A cationic liposome-DNA complexes adjuvant (JVRS-100) enhances the immunogenicity and cross-protective efficacy of Influenza pre-pandemic H5N1 vaccine in mice

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January 25 2013
Public Health needs for H5N1 vaccines

- The development of pre-pandemic vaccines for avian influenza viruses, that are both immunogenic and dose-sparing remains a high priority.
- In particular, vaccines which elicit broad cross-clade protection are needed to address antigenic and genetic diversity among circulating HPAI H5N1 viruses with pandemic potential.
- Therefore, a novel adjuvant, which not only can enhance immunogenicity but also can improve the quality of the immune response of the influenza vaccine is needed.

*The goal of this study is to test JVRS-100 (Colby Pharmaceutical Company) with an H5N1 vaccine in mice.*
Questions addressed

- Can JVRS-100 enhance the immunogenicity of clade 1 H5N1 vaccine in BALB/c mice?
- Can lower doses of adjuvanted vaccine generate cross-protective antibody responses (antigen dose-sparing)?
- Does the adjuvant enhance H5N1 cross-clade immunity and protection?
- Which IgG subclass and CD4+ T cells predominated in mice received the adjuvanted vaccine?
JVRS-100 was manufactured from cationic liposome (DOTIM/Cholesterol) with a double-stranded plasmid DNA (pMB75.6).

The liposomes are capable of adsorbing pDNA and antigens, and facilitate targeted delivery of them into APCs. Liposomes may also protect them from extracellular degradation.

The pDNA contains non-CpG and unmethylated CpG motifs, which are recognized by the immune system (TLR9, DNA sensors) and followed by the release of multiple cytokines and chemokines.
**Vaccination & Experimental Protocol**

**IMMUNIZATION**

BALB/c

**Day-0**

Injection (i.m)

Vaccine* 
0.03, 0.1, 0.3, or 1 (µg HA/mouse)

±

10 µg JVRS-100

**EVALUATION IN VITRO**

BALB/c

Sera

1 dose

3 w

2&14 m

HA-ELISA

HRBC HI

MN assay

Spleen

2 doses

10 d

Flow cytometric analysis

**EVALUATION IN VIVO**

BALB/c

Infection (i.n)

1 dose

3&14 m

1000 LD$_{50}$ in 50µl

-VN1203

-Turkey/15/06 (TK15)

-Anhui/1/05 (AH1)

* Vaccine=Split Vietnam/1203/04 (VN1203) clade-1 H5N1 vaccine commercial product from NIH resource
JVRS-100 enhances immunogenicity of clade-1 vaccine (antigen dose-sparing)

Serum antibody titers (Log$_2$)

<table>
<thead>
<tr>
<th>Doses of VN1203 vaccine (µg HA/mouse)</th>
<th>VN1203+JVRS-100</th>
<th>VN1203 alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td></td>
<td></td>
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</tbody>
</table>

GMT, *P < 0.01 compared to vaccine alone groups at the same dose of HA
JVRS-100 enhances cross-clade HI antibody responses

**Clade-1 virus**

**VN1203**

Doses of VN1203 vaccine (µg HA/mouse)

- 1
- 0.3
- 0.1

P <0.01 compared to vaccine alone groups at the same dose of HA
JVRS-100 enhances protective efficacy of clade 1 vaccine against HPAI H5N1 viruses

One dose of 0.3µg HA±JVRS

Challenge with 1000 LD$_{50}$

Virus titers

% Weight loss

% Survival

1000 LD$_{50}$ VN1203

$\ast$ $P < 0.05$ compared to vaccine alone group
JVRS-100 enhances cross-protective efficacies of clade 1 vaccine against clade 2 H5N1 viruses

*P <0.05 compared to vaccine alone group. †P <0.05 compared to adjuvant alone group.
JVRS-100 adjuvanted H5N1 vaccine induces long-term protective immunity in mice

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Antibody titers</th>
<th>Virus titer (Log_{10} EID_{50}/ml)</th>
<th>% Maximum Mean Weight loss</th>
<th>No. survival / total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI</td>
<td>IgG1</td>
<td>IgG2a</td>
<td>Lung</td>
</tr>
<tr>
<td>VN1203+JVRS</td>
<td>110†</td>
<td>8,444†</td>
<td>58,813†</td>
<td>2.3†</td>
</tr>
<tr>
<td>VN1203</td>
<td>16</td>
<td>459</td>
<td>&lt;100</td>
<td>7.3</td>
</tr>
<tr>
<td>JVRS</td>
<td>&lt;10</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>6.9</td>
</tr>
</tbody>
</table>

† $p < 0.05$ compared to vaccine alone group.
JVRS-100 mainly enhances Th1/IgG2a immune responses in mice

% CD69^+ IFN-γ^+ CD4^+ T cells

Antibody titers (log_2)

† P <0.05 compared to vaccine alone or vaccine with alum group

† P <0.01 compared to vaccine alone group

* IgG2a : IgG1 ratio of 12:1; p < 0.01
Summary and Conclusions

- JVRS-100 significantly improved immunogenicity to a clade 1 split H5N1 vaccine in mice.
  - Adjuvanted vaccine provided antigen-sparing and enhanced cross-clade antibody responses and cross-protection.
  - Formulation of vaccine with JVRS-100 significantly enhanced Th1/IgG2a immune responses.
  - Protective immunity induced by adjuvanted vaccine lasted for over one year without waning.

- Therefore, JVRS-100 warrants further investigation as a potential adjuvant for use in human vaccination against HPAI H5N1 virus with pandemic potential.
Future plan

The ability of JVRS-100 to enhance the immunogenicity and cross-protective efficacy of H5N1 vaccine will be evaluated in ferrets.
Acknowledgements

- **Influenza Division, CDC, USA**
  L. Dong, F. Liu, J. Belser, J. Patel, K. Hancock, J. Katz

- **Colby Pharmaceutical Company, CA, USA**
  J. Fairman

- **Juvaris BioTherapeutics, INC, CA, USA**
  T. Monath, J. Warner

- **Stanford University, CA, USA**
  D. Hong, D. Lewis

- **WHO Global Influenza Surveillance and Response System**