
<td>3843</td>
<td>44</td>
<td>1.14%</td>
</tr>
</tbody>
</table>

Phase III trial of Vero cell culture based seasonal influenza vaccine (2008-09 season), 18-49 year old

**Vaccine Efficacy of 71.5%**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th># with influenza TOTAL</th>
<th># with influenza MATCHED*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero-cell</td>
<td>3619</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Placebo</td>
<td>3617</td>
<td>80</td>
<td>60</td>
</tr>
</tbody>
</table>

VE for matched strains 78.5%

* Antigenically matched to vaccine strains


Recombinant Purified Protein

Purified HA protein

FluBlok®

- Baculovirus expression vector system
- Grown in *Spodoptera frugiperda* insect cells

Promoter drives high-yield of HA protein

HA protein forms “rosettes”

Incubation ~48-72h

High-yield
No egg products
Recombinant Purified Protein, Efficacy

Phase III trial of PSC rHA based seasonal influenza vaccine (2007-08 season), 18-49 year old subjects

**Vaccine Efficacy of 45%**

Vaccine Efficacy for matched strains 67%

<table>
<thead>
<tr>
<th>Culture-positive ILI</th>
<th>N</th>
<th># with influenza TOTAL</th>
<th># with influenza MATCHED*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flublok</td>
<td>2344</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>Placebo</td>
<td>2304</td>
<td>78</td>
<td>6</td>
</tr>
</tbody>
</table>

* Antigenically matched to vaccine strains

Treanor et al. *Vaccine* 2011; 29: 7733-9
Next Generation Influenza Vaccines

Traditional Approach

Newer Approaches

Future Approaches

Virus-like particles and Virosomes

“Universal” vaccine
MF59 Adjuvanted

Long-term Care Facilities in Italy
Iob et al. Epidemiol Infect 2005

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>ILI, n (%)</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>No vaccine</td>
<td>184</td>
<td>56 (30.4%)</td>
<td></td>
</tr>
<tr>
<td>TIV</td>
<td>1168</td>
<td>302 (25.9%)</td>
<td>25% (0-45)</td>
</tr>
<tr>
<td>TIV+MF59</td>
<td>926</td>
<td>174 (18.8%)</td>
<td>94% (47-100)</td>
</tr>
</tbody>
</table>

Children (6-72 mo) in Finland & Germany
Vesikari et al. NEJM 2011; 365:1406-16

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Flu*, n (%)</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>No vaccine</td>
<td>993</td>
<td>47 (4.7%)</td>
<td></td>
</tr>
<tr>
<td>TIV</td>
<td>1772</td>
<td>50 (2.8%)</td>
<td>43% (15-61)</td>
</tr>
<tr>
<td>TIV+MF59</td>
<td>1937</td>
<td>13 (0.7%)</td>
<td>86% (74-93)</td>
</tr>
</tbody>
</table>

* lab-confirmed influenza

Therefore, Adjuvants may be useful for:
- Improved immunogenicity & protection
- Dose-sparing regimens
- Eliciting earlier responses
- Broader responses (heterotypic)

Antibody repertoire (by phage clone distribution)
Surrender et al. Sci Trans Med 2011
“Universal” Vaccine

- Long lasting
- Cross-strain protection
- “common epitopes” or highly conserved structures

Potential Targets:
- M2 ectodomain
- Conserved epitopes of NP and M1
- Stalk portion of HA protein
Problem 4: What is correlate of protection?

Serum Antibodies against HA protein, traditionally by HAI Assay

Alternatives:
- Microneutralization Assay
- CMI

Prevention ILI among Military Recruits

<table>
<thead>
<tr>
<th>Pre-epidemic HAI Titer</th>
<th>N</th>
<th>ILI N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1:4</td>
<td>44</td>
<td>18 (43)</td>
</tr>
<tr>
<td>1:8</td>
<td>41</td>
<td>12 (29)</td>
</tr>
<tr>
<td>1:16</td>
<td>72</td>
<td>20 (28)</td>
</tr>
<tr>
<td>1:32</td>
<td>75</td>
<td>9 (9)</td>
</tr>
<tr>
<td>1:64</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

% Infection with A/H3N2 intranasal challenge

<table>
<thead>
<tr>
<th>Pre-Neut Ab Titer</th>
<th>HK</th>
<th>Eng</th>
<th>PC</th>
<th>Scot</th>
<th>Vic</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1:2</td>
<td>80%</td>
<td>60%</td>
<td>70%</td>
<td>73%</td>
<td>100%</td>
</tr>
<tr>
<td>4-8</td>
<td>62%</td>
<td>0%</td>
<td>25%</td>
<td>9%</td>
<td>50%</td>
</tr>
<tr>
<td>16-32</td>
<td>44%</td>
<td>0%</td>
<td>33%</td>
<td>14%</td>
<td>0%</td>
</tr>
<tr>
<td>64-128</td>
<td>21%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>256-512</td>
<td>6%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>≥ 1024</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

From Couch RB: FDA/NIH/WHO Workshop 2007
Problem 5: Predicting the next “pandemic”

Wild aquatic birds are natural reservoirs

- Asymptomatic infection (low pathogenic avian influenza A virus, LPAI)
- Severe disease (highly pathogenic avian influenza A virus, HPAI)

