Current Status of Development and Evaluation of Vero Cell-Derived Vaccines

Otfried Kistner

The First WHO Integrated Meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses
January 24 - 26, 2013, Hong Kong SAR, China
The following information and data has been presented at the “The First WHO Integrated Meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses” in Hong Kong SAR, China on January 24 - 26, 2013 and reflects Baxter’s then available knowledge.

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Overview

- Vero Cell Technology Platform
- Pandemic Vaccine Program: H5N1, H1N1pdm09, H9N2
- Seasonal Vaccine PREFLUCEL
- Summary & Conclusions
- References
Vero Cell Technology Platform
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Conventional Influenza Vaccine Production in Embryonated Hens’ Eggs

Embryonated eggs are stored for 10 to 12 days, constantly turned to ensure the healthy development of the embryos.

Courtesy: Solvay

Embryonated eggs at 10 to 12 days being inoculated by automated machinery. 1st larger needle (about 1 mm diameter) punches a hole in a shell and 2nd smaller needle injects a seed into the allantoic cavity of the egg followed by incubation for 2 to 3 days. It takes less than 10 seconds to inoculate a row of eggs.

Courtesy: Solvay

Eggs being candied to evaluate their quality: left – healthy egg – unhealthy one to be removed.

Courtesy: Solvay
Influenza has been a significant public health problem worldwide, with three pandemics during the past century. Immunization is the most effective measure to control an influenza pandemic. Since rapid production of large amounts of influenza vaccine depends on the availability of fertile hens’ eggs to grow the viruses, there is an urgent need for the development of alternative cell culture systems, which would allow rapid scale-up of production in the event of a pandemic. This WHO meeting discussed the results of studies from several laboratories on the cultivation of influenza viruses in stable cell lines, and made recommendations for further work.
Vero cells are an optimal basis for the production of vaccines:
- accepted by Regulatory Authorities in more than 60 countries worldwide
- used for the production of licensed vaccines for nearly 30 years, mainly polio and rabies; more recently Rota as well

Baxter has a fully characterized Vero Cell line:
- Baxter‘s Vero cell vaccines are licensed in the EU as well as in the US, Australia, New Zealand, Singapore, and Brazil

Use of a serum protein free medium

Potential for production of vaccines against a wide variety of viral diseases

Suitable for production of all types of viral vaccines:
- inactivated whole virus
- split
- subunit
- live-attenuated

Baxter’s Vero Cell Based Vaccine Production Facility in Bohumil, Czech Republic: Fermentation
Summary – Baxter’s Vero Cell Technology for Influenza Vaccines

- Fully characterized continuous cell line
- Serum protein free medium
- Well-established, highly standardized, robust and closed manufacturing process:
  - GMP production of more than 80 millions of doses of influenza vaccines with a total of 17 different seasonal (H1N1, H3N2, B) as well as pandemic (-like) H5N1, H1N1, and H9N2 WHO-recommended vaccine strains since 2003
- Allows for shorter and more flexible production cycle compared to traditional egg-based influenza vaccines
- Enables use of the original virus instead of reassortants
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Baxter’s Pandemic Influenza Vaccine Program

- State-of-the-Art Vero Cell Culture Technology: Independence from hen’s eggs
- Use of wild-type virus, not reverse genetics
- Use of whole virus, not split or subunit vaccine
- Not adjuvanted
- Proven for Production of
  - H5N1 A/Vietnam/1203/2004
  - H5N1 A/Indonesia/05/2005
  - H1N1pdm09 A/California/7/2009
  - H9N2 A/chicken/Hongkong/G9/97 (RG)
24 April 2009
First Report on „Swine Flu“

4 May 2009
Baxter receives the virus from US CDC

24 July 2009
Internal release of first batch

12 Aug 2009
First Baxter shipment to customers
H5N1 & H9N2 Clinical Development Program

- **EU Mock-up Licensure (A/Vietnam/1203/2004)** License
  - 4 Phase I/II/III studies including
    - dose finding
    - heterologous booster with Indonesia (clade 2.1) 12 – 17 month after primary immunization with Vietnam 1203 (clade 1)
    - study in younger (18-59 years) and elderly (60+ years) adults with 6, 12-15 and 24 month booster
    - supportive study with Indonesia

- **EU Pre-Pandemic Licensure (A/Vietnam/1203/2004)** License
  - 3 Phase I/II/III studies including
    - ped’s, adults, elderly, and risk groups
    - supportive Single Prime Boost study

- **Japan H5N1 Vaccine Licensure (A/Indonesia/05/2005)**
  - Phase II/III study in adults

- **US Studies**
  - 3 Phase I/II studies in adults with Vietnam 1203, RG Indonesia, RG H9N2

N > 6700 Subjects
Primary Objective
- To identify the immunogenicity and safety of different doses of adjuvanted and non-adjuvanted mock-up pandemic influenza vaccine

