Review of New Vaccine Platforms and Influenza Vaccine Pipeline

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Health and Human Services
Public health impact of influenza

• **Significant annual morbidity and mortality**
  — Globally 3-5 million severe illnesses and 0.25-0.5 million deaths

• **Groups at high risk of severe disease**
  — Very young and very old
  — Chronic underlying cardio-pulmonary disease
  — Pregnant women
  — Immunocompromised

• **Vaccination is the cornerstone of prevention**
  — Seasonal vaccines only 30% to 50% efficacy in older adults
  — Candidate pandemic vaccines are poorly immunogenic

• **Global shortfall of vaccine supply for a pandemic outbreak**
Current influenza vaccines

• Safe and efficacious
  – Established track record in most age groups

• Formulated / standardized on HA content to induce neutralizing antibodies

• Vulnerable to drift/shift of HA and NA
  – Antibodies target highly variable HA and NA regions
  – Protection following infection/vaccination is limited to specific strains
  – Little to no known cross-protection against drifted strains within subtypes or to other subtypes
Current influenza vaccines

• Long established production processes
  — Predominantly produced in embryonated eggs

• Require constant reformulation
  — Costly, time-consuming, year-round process
  • Surveillance, vaccine seed production, optimization of growth, reagent production, potency testing, stability, formulation, delivery
  • Repeated for northern and southern hemispheres

• Suffer from unpredictable yields and growth properties

• Poorly responsive to surge capacity for a pandemic outbreak
Goal: Develop technologies to address unmet needs

- Safety
- Capacity
- Low cost
- Broadly Reactive
- Rapid Response
- Simple Manufacture

Need for new high performance Platform Technologies
# Influenza Vaccine Landscape

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<th>Pre Clinical</th>
<th>Phase 1</th>
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<th>Phase 3</th>
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Updated: 07/07/2011
## Pre-Clinical Influenza Vaccine Landscape

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**Minimum of 26 technologies in pre-clinical development**
# Phase 1 Influenza Vaccine Landscape

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**Minimum of 25 technologies in Phase 1**
## Phase 2 Influenza Vaccine Landscape

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Minimum of 16 technologies in Phase 2
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### Minimum of 7 technologies in Phase 3
# Licensed Influenza Vaccine Landscape

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**Minimum of 18 vaccines licensed**

- Seasonal
- Pandemic
- Seasonal & Pandemic
- US License

*Updated: 07/07/2011*
Live-attenuated influenza viruses (LAIV)
Technology attributes - LAIV

- Nasal administration
- Efficacious, Strong safety record
- Antigen sparing (high yield, low-dose)
- Broadened immune response (mucosal, CMI)
- Produced in eggs or cell culture
- Rapid response time to produce vaccine
- Low cost
- Easily transferrable technology to developing country vaccine manufacturers
Technology Description: Cold-Adapted LAIV

Wild-type
- WHO recommended seasonal strain
- Virulent
- Infects nasal passage and respiratory tract

Cold-adapted
- Non virulent
- Master seed
- Infects nasal passages only

Wild-type virus provides genes coding the surface antigens, HA and NA. Stimulates protective immune response.

Attenuated vaccine
- Antigens from WT virus
- Replicates in nasal passages only

Master seed virus provides six genes required for influenza propagation
LAIV based on deletion of interferon antagonist NS1

NS1 inhibits the activity of innate immune responses\(^1\)

NS1 is required for efficient influenza replication

Technology challenges - LAIV

- Unknown correlates of immunity/efficacy
  - Regulatory challenge
- Reduced efficacy in non-naïve adults vs. TIV
- Current limitations on use in very young children and some high risk groups
- Limited use in pre-pandemic settings (fear of reassortment risk)
Recombinant virus-like particles (VLP)
Technology attributes - VLP

• Contain multiple influenza proteins to resemble virions with no genetic material
• Improved “strain-matching” ability
• Rapid production and response time
• High yields (continuous harvest)
• Produced in cell culture
• Broadened immune response (antigen presentation, cellular immunity)
• Easily transferrable technology
• Many varieties in development
Chimeric VLPs (Novavax, Ligocyte, others)

Multi-Antigen VLPs can be formed by simultaneous expression of core protein and antigens in insect cells.

