Re-engineering HA as a strategy to develop universal influenza vaccines

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Discovery Research
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Topics

- Background
- Target Product Profile
- Universal strategies
- Mapping the HA universe
- Consensus-based clustering (CBC’s)
- SMARt
Background

- **Universal influenza vaccine approaches have paradigm-shifting potential**
  - Goal is replacement of the seasonal vaccines

- **Recent developments in the field offering protection against disease**
  - Promising targets
  - Disruptive (change the influenza vaccination business model)

- **By contrast, the clinical benefit of the majority of ‘universal vaccine’ candidates in development (>40 candidates) limited to disease modulation**
  - ‘Adjunct’ positioning
  - M2e development efforts halted – expensive and incremental benefit

- **Approaches to universal flu vaccination have re-focused to sub-type universality**

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Project established focusing on universal vaccine candidates offering protection against influenza disease

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Strategy: Universal vaccines based on novel antigen constructs.

Opportunity: A universal vaccine that protects against disease represents a significant opportunity to alter the current influenza vaccine landscape.
**Universal Influenza vaccine targeting both A (H1 and H3) and B strains: Top-line TPP**

<table>
<thead>
<tr>
<th>Composition</th>
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| - Coverage of virus subtypes contained in regular QIV vaccines,
| - **One or more antigens** may be required per virus subtype
| - Assumption is that an **adjuvant** will be required |

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<tr>
<th>Indications</th>
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| - **Non-inferiority** to ‘standard-of-care’ flu vaccine at **time of launch** (replace seasonal vaccine)
| - ‘Standard-of-care’ at launch in the elderly segment is expected to be defined by ‘improved’ flu vaccines (e.g. HDIM), possibly necessitating modifications e.g. adjuvant
| - Protection against infection by a broad range of influenza virus subtypes and strains |

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<th>Target Popln.</th>
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<td>- All age groups as for regular influenza vaccine</td>
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<th>Target Markets</th>
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<td>- Global vaccine</td>
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<tr>
<th>Dosage &amp; Administration</th>
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| - IM (ID) administration
| - **Two or more** initial doses, followed by repeat vaccination at **intervals of several years** |

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<th>Safety &amp; Interactions</th>
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| - Systemic and local reaction profiles comparable to marketed adjuvanted seasonal vaccines
| - No clinically significant interference on co-administration with routine vaccinations |

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<th>Other Considerations</th>
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| - A **(pre-)pandemic** indication will also be targeted as a second step
| - Production should be **high-yielding, readily-scalable** using recombinant technologies permitting rapid and cost-effective production (‘state-of-the-art’ manufacturing systems)
| - The TPP fulfils a ‘True Universal’ vaccine, covering all virus types (‘**optimal TPP**’), and a ‘**Sub-type Universal**’ covering circulating virus types (i.e. H1, H3 and two B lineages; ‘**base case**’) |
Universal Influenza strategies

- **Antigen**
  - Re-engineered & immune-refocused HA
  - Sequence diversity
  - Validation of designs
    - Expression, folding & assessing breadth
  - Alternate antigens (tetrameric NA)

- **Adjuvants & delivery**
  - Breadth, magnitude and durability
  - Route
  - Antigen presentation (VLP, INV)

- **Immunogenicity / Efficacy**
  - Role of pre-existing immunity
  - Transmission

- **Clinical Research**
Strategy: HA as a Principal Component
Generating novel, re-engineered antigens that induce a neutralizing antibody response against a broad-range of viruses (H1 POC)

ANTIGENS

- Modified Full-Length HA
  (Re-engineered heads)
- HA-stem
  Headless-stem, chimeric HA, immune refocused HA
- NA tetramer
  (Univ. Ghent)

DELIVERY SYSTEMS
(Proprietary vectors)
- Split vaccine from Reverse Genetics
- Virus-Like Particles (VLPs)

Vaccine with improved breadth of neutralization (POC)

PROPRIETARY ADJUVANT
Scope of the problem: Breadth of Sequence Space for H1N1

- **H5N1**: 263 unique sequences
- **Pandemic – current**: 1956 unique sequences
- **1918 – 2009**: 1032 unique sequences

Overall Sequence Variation

**H5N1**

**H1N1**
Universe of H1N1 Hemagglutinin Sequences

H1 Strains to assess breadth (PRNT)

PRNT assays established for each cluster

- A/NewCaledonia/20/1999
- A/New Jersey
- A/Puerto Rico/8/1934
- A/Brazil/11/1978
- A/Texas/36/1991
- A/Brisbane/59/2007
- A/Solomon Islands/3/2006

