Nanoparticulate Vaccine Design:
The VesiVax® System

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Influenza

- Each year up to 20% of the world's population contracts influenza

- 250,000 to 500,000 people die annually from influenza-associated complications

- Pacific Bridge Life Sciences estimates the associated cost of influenza at $5.2B in 2004

- “Avian influenza” mortality rates greater than 50%
Influenza Virus Type A

- Enveloped RNA Virus
- Genome encodes 10 proteins
- Major Viral Envelope Proteins
  - Hemagglutinin (H)
  - Neuraminidase (N)
Survival Strategies Employed by the Influenza Virus

- **Antigenic Drift**
  - High mutation rates
  - RNA viruses lack proofreading capabilities
    - Often one mutation per genome copy
    - Evolutionary advantage
    - Active response to changes in environment and drug regimen
- **Antigenic Shift**
  - Shuffling of viral genes gives rise to “reassortants”
  - Recombination of H and N creates new strains
- **Infected for multiple hosts**
  - Humans, pigs, birds, horses, dogs, mice
- **Hardy**
  - Able to survive and retain virulence for up to 48 hours on hard non-porous surfaces
Antigenic Shift
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Influenza Vaccines

Time-intensive production process

• Generates inefficiencies that gives the virus an advantage

• Specific strains and virulence must be forecasted and produced well in advance of each flu season

• There is no feedback loop in this process, when the forecasts are inaccurate, supply chain is already committed, and course corrections can not be made

• Because the influenza virus changes constantly, clinical evaluation of new vaccines is impractical
The VesiVax® System

Designed to facilitate rapid vaccine development -
VesiVax® influenza vaccine targets the highly conserved M2 ectodomain segment (M2e)

HD = Hydrophobic domain
M2e = Antigen (~100/Liposome)
MPL = Adjuvant (~2500/Liposome)
### Vaccine Design

**Influenza type A M2e sequences**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>M2eA Sequence</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1N1</td>
<td>MSLLTEVETPTRNEWGCRCNDSSD</td>
<td>1918 – “Spanish”</td>
</tr>
<tr>
<td>H1N1</td>
<td>MSLLTEVETPIRNEWGCRCNGSSD</td>
<td>1934 – PR/8</td>
</tr>
<tr>
<td>H2N2</td>
<td>MSLLTEVETPIRNEWGCRCNDSSD</td>
<td>1957 – “Asian”</td>
</tr>
<tr>
<td>H3N2</td>
<td>MSLLTEVETPIRNEWGCRCNDSSD</td>
<td>1968 – “Hong Kong”</td>
</tr>
<tr>
<td>H5N1</td>
<td>MSLLTEVETLTRNGWECKCRDSSD</td>
<td>1997 – “Avian”</td>
</tr>
<tr>
<td>H9N2</td>
<td>MSLLTEVETPTRNGWECKCNDSSD</td>
<td>1999 – “Avian”</td>
</tr>
<tr>
<td>H5N1</td>
<td>MSLLTEVETPTRNEWECRCSDSSD</td>
<td>2004 – “Avian” X-88</td>
</tr>
<tr>
<td>H6N2</td>
<td>MSLLTEVETPIRNEWGCRCNDSSD</td>
<td></td>
</tr>
</tbody>
</table>

**H5N1** - First evidence of influenza virus transmitted from birds to humans. It is important to note that the majority of Influenza type A strains have high sequence homology for M2e. The conserved nature of M2e allows for the potential to create a vaccine that is effective against all strains.

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*VesiVax® Influenza Vaccine*
**VesiVax® Influenza Vaccine**

*Virally Infected Cells Display M2e*
VesiVax® Influenza Vaccine

Preparation of L-M2e-HD

![Image of molecular weight and diameter distribution](image-url)

![Image of IAVM2e1 and IAVM2e1-HD](image-url)

![Image of VesiVax® Influenza Vaccine](image-url)
VesiVax® Influenza Vaccine

Preliminary Formulation Screen

BALB/c mice (n=5)
Immunized twice (SubQ/IN)
**VesiVax® Influenza Vaccine**

**Dose Ranging Study**

- BALB/c mice (n=7)
- Immunized twice (SubQ/IN)
- Challenged with 10X LD50

Graph showing percent survival with different doses of vaccine:
- 15μg s.c./i.n.
- 10μg s.c./i.n.
- 5μg s.c./i.n.
- Control liposome
**VesiVax® Influenza Vaccine**