- Antigens traffic to lipid rafts on cell surface
- Gag protein buds out at lipid rafts to form VLPs

**Triple Expression Vector**

- M1/gag
- NA
- HA

**Baculovirus**

**Insect Cell Expression**

- NA Antigen
- HA Antigen
- M1/gag
Lentiviral “triple” transduction
(Lentigen)
Genes for each product subunit are inserted into sibling strains which are then fused and express a VLP.

Photos by Patrick Hickey, Nick Read, Dave Jacobson
Technology challenges - VLP

• Early to mid stage development
• Regulatory hurdles for novel technologies (substrates)
• Scalability of process
• Stability of particles
• Safety (removal of vectors, host cell components, nucleic acid)
• Low immunogenicity (may need adjuvants)
• Young companies/development teams
• Funding
Plant-based expression systems
Plant-based expression systems

Historical Challenges
- Long lead time
- Low yield
- Non-uniform expression
- Gene silencing
- Containment
- Regulatory issues
- Commercialization
Plant-based expression systems

- Proprietary platform for the production recombinant proteins based on transient expression technology in plants (*N. benthamiana*)

- Fast and inexpensive
  - From plant to vaccine in 5 days
  - Can commence production of any new pandemic strain in 1 month
  - Substrate easy to supply (plants in greenhouses)
  - Simple process and manufacturing facilities
Rapid protein production in plants

Sequence information

- Sequence Optimization: 1 day

Molecular Biology

- Cloning: ~4 days
- Grow agrobacteria: 2 days
- Target accumulation: ~5 days
- Target purification: ~5 days

Biomass Production

Total time = 36 days
Vaccine attributes - Plants

- Rapid production – 8 weeks from sequence to release
- Suitable for mix and matching of subunit vaccine components at short notice (customizable)
- Animal cell, microbial pathogen and animal virus free: implying safe host cell system
- High yields
- Low production cost: at current stage of development estimated to be <$1 per dose under cGMP production
- Highly scalable (either in greenhouses or “growth rooms”)
- Easily transferrable technology
Technology challenges - Plants

• Early stage development
• Regulatory hurdles for novel technologies
• Scalability
• Stability of target proteins
• Safety (removal of vectors, host cell components, allergic reactions)
• Low immunogenicity (may need adjuvants)
• Young companies/development teams
• Funding
“Universal” influenza vaccines
Universal influenza vaccine

- “Heterotypic” or “Heterosubtypic”
- A single influenza vaccine that would provide “protection” against any given subtype of influenza A
- Could be used for several influenza seasons before reformulation
  - Reduce annual “guesswork” for strain selection
  - Reduce production costs (thus vaccine costs)
  - Reduce vaccine “mismatches”
  - Reduce the potential for vaccine shortages
  - Increase the global supply of vaccine
- Could be stockpiled for epidemics/pandemics
- Surge capacity
  - Rapid scale-up, reduce production bottlenecks
Universal influenza vaccine

• Target conserved proteins or cross-reactive epitopes
  — Less sensitive to antigenic drift

• Identify less immunodominant, but more cross-reactive B and T cell epitopes on HA, NA and conserved proteins to “engineer” sequences that would direct the immune response to:
  — Induce humoral and/or cellular immunity

• Utilize recombinant technologies to optimize expression and delivery/uptake of the antigen

• Live virus vectors may offer advantage of inducing broader immunity
Is it possible to:

- Identify less dominant, yet more broadly reactive epitopes
- Engineer HA and/or NA genes to direct immune response
- Incorporate into vectored vaccine along with conserved Ags

Adapted from: Paul Lewis, MD
Oregon State Public Health
HA: surface, immunogenic
Highly variable. Drift. Shift.

NA: surface, immunogenic
Variable. Drift. Shift.