Subjects
- 270 subjects aged 18 to 45 years, healthy volunteers

Vaccination
- 2 vaccinations on day 0 and day 21 (A/Vietnam/1203/2004 vaccine)

Six arm study (45 subjects / arm)
- 3.75, 7.5, 15 and 30 µg with aluminum adjuvant
- 7.5 and 15 µg non-adjuvanted

Study sites
- Austria and Singapore
H5N1 Seroprotection Phase I/II Dose-Escalation Study
Seroprotection Rate (MN) against A/Vietnam/1203/2004

Primary Objective

- Assess immunogenicity and safety of a heterologous booster 12 to 17 months after two-dose priming vaccination
- Assess antibody persistence 180 days after a heterologous booster vaccination

Subjects

77 (out of 141 eligible) subjects

Vaccination

Booster vaccination with 7.5 μg HA A/Indonesia/05/2005 at 12-17 months after first vaccination in Phase I/II (A/Vietnam/1203/2004)

Study site

Austria
Phase II Study – 12 to 17 Months Booster with Indonesia Vaccine Seroprotection Rate (MN) against Indonesia

Percentage of subjects with neutralising antibody response (MN titer ≥20)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 21</th>
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</thead>
<tbody>
<tr>
<td>3.75µg adjuv.</td>
<td>81%</td>
<td>94%</td>
<td>100%</td>
</tr>
<tr>
<td>N=17</td>
<td></td>
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<td></td>
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<tr>
<td>7.5µg adjuv.</td>
<td>93%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>N=15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5µg</td>
<td>91%</td>
<td>91%</td>
<td>92%</td>
</tr>
<tr>
<td>N=12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15µg adjuv.</td>
<td>100%</td>
<td>100%</td>
<td>92%</td>
</tr>
<tr>
<td>N=13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15µg</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>N=8</td>
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<tr>
<td>30µg adjuv.</td>
<td>88%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>N=12</td>
<td></td>
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</tbody>
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Phase II Study – 12 to 17 Months Booster with Indonesia Vaccine

Heterologous Seroprotection (MN) against Different Clades

21 days post booster

% Subjects with MN titer ≥1:20

- 3.75 µg adj.
- 7.5 µg adj.
- 7.5 µg
- 15 µg adj.
- 15 µg
- 30 µg adj.

N=17  N=15  N=12  N=13  N=7  N=12

Indonesia/05/2005 Clade 2.1
Vietnam/1203/2004 Clade 1
turkey/Turkey/1/2005 Clade 2.2
Anhui/1/2005 Clade 2.3

Baxter’s Vero cell derived whole virus H5N1 vaccines against A/Vietnam/1203/2004 and A/Indonesia/05/2005 demonstrate excellent tolerability profile that is highly consistent across all clinical studies.

Safety profiles in healthy populations and risk groups (elderly adults, chronically ill, immunocompromised) are comparable.

The vaccine was safe and well tolerated in all three pediatric age strata i.e. 6 – 35 months, 3 – 8 years, and 9 – 17 years.

Systemic and local reaction rates were low and predominantly mild and transient.
Baxter’s H5N1 Clinical Development Program –
Conclusions "Immunogenicity"

- Vaccine doses as low as 3.75 µg or 7.5 µg are highly immunogenic following two dose immunization, as shown in the MN and the SRH test.
- Non-adjuvanted formulations are more immunogenic than the adjuvanted ones.
- Vaccine induced substantial immune responses in immunocompromised and chronically ill individuals.
- Vaccine shows cross-neutralization against widely divergent H5N1 strains.
- Vaccine is capable of inducing long-lasting cross-clade immunological memory response that can be effectively boosted up to 24 months following priming.
- Similarly strong booster response is achieved following either a two dose or single dose priming schedule.
- Vaccine induced same level of cross-clade reactive CD4+ T-cell and memory B-cell responses adults (18–59 years) and elderly (≥60 years).
- Vaccine induces effectively Neuraminidase-inhibiting (NAI) antibodies.

A single heterologous boost with the clade 2.1 vaccine Indonesia 12 – 17 months after the primary immunization with the clade 1 vaccine Vietnam 1203 resulted in the rapid induction of very high titers against the initial vaccine and the booster strain.

Seroneutralization rates between 90% and 100% against the priming vaccine and the booster vaccine were already obtained 7 days after the booster vaccination.

This heterologous prime-boost effect with the Indonesia vaccine after the primary immunization with the Vietnam 1203 vaccine has been confirmed in a Phase III study in two different age groups, in younger (18 – 59 years) and older (60+ years) adults.