Color by Year:
- 2010 - 2011
- 2009 - 2010
- 2008 - 2009
- 2008 - 2008
- 2003 - 2006
- 1998 - 2002
- 1990 - 1990
- 1980 - 1990
- 1970 - 1980
- 1960 - 1970
- 1950 - 1960
- 1940 - 1950
- 1930 - 1940
- 1918 - 1930

Shape by Region:
- Africa
- Asia
- Europe
- Middle East
- Oceania
- The Americas
Strategy 1: Cluster-Based Consensus (CBC)

- Identification of most common amino acids at each position within and between H1N1 clusters followed by structure-guided optimization

* Denotes where cluster consensus matches circulating virus sequence
CBC designs recognized by neutralizing mAb’s (analsogs)

- Re-engineered HA sequence retains proper folding of head and stem, as shown by binding of neutralizing mAbs (FACS) (and sialic acid on RBCs)

![Graphs showing MFI values for different strains](image)

Krause et al. 2011

Krause et al. 2011

Okuno et al. 1993

Whittle et al. 2011

Epitope shared with neutralized strains

CBC Tier 1

CBC Tier 2

CBC Tier 3

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☆ Epitope shared with neutralized strains
Viable Influenza viruses rescued containing Consensus (CBC) HA’s

PR8 Backbone

HA + Consensus/SMARt HA → Rescued virus with Consensus HA

Rescued viruses

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<th>Tier</th>
<th>Cluster</th>
<th>HA titer</th>
<th>Plaque Assay</th>
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<tr>
<td>Tier 1</td>
<td>4</td>
<td>32</td>
<td>&gt;1x10^6</td>
</tr>
<tr>
<td>Tier 1</td>
<td>5</td>
<td>64</td>
<td>4.7x10^5</td>
</tr>
<tr>
<td>Tier 2</td>
<td>345</td>
<td>128</td>
<td>6.6x10^5</td>
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PR8 Backbone
Breadth of neutralization (PRNT) induced by Tier1 (cluster 4) virus similar to NC

** P<0.01 Tukey-Kramer HSD test
Breadth of neutralization induced by Tier2 (clusters 345) virus similar to NC

Heat map of sequence differences at key epitopes/antigenic regions

*** P<0.001 Tukey-Kramer HSD test

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HAI confirms limited breadth of initial prototypes

Live WT virus (IN) versus live CBC Tier viruses (IN) day 35 (2x)

HAI titer

Live virus (IN; 1 x 10^5 pfu) CBC Tier virus

anti-California (pools)
anti-New Jersey (pools)
anti-PR (pools)
anti-Texas (pools)
anti-New Caledonia (pools)
anti-Brisbane (pools)
anti-Solomon Island (pools)
Expression and folding screen with mAbs have yielded multiple SMARt candidates

- Broadly neutralizing anti-stem & anti-head mAbs demonstrate surface localization and folding
- VLPs prepared of SMARt HA candidates and assessing breadth of neutralizing antibody response with and without adjuvant

**SMARt DESIGN**

Neutralizing Epitope #1
Influenza strain 1

Neutralizing Epitope #2
Influenza strain 2

Neutralizing Epitope #3
Influenza strain 3

Neutralizing Epitope #4
Influenza strain 4

Single HA molecule containing repertoires of neutralizing epitopes. Epitopes computationally assembled from diverse strains and carefully selected to collectively elicit broadly neutralizing antibodies

**Strategy 2: Structural Mapping of Antigenic Repertoires**
Mosaic SMARt designs: Differences to NC99 template sequence

• Surface representation of trimeric HA with monomers colored blue green and gray. Amino acid differences between the designed molecule and the New Caledonia reference are highlighted in red. Fab fragments are docked onto the locations of three known neutralizing epitopes on the surface of HA: CH65, HC45 and FI6.
SMARt designs appear well-folded and are functional

- Modification of HA sequence by SMARt method still retains proper folding of head and stem, as shown by binding of neutralizing mAbs (FACS)

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**NC1999 reference seq.**

- Epitope shared with neutralized strains
SMARt HA proteins bind sialic acid on RBCs

- Assay performed using Guinea Pig RBCs
- Immunogenicity and breadth of neutralization studies on-going
Conclusions

- Implemented strategy to evaluate re-engineered HA for increased breadth
  - Mapped the evolution of influenza viruses
  - Established bioinformatics approach to screen novel HA candidates
  - Generated full-length consensus and mosaic HA that are properly folded & functionally active
  - Viable viruses rescued with CBC HA’s validates modeling approach
  - Initial CBC designs (closely grouped clusters) did not increase breadth over wild type but approaches looking to increase breadth across multiple clusters appear promising (SMARt)
    - NC99 is a broadly protective virus

- Test novel SMARt and Tier 3 CBC’s in-vivo (+/- adjuvant)
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