Computationally Optimized Broadly Reactive Antigen (COBRA): A novel strategy for developing a broadly reactive vaccine against emerging H5N1 influenza

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Overview

- A goal of influenza vaccine development is the elicitation of cross-protective immunity. It is currently impossible to predict which antigenic variants may emerge and therefore an ideal vaccine will elicit immunity to most potential variants.

- H5N1 Influenza. Support from NIAID and PATH Vaccine Solutions.

- Seasonal Influenza (H1N1, H3N2, and B Influenza) in partnership with Sanofi-Pasteur.

- Strategies are under development using various influenza immunogens.

- Hemagglutinin (HA).

Developing a cross-reactive vaccine that elicits cross-protective immunity to multiple clades may provide more promise than a vaccine that is unable to elicit immunity across current clades. Current vaccine strategies have met this goal with mixed success.
Influenza Viruses

Influenza Pandemics

- Occasionally emerge (10-50 yrs)
- Genetic reassortment
- Antigenic shift
- World Health Organization Criteria
  - Highly pathogenic
  - Novel subtype
  - Efficient human to human transmission

H5N1 Background

- Initially emerged in poultry and humans in 1997
- Diversity within subtype
  - 10 phylogenetic clades
  - Geographically distinct
  - Human infections from clades 0, 1, 2 and 7
Clade 2: Westward Bound

Areas with confirmed human cases of H5N1 avian influenza since 2003

- Clade 1
- Clade 2.1
- Clade 2.2
- Clade 2.3
- Clade 7

* All dates refer to onset of illness

Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization
H5N1 Vaccines

- High virulence complicates traditional influenza vaccine production strategies
  - Wild-type viruses do not grow to high titer in eggs
  - High pathogenicity mandates ABSL-3 manufacturing facilities
- H5N1 HA is poorly immunogenic
  - Larger doses than seasonal vaccine
  - Adjuvant inclusion
- Poor cross-reactivity between clades
  - Current FDA approved vaccine is from clade 1
  - Predict pandemic clade?
  - Increase breadth of vaccines?
How do we overcome viral diversity?
Broadening Strategies

- **Traditional (Standard)**
  - Polyvalent
    - Mix multiple antigens into a single formulation
    - Seasonal flu, HPV, pneumococcal
    - Breadth limited to that of components

- **New Generation**
  - Centralized
    - Utilize sequencing efforts
    - Capture multiple antigenic features in single molecule
    - Not naturally occurring
Centralized Sequences

- Synthetic sequences that represent a given population
- Three methods for generating centralized sequences
  1. **Ancestral**: the most recent common ancestor
  2. **Center of the tree**: the phylogenetic point that is equidistant from all input sequences
  3. **Consensus**: the most common amino acid at each position

Goals

- Design and characterize centralized antigen to address diversity within clade 2
  - Diverse
  - Prevalent
  - Spreading west
- Compare centralized antigen and polyvalent vaccine approaches
- Evaluate protective mechanism
Antigen Design

- Computationally Optimized Broadly Reactive Antigen (COBRA)
  - Align amino acid sequences from Clade 2 human isolates
  - Assemble ‘Layered’ Consensus
  - Limit sampling bias

- Confirm presence of conserved linear epitopes
  - (Immune epitope database; www.immuneepitope.org)
Recombinant Virus-like Particle Candidate Vaccine

- Exact genetic match
- Correct 3D configuration of HA/NA proteins
- Efficient insect cell-based production
- No safety risks associated with live virus
Mouse Vaccine Study: Study Design

- Goals:
  - Compare VLPs with COBRA HA to VLPs with Clade 2.2 HA
  - Challenge with HPAI clade 2 H5N1 virus
    - Morbidity/mortality
    - Lung titers at D1, D3, D5 post-challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Description</th>
<th>HA Dose</th>
<th>Adjuvant</th>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>COBRA</td>
<td>3ug</td>
<td>Imject</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Clade 2.2</td>
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<tr>
<td>3</td>
<td>5</td>
<td>Mock</td>
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<td>Imject</td>
</tr>
</tbody>
</table>

Procedure:
- Prime
- Boost/Bleed
- Bleed
- Challenge

Week:
- 0
- 3
- 5
- 6

Giles and Ross. 2011. Vaccine. 29:3043-54
Mouse Antibody Responses

A. Total IgG (End Point Titers)

B. Receptor Blocking Antibody (HAI Titers)

Giles and Ross. 2011. Vaccine. 29:3043-54
Mouse Clinical Signs

Challenged with A/Whooperswan/Monoglia/244/2005; Clade 2.2.