M2e: surface, immunogenic??
Fairly conserved. Ab-mediated.
Protective? Reduce severity.

NP (nucleoprotein): internal
Highly conserved.
Induces CMI. Reduce severity?

Matrix: internal
Highly conserved.
Induces CMI.

Adapted from: Paul Lewis, MD
Oregon State Public Health
HA Stalk or Fusion Peptide
Highly conserved.
Transiently accessible on infected cell surface.

Adapted from: Paul Lewis, MD
Oregon State Public Health
“Universal” vaccines have “Universal” challenges

• Often require alternate development/release assays
  — Most regulators are accustomed to SRID or SRH

• Often induce an immune response to something other than the HA protein
  — Most regulators are accustomed to HAI antibodies for licensure

• May require large scale efficacy trials or other “creative” clinical development plans

• Are not always as “Universal” as they claim
  — A single amino acid change can render ineffective
Developing Country Vaccine Landscape

### Pre Clinical

- **Egg-based inactivated**
  - [Image](#)
  - [Image](#)
  - [Image](#)
  - [Image](#)
  - [Image](#)
- **Cell-culture inactivated**
  - [Image](#)
  - [Image](#)
- **Live**
  - [Image](#)
  - [Image](#)
- **Recombinant SUVs and VLPs**

### Phase 1

- [Image](#)
- [Image](#)
- [Image](#)
- [Image](#)

### Phase 2

- [Image](#)
- [Image](#)
- [Image](#)
- [Image](#)

### Phase 3

- [Image](#)
- [Image](#)
- [Image](#)
- [Image](#)

### Market Approved

- [Image](#)
- [Image](#)
- [Image](#)
- [Image](#)

### Universal

- **Seasonal**
- **Pandemic**

### Viral Vectors

- **Seasonal & Pandemic**
- **US License**

### DNA

Updated: 07/07/2011
Technology
LAIV
- Recombined, cold-adapted virus
- Trivalent
- $10^{6.5-7.5}$ fluorescence focusing units/dose, per strain

Manufacturing Platform†
Expressed and purified from Eggs

Clinical Results
Complete Phase III
- Indicated for ages 2-49

Status†
Market Licensed
- Administration:
  - Single dose syringe,
  - Nasal route 0.1 mL/nostril
- Ages 2-49

WT, Virulent
Cold Adapted, Non-Virulent

Temperature sensitive
Non-Virulent
Immunogenic

Technology
Universal Flu Vaccine
• Multimeric-001: Nine conserved epitopes from HA, NP and M proteins
• Several repetitions of linear influenza epitopes from H1N1, H4N2 and avian H5N1
• Stimulates production of antibodies and INF-γ and IL-2 in mice and humans

Manufacturing Platform†
Expressed and purified from *Escherichia coli*
Production cycle requires 6-8 weeks.

Clinical Results
Phase III anticipated to start 2012†

Phase IIa complete 06/16/2011‡
Randomized, double blind, placebo controlled, 200 healthy volunteers
• Safe, well tolerated.
• Met primary safety and immunogenicity endpoints
• Met secondary immunogenicity endpoints
• Antibody and cellular mediated immune responses
• Co-administration with 50% TIV dose produced higher HAI titers than TIV alone

‡Ben-Yedidia, T. The Pursuit of Alternative Influenza Vaccines: Where are we now, what challenges remain?. Presentation at BIO 2011, Washington, DC
Technology
Universal, Recombinant Flu Vaccine
• Recombinant expression of M2e

Pre-Clinical Results
15 µg s.c./i.n. protected 6/7 BALB/c mice from 10LD_{50} challenge

News
15 µg s.c./i.n. protected 6/7 BALB/c mice from 10LD_{50} challenge

Manufacturing Platform†

Figure References:
**Technology**

**Live Attenuated Influenza Virus**
- Deleted NS1 gene prevents viral inhibition of cellular defenses
  - Limits INF-β production and induction
  - Blocks 2’-5’-oligoadenylate synthetase (OAS) and dsRNA-dependent serine/threonine protein kinase R (PKR) — key regulators of viral transcription/translation
  - Agonizes putative RNAi host-cell defenses
- Intranasal administration
- Replication defective — no shedding detected

