Pre-pandemic Influenza Vaccines: Breaking the Immunogenicity Barriers and Broadening Immune Responses with Adjuvants and Formulations

Dr. Prakash Sambhara, D.V.M., Ph.D., Centers for Disease Control & Prevention
Atlanta GA 30333
Current situation with Avian Influenza H5N1 viruses

• H5N1 viruses detected in birds in 65 countries

• 15 countries reported over 300 human cases with about 60% case fatality due to H5N1 virus infection

• Virus has drifted into genetically distinct clades (0-9) with multiple subclades in each clade

• Currently licensed tri-valent seasonal vaccines consisting of H1N1, H3N2, and B components do not provide protection against H5N1 viruses

Preventive vaccination is the most cost-effective intervention strategy
Vaccine Technologies

Pre-Pandemic Influenza Vaccines
- Cell-derived: Wild-type seed virus, Whole inactivated, RG-seed virus, inactivated, Detergent Split
  - Baxter, Novartis, GSK, Sanofi, Solvay, Crucell, Green Hills Biotech
- Egg-derived: RG-seed virus, Whole inactivated, Detergent split, Cold-adapted
  - Sinovac, Omnivest, Novartis, GSK, Sanofi, Crucell, Medimmune
- Recombinant proteins: Insect, Plant, or Algae, Insect/mammalian/Plant, fungi derived
  - protein Sciences, Fraunhofer CMB, Globeimmune, Neurogenisis
- Virus-like Particles (VLP): Insect/mammalian/Plant, Fungi derived
  - Novavax, Ligocyte, Medicago, Technovax, Epixis
- M2e Vaccine and NP
  - Acambis, Vaccinnate, Dynavax, Merck
- DNA Vaccine
  - Vical, Inovio, VRC
- Viral Vectors: Adeno, Pox, VSV, RNA replicon
  - PaxVax, Vaxin, VRC, GenVec, AlphaVax, Vaxart
Egg-derived rgA/VN/1203/04 without adjuvant
Figure: “Target” paradigm of H5N1 pandemic vaccine development
Challenges: Vaccine availability in a pandemic

• Why Avian HA is NOT very immunogenic?

• Lack of manufacturing capacity to make enough doses of vaccine for 1.2 billion people who may be at risk

• H5N1 viruses are highly lethal to poultry and ensuring the availability of eggs for vaccine production becomes a problem

• Production capacity of egg-independent vaccine strategies

• Availability of vaccine to the developing world at an affordable price

• Short timeframe to make a vaccine during a pandemic

• Dose-sparing with novel adjuvants and formulations
Pioneers in Adjuvant Research

Gaston Ramon (1886-1963)

**Adjuvants:** Breadcrumbs, Agar, Tapioca, Starch, Oil lecithin

**Antigens:** diphtheria and tetanus toxins

Alexander Thomas Glenny (1882 - 1965)

**Alum** adsorption of Toxoid

Jules T. Freund (1890 – 1960)

**Oil-in-water emulsion**
Complete and incomplete adjuvants
Ideal Adjuvant/Formulation

1. Safe (alone or in combination, potential risk and benefit)
2. Biodegradable
3. Induce robust immune response
4. Chemically and biologically well defined
5. Antigen dose-sparing
6. Stable
7. Affordable
8. Induces humoral and cellular immunity
Pre-pandemic influenza Vaccines

Egg-derived rgH5N1 vaccines with Novel Adjuvants

GSK
• 2 doses of 3.8µg with ASO3 induced about 80% seroconversion
  • Cross-reactivity with clade 2 viruses (published)

Sanofi-Pasteur:
• 2 doses of 1.9µg or 3.75µg with AFO3 induced seroconversion in more than 70% and 80% of the vaccinees
  • Cross-reactivity with clade 2 viruses (published)

Novartis: MF59 adjuvant showed significant seroprotection rates and cross-clade reactivity
Bird flu vaccine to hit the shelves

Europe approves pandemic vaccine; countries must decide own strategies.

Tony Scully

GlaxoSmithKline hopes the vaccine will at least buy governments some time in the event of a bird flu pandemic. The European Commission has approved a new vaccine against the H5N1 bird flu virus — the first vaccine designed to ward off a future pandemic. But how the drug, called Prepandrix, will be deployed by national governments remains unclear. The vaccine, produced by the UK drug giant GlaxoSmithKline, is aimed at the H5N1 strain currently circulating in birds as epidemiologists think that this is the most likely strain to cause a human pandemic. H5N1, which originated in south-east Asia and is carried by migrating birds and domestic poultry, has caused 382 human cases and 241 deaths worldwide since 2003. Prepandrix targets an antigen from an H5N1 strain called A/Vietnam/1194/04, which has been detected in birds in Asia, Europe and Africa.
Cross clade (2 and 3) neutralizing immune responses

Alum did not improve the antibody response

7.5 µg and 15 µg of hemagglutinin antigen without adjuvant produced the maximum responses.

Is glycosylation pattern influencing the immunogenicity?

Virion RNA is mediating the adjuvant effect?
Egg-independent strategy

H5N1 A/Indonesia/05/2005 (clade 2) VLP Vaccine: Results from a Phase I/IIa Study

2 doses of vaccine

Percentage of subjects with virus neutralization titer >1:20 (>4-fold rise in titer)

15 µg – 72%
45 µg – 73%
90 µg – 94%
Placebo – 0%

Cross-clade neutralization?
Adjuvant may achieve further dose-sparing
Pathogen Sensors of Innate Immune System

**Extracellular-Soluble**
- Collectins and Ficolins
  - SP A & D
  - MBL
  - Ficolins L, M, and H
- Pentraxins
  - PTX3, PTX4, NPTX1, NPTX2
  - CRP, SAP
- PLUNC
  - BPI
  - LPS-binding protein
- Complement

**Membrane-bound**
- Toll-like Receptors
  - TLRs 1,2: 2,6: 4,5
- Lectin-like receptors
  - Mannose Receptor
  - DC-SIGN
  - SIGNR1
  - Langerin
  - Dectin-1 & 2
- Scavenger Receptors
  - SR-AI, SR-AII, SR-AI, SR-AII
  - MARCO
- Integrins
  - Mac-1

**Vesicular**
- Toll-like Receptors
  - TLR-3
  - TLR-7/8
  - TLR-9

**Cytoplasmic**
- RNA Sensors
  - RIG-I like receptors
    - RIG-I
    - MDA5
- Peptidoglycan Sensors
  - NOD-like Receptors
    - NOD1 & 2
    - Ipaf
    - cryopyrin
- DNA Sensors
  - DAI (DLM-1/ZBP1)
Oil-in-water emulsion for parenteral administration

Preliminary data
Study Design

Adjuvant Base
- **Water phase**: PBS and Tween
- **Oil phase**: Squalene, alpha-tocopherol
- **PAMPs**: Pam3CSK4, poly dAdT, LPS

**Vaccine**: rgA/VN/1203/04xA/PR/8/34.  
Dose range: 3, 1, 0.3, and 0.1 µg of HA  
PAMPs: 10ηg

**Immunizations**: 2 doses at 4 weeks apart
Imunogenicity 21-days post-booster in mice

Vaccine 0.1µg of HA
TLR ligands 10ηg

*,#,@ indicate statistical significance p<0.01 to <0.001
Ongoing studies......

• Immunological assessment: Humoral and cellular
• Protection against challenge
• Adjuvant combinations
• Alternate routes