Development of MDCK Cell-Derived Influenza H7N9 Vaccines

Funded by NHRI and Taiwan MOHW

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On behalf of Novel Influenza Vaccine Research Group
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Zhunan, Taiwan
## Pipeline of Product Development in NIIDV, NHRI, Taiwan

<table>
<thead>
<tr>
<th>Group</th>
<th>Product</th>
<th>R&amp;D</th>
<th>IND</th>
<th>Clinical Trial</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Government Contract Production</td>
<td>BCG (licensed from Taiwan CDC)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Anti-venom horse sera (Taiwan CDC)</td>
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<tr>
<td>II. National Security Project</td>
<td>Influenza H5N1</td>
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<tr>
<td></td>
<td>Influenza H7N9</td>
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<td></td>
<td>Influenza H5N2</td>
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<tr>
<td></td>
<td>Enterovirus 71 (B4)</td>
<td></td>
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<tr>
<td></td>
<td>High-growth EV71 (B5)</td>
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<tr>
<td>III. PI-initiated project</td>
<td>Meningococcus B</td>
<td></td>
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<td></td>
<td>Adeno-vector RSV</td>
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<td></td>
<td>Therapeutic HPV vaccine</td>
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</tbody>
</table>
Timeline of Influenza Viruses in Humans

- **1918**: Spanish Influenza
- **1957**: Asian Influenza
- **1968**: Russian Influenza
- **1977**: Avian Influenza
- **1997**: Swine Influenza
- **1998/9**: Hong Kong Influenza
- **2003**: Swine Influenza
- **2009**: Pandemic vaccines

### Major Influenza Types

- H1
- H2
- H3
- H5
- H7
- H9

### Vaccines

- Seasonal vaccines
- Pandemic vaccines

### Lineages

- Spanish Influenza (two lineages)
Expectations for an Influenza Risk Assessment

Viruses - Highest Priority

H5N1 < H7N9

Viruses - Increasing Priority

H5N6

H9N2

American-lineage H5N2

High Ag Yield Reassortants, Lab Studies

Safety Studies

Production Scale-Up

Phase I Clinical

Vaccine Development

Taiwan Influenza Risk Assessment Network (TIRAN):
AHRI, Taiwan CDC, DOD, NHRI

Courtesy of Dr. Ruben Donis, US CDC
Control strategies in China, 2017: 1) promoting large-scale farming and centralized slaughtering, 2) improving poultry product cold chain transportation and storage at markets, 3) routine live poultry market closures with cleaning and disinfection, and 4) a national poultry vaccination program.
Viral and epidemiologic features identified during the fifth epidemic of Asian H7N9 in China (MMWR, 8 Sep 2017)

- Infections in humans and poultry were reported from most areas of China, including provinces bordering other countries.

- The risk to the general public is very low and most human infections were, and continue to be, associated with poultry exposure.

- During the fifth epidemic, mutations were detected among some Asian H7N9 viruses, identifying the emergence of high pathogenic avian influenza (HPAI) viruses as well as viruses with reduced susceptibility to influenza antivirals.

- The fifth-epidemic viruses diverged genetically into two separate lineages (Pearl River Delta lineage and Yangtze River Delta lineage), with Yangtze River Delta lineage viruses emerging as antigenically different compared with those from earlier epidemics.

- US CDC is working with partners to enhance surveillance for Asian H7N9 viruses in humans and poultry, to improve laboratory capability to detect and characterize H7N9 viruses, and to develop, test and distribute new candidate vaccine viruses (CVV).
Generation of Influenza Vaccine Seed Viruses

Classical reassortment

Ab Selection (1/2^8 or 1/256)

Cloning

Donor strain

+ 6:2 reassortant Vaccine

Donor strain

RT-PCR

Donor strain

Transfection Vero cells

RT-PCR

6:2 reassortant Vaccine

Mass production

Wt

NIBRG-14 vaccine strain from UK
NIBSC: NA & modified HA from A/Vietnam/1194/2004 (H5N1) and the other 6 gene segments from egg-adapted A/PR/8/1934 (H1N1)
Production of Influenza Vaccines

Egg-Based

- Advantage: history of success, low technology
- Disadvantage: hard to scale up, biosafety concern, labor-intensive, egg supply during pandemics caused by HPAI

Cell-Based

- Vero cells
- MDCK cells
- Insect cells (rHA, 2013, USA)
- Duck cells (H5 in 2014, Japan)
Progress in pre-pandemic & pandemic vaccine development