**Pre-Clinical Results**

Protective in ferrets and mice

**Clinical Results**

H1N1 n=96: Safe and immunogenic, local and systemic immunity, cross-reactive antibodies

H5N1 n=72: Safe and immunogenic, >70% seroconversion after first immunization; local and systemic immunity

Seasonal Trivalent: (H1, H3, B) n=90: In progress. Finalized Q1, 2011

Research in progress

• Live-attenuated influenza viruses (LAIV)
• DNA
  — Plasmid-based, single or multiple gene combinations, in vivo electroporation
• Recombinant subunit expression systems
  — Baculovirus, yeast, tobacco plants, fungi, pseudomonas
• Virus-like particles (VLP)
  — Lentivirus, baculovirus, fungi, plants
• Live-virus vectors
  — Adenovirus, MVA, alphavirus, poxvirus
• Peptides
  — Synthesized multigenic (conserved CTL epitopes, variable regions)
Overall challenges

• Safety
  — New carriers, vectors, fusion proteins, substrates, adjuvants

• Scalability

• Formulation and potency determination
  — Standardizing and stabilizing the protein
  — Each new substrate may require new, specialized release assays

• Complicated/uncertain regulatory pathways

• Funding

New vaccine development is a time-consuming and expensive process
Summary

• The landscape of new influenza vaccine development is active and rapidly evolving
  ─ Notable changes in the emergence of DCVMs and LAIV
  ─ Next horizon likely to be cell-based vaccines, followed by recombinant technologies as remaining challenges are addressed

• Significant challenges ahead
  ─ Safety, immunogenicity, scalability and regulatory science

• Ongoing effort and support is needed to accelerate development of innovative technologies

• Recommend support of new technologies and platform approaches that move the field toward a “universal” (broadly reactive) solution
THANK YOU
## Stage of development – LAIV

<table>
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<tr>
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<th>DEVELOPMENT</th>
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<tbody>
<tr>
<td><strong>Lab</strong></td>
<td><strong>PrCl</strong></td>
</tr>
<tr>
<td>Design</td>
<td>Const</td>
</tr>
</tbody>
</table>

- **Pandemic**
  - IEM/Microgen (H1N1)
  - MedImmune (H5N2)
  - Nobilon/Merck
  - SIIL (H1N1)

- **Seasonal**
  - SIIL (H1N1)
  - Vivaldi
  - Nobilon/Merck
  - MedImmune (H1N1)
**Stage of development – VLPs**

<table>
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<th>Ph 2</th>
<th>Ph 3</th>
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<td>Const</td>
<td>Immuno</td>
<td>Challenge</td>
<td>GMP</td>
<td>Tox</td>
</tr>
</tbody>
</table>

- **RESEARCH**
  - Neugenesis
  - Lentigen
  - GlobeImmune
  - Zeta
  - Technovax
  - Ligocyte

- **DEVELOPMENT**
  - Pandemic/Seasonal
  - Novavax
## Stage of development – Plants

### RESEARCH vs DEVELOPMENT

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</tr>
</tbody>
</table>

**Phases**
- **Ph 1**: GMP, Tox, Ph 1
- **Ph 2**: Ph 2
- **Ph 3**: Ph 3
- **MA**: MA

**Institutes**
- **Kentucky Bio**: Pandemic/Seasonal
- **Caliber Bio**: Pandemic/Seasonal
- **Fraunhofer**: Pandemic/Seasonal
- **Medicago**: Pandemic/Seasonal
Influenza virion

Enveloped virion

Genome is segmented, negative single-strand RNA

Categorized into **serotypes** based upon surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins

HA: 16 serotypes, surface antigen Immunogenic, highly variable Drift. Shift.

NA: 9 serotypes, surface antigen Immunogenic, variable Drift. Shift.

Adapted from: Paul Lewis, MD
Oregon State Public Health