**Maximal Viral Challenge**

BALB/c mice (n=7)
Immunized twice (SubQ/IN)
Challenged with X-88
**Passive Transfer of Immunity**

**VesiVax® Influenza Vaccine**

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### Immunized Mouse Sera

<table>
<thead>
<tr>
<th>Immunized Mouse Sera</th>
<th>Coating Antigen</th>
<th>IgG Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-IAVM2e1-HD</td>
<td>IAVM2e1</td>
<td>13,312</td>
</tr>
<tr>
<td>L-IAVM2e1-HD</td>
<td>IAVM2e1</td>
<td>5,120</td>
</tr>
<tr>
<td>Sham-L</td>
<td>IAVM2e1</td>
<td>0</td>
</tr>
<tr>
<td>UV Irradiated IAV</td>
<td>1:32 Irr. IAV</td>
<td>81,920</td>
</tr>
<tr>
<td>UV Irradiated IAV</td>
<td>IAVM2e1</td>
<td>256</td>
</tr>
<tr>
<td>PBS</td>
<td>1:32 Irr. IAV</td>
<td>0</td>
</tr>
<tr>
<td>PBS</td>
<td>IAVM2e1</td>
<td>0</td>
</tr>
</tbody>
</table>
VesiVax® Influenza Vaccine

Cross-Protection of M2e

Days Post-challenge

Percent Survival

- L-IAVM2e1-HD
- L-Sham
# VesiVax® Influenza Vaccine

## Reduction of Viral Burden

<table>
<thead>
<tr>
<th>Immunization</th>
<th>M2e1</th>
<th>M2e2</th>
<th>M2e3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge</td>
<td>H1N1</td>
<td>H5N1</td>
<td>H9N2</td>
</tr>
<tr>
<td>N-fold Reduction</td>
<td>&gt;300</td>
<td>&gt;10</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>
VesiVax® Influenza Vaccine

**M2 as a target for vaccine development**
- Evolutionarily conserved
- *Not as susceptible to the high mutation and reassortment rates observed with the H and N epitopes*
- *Present on the surface of viruses and infected cells*

**Is M2 a good flu vaccine target?**
- *Significant protection observed for epidemic and pandemic strains of influenza*
- *Cross protection against strains with the same M2 demonstrated*
- *The data suggests that M2 mediated immunity is antibody dependent*
- *M2 specific antibodies recognize M2 on the surface of infected cells and on the virus*
VesiVax® Vaccination Studies

Protection from severe infection observed

- Against viral and bacterial pathogens
- In different species and strains of animals
- In both sexes
- In short and long term studies
- In adults and young animals
- Through different routes of vaccination

Assays of immunological response parameters demonstrate

- Antibody titers increase >30x over placebo
- Antigen specific proliferation of immune cells increase >10x
- Key cytokine levels increase by >10x over placebo

No significant side effects observed
### The VesiVax® Advantage

**VesiVax® Influenza Vaccine**
- Recombinant DNA system allows “cut & paste” design of M2e antigens
- Flexible design facilitates rapid engineering of new influenza vaccines
- Routine scale-up procedure
- Production simplified
- Minimal biohazard (BL1)
- Selective antigen display (M2e)
- Reduced possibility of side effects
- No risk of infection

**Influenza Virus Vaccine**
- Pathogen-based vaccines are not amenable to rapid development
- Time and labor intensive manufacturing process
- Complex production procedures (eggs)
- Biohazard requirements (BL2-BL4)
- Non-selective antigen display
- Inflammation at the site of injection
- Increased possibility of clinical complications
Implications

VesiVax® technology

• Represents a leap forward in vaccine development and production
• Demonstrated efficacy with Influenza
• Demonstrated efficacy with HSV2
  – Significantly shortens time of vaccine production
  – Can potentially respond to new pathogens in weeks, not months
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Questions