Egg-based technology

- Inactivated vaccines
  - Whole virus
  - Split virus
  - Embryonated egg
  - Subunit
  - Live attenuated

- Adjuvants

- Other immune-enhancing mechanisms
  - Such as virosomes

Methods of administration:
- Injection
- Intranasal
- Patch
- Intradermal

IFPMA IVS Tech Briefing, Nov. 20, 2007
Milestones of Cell-based Influenza H7N9 Vaccine in NHRI, Since May 2013

1. Virus adaptation in MDCK and Vero cells
2. Validation of master virus bank
3. Process development: disposable bioreactor and liquid chromatography
4. Obtaining potency assay (SRID) reagents
5. Mice immunogenicity
6. Ferret immunogenicity and protection study
7. Preclinical toxicology in rats and rabbits
8. Tech transfer to an industry partner (Medigen Vaccinology, Inc., April 2014)
9. Conduct phase I & II clinical trials (April 2015~1Q2017)
10. Taipei Biotech Award (台北生技銅牌獎), July 2016
11. Give a talk in the DCVMN’s Annual Meeting, Sep 2017
Ferret Study (immunogenicity & protection)

Materials & Methods:
- Age: 4~8 months of age (4 ferrets / group)
- Time: Nov 18 ~ Dec 30 (vaccinated at day 0 and 14, challenged at day 28, sacrificed at day 31 and 42)
- Control group: vaccine solvent (PBS) + adjuvant
- Vaccine: inactivated H7N9 whole virus vaccines
- Challenge study: A/Anhui/1/2013 (H7N9) in BSL-3

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA (ug)</td>
<td>0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Alum (ug)</td>
<td>300</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>HI (Nt) GMT</td>
<td>&lt;10 (&lt;40)</td>
<td>&lt;10 (&lt;40)</td>
<td>48 (48)</td>
</tr>
<tr>
<td>Post dose 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI (Nt) GMT</td>
<td>&lt;10 (&lt;40)</td>
<td>17 (80)</td>
<td>190 (640)</td>
</tr>
<tr>
<td>Post dose 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chia et al. PLoS One 2015
Pathological Examination in Lung Tissues of Ferrets Infected with Influenza A/Anhui/1/2013 (H7N9)

Group 1: Alum only  
F180 (DPI 3)

Group 2: HA only  
F187 (DPI 3)

Group 3: HA+ Alum  
F191 (DPI 3)
Clinical Trials of MDCK Cell-based Influenza H7N9 Vaccines in Taiwan (Medigen Vaccine Biologics Co.)
(Phase I & II in 200 Healthy Adults, NCT02464163)

<table>
<thead>
<tr>
<th>Group</th>
<th>HA dosage</th>
<th>Alum hydroxide</th>
<th>N</th>
<th>Post dose 2 HI antibody GMT, titer&gt;=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15 μg</td>
<td>0</td>
<td>45</td>
<td>24, 42%</td>
</tr>
<tr>
<td>II</td>
<td>15 μg</td>
<td>300 μg</td>
<td>48</td>
<td>22, 40%</td>
</tr>
<tr>
<td>III</td>
<td>30 μg</td>
<td>0</td>
<td>49</td>
<td>33, 51%</td>
</tr>
<tr>
<td>IV</td>
<td>30 μg</td>
<td>300 μg</td>
<td>48</td>
<td>36, 65%</td>
</tr>
</tbody>
</table>