Domestic Poultry
Avian Influenza

- H5N1 (1997 & 2003-ongoing)
- H7N9 (2013-ongoing)
- H7N7 (2003-15)
- H9N2 (2009)
- H7N3 (2012)
- H10N8 (2013)
Epidemiology of Pandemic Influenza

- Rapid surge of cases
- Second “wave” more severe

- “W-shaped” curve

- General: Unpredictable severity, mortality, patterns of spread
ACIP Recommendations

• Routine annual influenza vaccination of all persons aged ≥6 months is recommended

• Highest Risk:
  – Children 6 – 59 months
  – Adults 65 years or older
  – Persons with underlying conditions that predispose to more severe disease (includes pregnant women)

• Healthcare workers or others in direct contact with high-risk persons
## Flu Vaccines, Choices

<table>
<thead>
<tr>
<th>Route</th>
<th>Live Attenuated</th>
<th>Standard Inactive</th>
<th>High-dose</th>
<th>Cell-culture</th>
<th>Recomb</th>
<th>Intraderm</th>
<th>MF-59</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intranasal</td>
<td>IM</td>
<td>IM</td>
<td>IM</td>
<td>IM</td>
<td>ID</td>
<td>IM</td>
</tr>
<tr>
<td>Frequency</td>
<td>Annual</td>
<td>Annual</td>
<td>Annual</td>
<td>Annual</td>
<td>Annual</td>
<td>Annual</td>
<td>Annual</td>
</tr>
<tr>
<td>Approved Ages</td>
<td>2 – 49 yr</td>
<td>≥ 6 mon</td>
<td>≥ 65 yr</td>
<td>4 – 17 yr</td>
<td>≥ 18 yr</td>
<td>18-64 yr</td>
<td>≥ 65 yr</td>
</tr>
<tr>
<td>HA (μg/strain)</td>
<td>15</td>
<td>15</td>
<td>60</td>
<td>15</td>
<td>45</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Substrate</td>
<td>eggs</td>
<td>eggs</td>
<td>eggs</td>
<td>Cell-culture</td>
<td>Cell-culture</td>
<td>eggs</td>
<td>eggs</td>
</tr>
<tr>
<td>Common Side Effects</td>
<td>Sore throat, nasal congest</td>
<td>Inject site rxn</td>
<td>More inject site rxn</td>
<td>Inject site rxn</td>
<td>Inject site rxn</td>
<td>Erythema, inject site rxn</td>
<td>More inject site rxn</td>
</tr>
</tbody>
</table>

June 22, 2016: ACIP votes down use of LAIV for 2016-17 season
LAI V efficacy in children

CDC data slide has been deleted on purpose
LAIV against H3N2

CDC data slide has been deleted on purpose
Possible reasons for LAIV-4 Poor Performance

• Suboptimal performance (reduced fitness)
  – of A/Bolivia/559/2013 (H1N1)pdm09 HA vaccine component

• Potential interference among viruses in quadrivalent (two B components)

• Reduced immunogenicity due to more highly vaccinated population (compared to earlier years when vaccination uptake was lower)

LAIV controversy

- Canadian RCT (Hutterite community) LAIV 76.9% vs. IIV 72.3% efficacy

- Canadian NACI, LAIV can be used in children 2-17 years
- UK PHE, LAIV is the preferred vaccine for children 2-17 years
Sub-optimal Protection for Older Adults

• <60% vaccine efficacy

<table>
<thead>
<tr>
<th>Laboratory-confirmed influenza illness</th>
<th>Seroprotection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaccine group</strong></td>
<td><strong>Placebo group</strong></td>
</tr>
<tr>
<td>All ages</td>
<td>16/927 (1.7%)</td>
</tr>
<tr>
<td>60-69 years</td>
<td>12/649 (1.8%)</td>
</tr>
<tr>
<td>70 years and above</td>
<td>4/278 (1.4%)</td>
</tr>
</tbody>
</table>

• Can’t we do better?
  – Prevention illness, hospitalizations, death?
  – Longer lasting
  – Broad protection
  – Safer, less reactions
  – Easier, faster to manufacture

Thijs C. Lancet 2008; 8:460
Cost-Benefit

Would you want 99.9% Wilbur’s net worth or 30% of Bill Gate’s net worth?

• There is still great potential for significant public health impact for a vaccine with sub-optimal efficacy
Flu Vaccines for Older Adults

High-dose vaccine
• 24.2% relative efficacy, LCI\(^1\)
• 24% efficacy, Death\(^2\)
• 22.1% efficacy, Hospitalizations\(^2-3\)
• 22% efficacy, ILI\(^2-3\)

MF59 adjuvanted vaccine
• 60% effectiveness, LCI\(^4\)
• 51% efficacy, Hospitalizations\(^4\)
• 87% efficacy, acute coronary events\(^5\)
• 93% efficacy, cerebrovascular events\(^5\)
• 94% efficacy, ILI (long-term care facilities)\(^6\)

\(^1\) DiazGranados et al. NEJM 2014; 371: 635-45  
\(^2\) Shay et al. JID 2017; 215: 510-17  
\(^3\) Izurieta et al. Lancet ID 2015; 15: 293-300  
\(^4\) Domnich et al. Vaccine 2017; 35: 513-20  
\(^6\) Iob et al. Epidemiol Infect 2005; 133: 687-93
Summary

• Influenza viruses are difficult to predict
• Manufacture of influenza vaccines has expanded dramatically
• “Better” vaccines are needed
• Existing vaccines should be used