Giles and Ross. 2011. Vaccine. 29:3043-54
Mouse COBRA vs. Polyvalent Immunogenicity

8-12 week
BALB/c
3ug HA + Alum

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<th>Adjuvant</th>
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<tr>
<td>1</td>
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<tr>
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<tr>
<td>6</td>
<td>5</td>
<td>Mock</td>
<td>-</td>
<td>Imject</td>
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</tbody>
</table>

Mouse H5N1 (Clade 2.2) Challenge

Challenge virus: Clade 2.2
(A/Whooper Swan/Mongolia/244/2005)

0 3 5 6
8-12 week
BALB/c 3ug HA + Alum

Mouse Lung Viral Titers

Lung Viral Titers

PFU/g tissue

COBRA
Polyvalent
Mock

Day Post Infection

Day 1 Day 3 Day 5

10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9

Mouse Lung Viral Titers

COBRA
Clade 2.1
Clade 2.2
Clade 2.3

PFU/g tissue

Mock

Day Post Infection

Day 1 Day 3 Day 5

10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9

Limit of detection

Mouse H5N1 (Clade 1) Challenge

Challenged with A/Vietnam/1203/2004, Clade 1

Lung Viral Titers

3 Days Post Infection

PFU/g tissue

- 10^2
- 10^3
- 10^4
- 10^5
- 10^6
- 10^7
- 10^8
- 10^9

COBRA
Polyvalent
Mock

Ferret Immunogenicity and Protection

Total IgG (End Point Titers)

Receptor Blocking Antibody (HAI Titers)

Nasal Wash Viral Titers

Monkey Study

Cynomolgus Macaques
Route: IM

Immunogen: VLP
VLP Dose: 15ug
Adjuvant: Imject

Vaccination: Week 0, 3, 6

Challenge: WS/05; Clade 2.2

<table>
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<th>Group n=7</th>
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<tbody>
<tr>
<td>WS/05 VLP</td>
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<tr>
<td>COBRA-2 VLP</td>
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<tr>
<td>Unvaccinated</td>
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</table>

Vaccination Regimen

0  3  6  9  11

Week

Challenge

Tissues
Nasal Washes
RNA for Microarray
Histopathology
Gross Pathology
Immunology

NHP Immunogenicity Breadth (HAI)

- Clade 0 (HK/483/97)
- Clade 1 (HK/213/03)
- Clade 1 (VN/1203/04)
- Clade 2.1.1 (Dk/HU/02)
- Clade 2.1.3 (IN/05/05)
- Clade 2.2.1 (Eg/321/07)
- Clade 2.2.1 (Eg/3300/08)
- Clade 2.2.2 (Tk/EG/07)
- Clade 2.2.2 (Tk/Tk/05)
- Clade 2.2.2 (WS/05)
- Clade 2.2.2 (BHG/1/05)
- Clade 2.3.2 (CMP/HK/07)
- Clade 2.3.2 (Buz/Bul/10)
- Clade 2.3.4 (JWE/1038/06)
- Clade 2.3.4 (AN/1/05)
- Clade 4 (Gs/1175/06)
- Clade 7 (Ck/VN/08)
- Clade 2.0 COBRA

- COBRA VLP
- Whooper Swan Clade 2.2 VLP
- Mock (Alum Only)

Receptor Blocking Antibody (HAI Titers)

Data Summary

- COBRA sequence is a functional protein
- COBRA vaccine is immunogenic
- COBRA vaccine protects from H5N1 viral challenge
  - All vaccinated animals were completely protected from morbidity and mortality
  - COBRA vaccinated animals have decreased viral replication
**COBRA**

- Expand these results using second generation H5N1 COBRA to capture isolates.
- Development of H1N1 and seasonal influenza.
- Capture multiple epitopes in a single immunogen to elicit a broadly reactive polyclonal antibody response to HA.
- Can this work for Seasonal Influenza?
H1N1 Antigenic Timeline