Wu et al. Vaccine 2017
## Clinical Trials of Influenza H7N9 Vaccine Candidates

<table>
<thead>
<tr>
<th>Vaccine Antigens</th>
<th>Sponsor</th>
<th>Adjuvant</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>類病毒顆粒流感疫苗 (VLP)</td>
<td>Novavax</td>
<td>ISCO</td>
<td>Phase II</td>
</tr>
<tr>
<td>次單位疫苗 (MDCK cell-derived H7N9 subunit Vaccine)</td>
<td>Novartis Vaccines</td>
<td>MF59</td>
<td>Phase I</td>
</tr>
<tr>
<td>去活化流感病毒裂解疫苗 (Egg-derived H7N9 Split Vaccine manufactured by Sanofi)</td>
<td>National Institute of Allergy and Infectious Diseases (NIAID), NIH</td>
<td>MF59 or AS03</td>
<td>Phase II</td>
</tr>
<tr>
<td>去活化流感病毒裂解疫苗 (Egg-derived H7N9 Split Vaccine)</td>
<td>GSK</td>
<td>AS03</td>
<td>Phase I</td>
</tr>
<tr>
<td>去活化流感病毒裂解疫苗 (Egg-derived H7N9 Split Vaccine)</td>
<td>AdImmune (國光)</td>
<td>Alum</td>
<td>Phase II</td>
</tr>
<tr>
<td>去活化全流感病毒疫苗 (MDCK cell-derived inactivated whole virion Vaccine)</td>
<td>Medigen Vaccinology (基亞疫苗)</td>
<td>Alum</td>
<td>Phase II</td>
</tr>
<tr>
<td>活性減毒疫苗 (egg-derived LAIV)+去活化流感病毒次單位疫苗</td>
<td>NIAID, NIH</td>
<td>None</td>
<td>Phase I</td>
</tr>
<tr>
<td>活性減毒疫苗 (egg-derived LAIV)</td>
<td>俄國流感研究中心</td>
<td>None</td>
<td>Phase I</td>
</tr>
<tr>
<td>DNA疫苗)+去活化流感病毒次單位疫苗</td>
<td>NIAID, NIH</td>
<td>None</td>
<td>Phase I</td>
</tr>
<tr>
<td>H7重組蛋白疫苗</td>
<td>Protein Sciences Corporation</td>
<td>2% SE</td>
<td>Phase II</td>
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</table>
Clinical Evaluation of Influenza H7N9 Vaccines Worldwide

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Production platform</th>
<th>Antigen type</th>
<th>Adjuvant</th>
<th>Antibody titers in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Egg</td>
<td>Split</td>
<td>None</td>
<td>Low</td>
</tr>
<tr>
<td>2</td>
<td>Egg</td>
<td>Split</td>
<td>AS03</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td>Egg</td>
<td>Split</td>
<td>MF59</td>
<td>Intermediate</td>
</tr>
<tr>
<td>4</td>
<td>Suspensive MDCK cell</td>
<td>Subunit</td>
<td>MF59</td>
<td>Intermediate</td>
</tr>
<tr>
<td>5</td>
<td>Suspensive MDCK cell</td>
<td>Subunit</td>
<td>None</td>
<td>Low</td>
</tr>
<tr>
<td>5</td>
<td>Adherent MDCK cell</td>
<td>Virion</td>
<td>None</td>
<td>Intermediate</td>
</tr>
<tr>
<td>6</td>
<td>Adherent MDCK cell</td>
<td>Virion</td>
<td>Al(OH)_3</td>
<td>Intermediate</td>
</tr>
<tr>
<td>7</td>
<td>Insect cell</td>
<td>VLP</td>
<td>None</td>
<td>Low</td>
</tr>
<tr>
<td>8</td>
<td>Insect cell</td>
<td>VLP</td>
<td>ISCO</td>
<td>High</td>
</tr>
<tr>
<td>9</td>
<td>Egg</td>
<td>Live virus</td>
<td>None</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

More formulations: inactivated whole virion and adjuvants.
Conclusions

1. Based on published clinical studies, influenza H7N9 inactivated whole virus antigens seem to be more immunogenic than VLP, split and subunit antigens.

2. Influenza H7N9 inactivated whole virus antigens formulated with emulsion-based adjuvants (MF59 or PELC) are highly immunogenic in ferrets and clinical evaluations are warrant.

3. To encourage development of pandemic influenza vaccines, national stockpile policy for pandemic influenza vaccines is necessary.

4. Since influenza pandemic is not predictable, it is desirable to separately stockpile vaccine antigens and adjuvants.
Development of Influenza H5N2 and H7N9 Vaccines: teams and advisors

Nov 2012 Influenza Symposium

10 April 2013 Vaccine Strain Selection Meeting

26 March 2014 Advisor Meeting

17 Oct 2014 Advisor Meeting
Acknowledgements

- Scientific Advisors, NIIDV, NHRI, Taiwan
- IPM, National Defense Medical Center, Taiwan
- AHRI, COA, Taiwan
- MOHW, Taiwan
- CDC, Taiwan
- MOST, Taiwan
- FDA, Taiwan
- CDE, Taiwan
- NIBSC, UK
- NIID, Japan
- US CDC
- US FDA


Postdoc positions are available. Please contact minshi@nhri.org.tw.