The rapid induction of a protective immune response was not only achieved against the clade 1 strain Vietnam 1203 and the clade 2.1 strain Indonesia, but also against representative viruses of clade 2.2 (turkey/Turkey) and 2.3 (Anhui) not being covered by the vaccination strategy; indicative of cross-protective memory.

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Clinical Studies with Seasonal Vero Influenza Vaccines (Inactivated Split TIV‘s)


- Safety and immunogenicity in all age groups from 18 years and risk groups
- Efficacy in young and healthy adults from 18 – 49 years
- 15,045 subjects
  - Vero Vaccine  9001
  - Egg Vaccine  599
  - Placebo  5461
Conclusions – **Efficacy**

**Primary Endpoint of Vaccine Efficacy Met**

- Overall vaccine efficacy against matching strains is 78.5% (CI 60.8 – 88.2%)
- Overall vaccine efficacy against matching and non-matching influenza strains is 71.5%
- Protective Efficacy against all circulating viruses proven over the whole influenza season 2008/2009

**Reduction of Disease Symptoms**

- The vaccination significantly reduces the duration and severity of disease symptoms in those individuals in which infection is not prevented


Ehrlich et al. A Cell Culture-Derived Influenza Vaccine Provides Consistent Protection Against Infection and Reduces the Duration and Severity of Disease in Infected Individuals. Clinical Infectious Diseases 2012, 54: 946-954
Conclusions – **Immunogenicity**

 **Consistent Immunogenicity**

with regard to achieving all 9 of 9 CHMP criteria for rate of seroprotection, rate of seroconversion and GM-fold increase confirming data from 3 Phase III Studies with more than 8000 younger and elderly adults, i.e. 27 out of 27 criteria

 **Correlate of protection**

Vaccine efficacy correlates with an HI titer of ≥ 15, no added benefit at HI titer > 30

Ehrlich et al. Clinical development of a Vero cell culture-derived seasonal influenza vaccine. Vaccine 2012, **30**: 4377-4386
Overview

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Overall Summary – Pandemic Influenza Vaccines

- The combination of

  - Preclinical Data
    - H5N1 protection studies in mouse and ferret challenge studies (lethal model)
    - H5N1 passive transfer studies using sera from human studies (lethal model)
    - H1N1 protection studies in mice and ferrets (non-lethal models)
    - H1N1 passive transfer studies with human sera in transgenic mice (lethal model)
    - H9N2 protection studies in mice (non-lethal model)

  - Clinical Data
    - H5N1 immunisation (priming and booster studies) – humoral and cellular data
    - H1N1 primary immunisation studies
    - H1N1 efficacy studies
    - H9N2 primary immunisation studies

Indicate that Vero cell derived whole virus pandemic vaccines are highly protective
12 weeks between receipt of a pandemic virus and availability of internally and externally released vaccine lots

No adjuvant required – whole virus structure may work as an adjuvant itself

Cross- Protection against all relevant H5N1 clades which cause frequent human infections by heterologous Prime-Boost vaccinations

Pandemic (-like) H5N1 and H1N1 vaccines are safe and immunogenic in all age groups from 6 months of age onwards

Seasonal vaccine has shown high efficacy against matching and non-matching strains over the whole influenza season 2008/2009

EMA / FDA accepted protective HI titer of 1:40 confirmed with a Vero-derived seasonal influenza vaccine (Preflucel)
Baxter’s Licensed Influenza Vaccines

**Pandemic Influenza Vaccine H5N1 Baxter**
- H5N1 licensed in EU, CH, Australia, NZ, Singapore

**Pandemic Mock-Up Vaccine**
- H1N1pdm09 licensed in EU, Australia, NZ, Brazil

**Pre-pandemic H5N1 Vaccine (A/Vietnam/1203/2004)**
- Licensed in EU

**Seasonal Influenza Vaccine**
- Licensed in 15 European countries and Brazil
- Submitted for licensure in several Asian-Pacific and Latin-American countries
## Acknowledgements

**Austria**  
Markus Müller  
Herwig Kollaritsch  
Franz Ambrosch  
Katharina Riedl  
Reinhard Lober  
Fritz Pinl

**Singapore**  
Paul A Tambyah  
Dale Fisher  
Helen Oh  

**Germany**  
Petra Staubach  
Frank Wagner  
Bernhard Schmitt

**CRO**  
PPD Asia

**Hong Kong**  
KY Yuen  
Malik Peiris  
Paul KS Chan  
David Hui  
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**Baxter**  
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This project has been funded in whole or in part with Federal Funds from the Office of Public Health Emergency Preparedness, Office of Research and Development Coordination, under Contract No HHSO100200600013C to DynPort Vaccine Company LLC CSC.
Vero Cell Technology Platform

Pandemic Vaccine Program: H5N1, H1N1pdm09, H9N2

Seasonal Vaccine PREFLUCEL

Summary & Conclusions

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Vaccine 2012 30: 5956-5966

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