1918  PR/34  Weiss/43  FM/47  Den/57

CA/78  Sing/86  TX/91  NC/99  SI/06  Bris/07  CA/09

Individual Infections

PR/34

FM/47

Sequential Infections

PR/34  FM/47  Den/57  Novel H1N1 CA/09

Aerosol Transmission

Contact Transmission

Determine: Clinical Signs, Weight Loss, Viral Titers, & Transmission

# Experimental Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Initial Virus Infection</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>A/PR/8/34; A/FM/1/47; A/Denver/1/57</td>
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<tr>
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<td>4</td>
<td>A/Texas/36/91; A/NC/20/99; A/Brisbane/59/07</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>A/PR/8/34</td>
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<tr>
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<td>A/FM/1/47</td>
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<td>5</td>
<td>4</td>
<td>A/Denver/1/57</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>A/Texas/36/91</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>A/NC/20/99</td>
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<tr>
<td>8</td>
<td>4</td>
<td>A/Bris/59/07</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>A/Cal/07/09</td>
</tr>
</tbody>
</table>

- Ferrets will be nasal washed at Day 1, 2, 3, 5, 7 for viral load analysis
- Ferrets will be bled Day 14 post infection; bled every month for 4 months
- 2 Ferrets from groups 3-9 were infected with A/Cal/07/09 at 4 months
  - Nasal Wash at Day 1, 3, 5, 7 for viral load
  - Bled at D7; 14; 28 post infection
- Remaining Ferrets will be monitored until antibody titer.
- 2 ferrets from group 1 and 2 will be infected with next virus at 4 months
  - Nasal Wash at Days 1, 3, 5, 7 for viral load
  - Bled at D7; 14; 28 post infection
- Remaining Ferrets will be monitored until antibody titer

Weight and Viral Titers
Direct Infection with novel H1N1 (A/California/07/2009)

A. Direct Infection

- Naive
- A/PR/8/1934
- A/FM/1/1947
- A/Den/1/1957
- Historical Sequential

B. Direct Infection

- Naive
- A/PR/8/1934
- A/FM/1/1947
- A/Den/1/1957
- Historical Sequential

C. Direct Infection

- Naive
- A/TX/36/1991
- A/NC/20/1999
- A/Bris/59/2007
- Modern Sequential

D. Direct Infection

- Naive
- A/TX/20/1991
- A/NC/20/1999
- A/Bris/59/2007
- Modern Sequential

**HAI Titers**

**Individual Infections**

HAI Titers
Sequential Infections

A. Historical Group

B. Modern Group

Infection with A/PR/8/34
Infection with A/FM/1/47
Infection with A/Denver/1/57
Infection with A/California/07/09

Infection with A/Texas/36/91
Infection with A/NC/20/99
Infection with A/Brisbane/59/07
Infection with A/California/07/09

Neutralization Titers

A. Sera from Infected Ferrets

B. Sera from Infected Ferrets

HAI and Neutralization Titers
Mixing Individual Serum Samples

A. HAI Titer Log2
   - Historical Mixed Sera
   - Modern Mixed Sera

B. Neutralization Titer Log2
   - Historical Mix
   - Modern Mix
   - Sera from Infected Ferrets

C. HAI Titer Log2
   - PR/34-Den/57
   - PR/34-Bris/07

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- Sholmo Ta’asan
- Gilles Clermont
- Takis Benos
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- Jerry Nau
- Russell Salter
## National & International Collaborations

### Influenza-Related

- Rick Bright - **HHS-BARDA**
- David Lipmam & Joshua Cherry - **NCBI**
- Terry Tumpey, Thomas Rowe, Ruben Donis - **CDC**
- Annie DeGroot - **Univ. Rhode Island/Epivax**
- Jay Kolls - **LSU**
- Shabaana Khader - **CHP**
- Nikolai Petrovsky - **Flinders Med Ctr/Vaxxine**
- David Kelvin - **UHN Toronto**
- Hana Golding - **FDA/CBER**
- John Hiscott - **VGTI-FL**
- Dave Wentworth - **JCVI**

### Non-Influenza-Related

- James Smith, Janet McNichols - **CDC**
- Mark Heise - **Univ. North Carolina**
- Robert Doms - **Univ. Pennsylvania**
- David Weiner - **Univ. Pennsylvania**
- William Wilson - **USDA-ABADRU/CGAHR**
- Michael Diamond - **Washington Univ.**
- Shan Lu - **UMass**

### Influenza Projects Supported by:

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- **NSF**
- **DoD**
- **PATH Vaccine Solutions**
- **Sanofi-Pasteur**
Questions?