Immunological evaluation of next-generation influenza vaccines

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Today’s presentation

• Evaluation of current influenza vaccines
• European regulatory guidelines
• Evaluation of novel vaccines
• Standardisation of immunoassays
Evaluation of current influenza vaccines

Serologic analysis established and routinely performed:
HI, SRH, VN

Correlates of protection (CoP):
HI ≥ 40 associated with >50% reduction of the risk of influenza infection or influenza disease

So-called CHMP criteria¹:

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<tr>
<th></th>
<th>18 – 60 years of age</th>
<th>&gt; 60 years of age</th>
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</thead>
<tbody>
<tr>
<td>Seroconversion/significant increase</td>
<td>neg → ≥ 40; ≥ 4 fold increase</td>
<td>&gt; 40%</td>
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<tr>
<td>Mean geometric increase</td>
<td></td>
<td>&gt;2.5</td>
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<tr>
<td>Seroprotection</td>
<td>HI: ≥ 40&lt;br&gt;SRH: &gt; 25mm²</td>
<td>&gt;70%</td>
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¹ Note for guidance on harmonisation of requirements for influenza vaccines, 1997, EMA, CPMP/BWP/214/96
Evaluation of current influenza vaccines (2)

CHMP criteria widely used, including for decisions on marketing authorisation/licensure of vaccines

Issues with existing CoP and CHMP criteria:
• Lack of standardisation of assays
• CoP not established for all age groups/risk groups (particularly elderly and unprimed individuals)
• No CoP for virus neutralisation assays

EMA has withdrawn previous guidance\(^1\) – CHMP criteria not applicable in EU anymore

\(^1\) Explanatory note on the withdrawal of the note for guidance on harmonisation of requirements for influenza vaccines, 2014, EMA/CHMP/VWP/40560/2014
New European non-clinical and clinical guideline

Published 21 July; to come into effect 1 February 2017

- No confirmed immunological correlate of protection
- However, for new inactivated vaccines, comparison with authorised vaccine possible – demonstration of non-inferior immune responses in population sub-groups
  - Comparator vaccine: should be authorised (in the EU), similar manufacturing process, at least some data available on effectiveness
- For young children (6 – 36 months): vaccine efficacy trial required
- For inactivated adjuvanted vaccines, superiority should be demonstrated
- For authorisation of LAIV, clinical efficacy needed

New European non-clinical and clinical guideline – immunological methods

Methods to measure antibodies:
- HI, SRH
- VN – ‘essential that neutralizing antibody titres are determined in all studies’
- For consideration:
  - Anti-NA antibodies
  - Antibody kinetics
- For zoonotic strains:
  - Cross-reactivity
  - Cross-priming
  - Cross-protection

Cell-mediated immunity:
- Measurement of CMI ‘encouraged’; particularly informative in the elderly
  - Quantity and quality of T cell responses
  - Activation of memory B cells
New European non-clinical and clinical guideline (3)

New guidance relevant to:

- LAIV
- Inactivated split or subunit vaccines and inactivated whole virion vaccines
- Vaccines that contain adjuvants

Principles of the requirements also applicable to:

- Inactivated vaccines that contain alternative vaccine antigens
- Vaccines that contain recombinant surface antigens
- DNA vaccines expressing surface antigen(s)
- Virus-like particle (VLP)-based vaccines
Evaluation of novel vaccines that aim to provide broad protection

Underlying principles vary widely between vaccine approaches

Vaccines inducing antibodies:

- Use of adjuvants to broaden humoral immune response
- Other strategies to induce broader humoral immune response directed against HA head
- Vaccines inducing anti-HA stem antibodies
- Vaccines inducing antibodies targeting non-HA antigens (NA, M2e)

- Classical serologic analyses may be appropriate for some vaccines
- New or modified assays for antibodies may be required
- Assays measuring activities of antibodies in conjunction with effector cells (eg ADCC)
Evaluation of novel vaccines that aim to provide broad protection (2)

Vaccines inducing CMI:
• Variety of assays available and in use
• Characterisation of T and B cell responses
• May be combined with assays measuring antibodies (if appropriate)

Issue:
• No correlate of protection known for any assays other than HI and SRH
• Manufacturers/developers need to generate data to enable definition of new CoPs
• Focus on primary evaluation criteria for vaccine of interest
• Exploratory assessments to define the immune profile of the vaccine candidate
Standardisation of immunoassays for evaluation of novel vaccines

Why standardise?
• To obtain reliable results
• To make results comparable between studies and laboratories
  • May help to define CoP

Approaches:
• Harmonisation of assay protocols
• Use of standards
• Regular assessment of labs through proficiency testing
• Alternative: Testing (or re-testing) in centralised expert laboratory
Harmonisation of assay protocols

- Harmonisation of assay procedures is feasible
- Harmonisation/standardisation of critical reagents may be harder (or impossible)
- Aspects to be considered in harmonisation:
  - Type of sample
  - Sample storage and preparation
  - Supply of critical reagents, preparation of critical reagents
  - Experimental procedure
  - Method of acquiring read-out (manual, instrumentation)
- Harmonisation of methods does not always lead to improved agreement between laboratories:
  - E.g. EDQM study for HI and SRH, 2007 – 2009: common SOPs for both methods did not lead to good agreement between labs

1 Wood et al, Pharmeur Bio Sci Notes, 2011, 1:36–54
Use of biological standards

• Biological activity can only be measured in a biological assay
  SI units (eg mass) do not capture the activity of a biological analyte
• International Unit:
  A fraction of a vial of a biologically active substance, arbitrarily defined as having the biological activity of 1 IU
• Method not defined
  Use of standard permits measurement in IU, irrespective of method (within limits)
• Different levels/types of standard
  – WHO International Standards – highest level, defines the IU
  – National/regional/pharmacopoeial standards
  – Working reagents
  – In-house standards
  – Run controls
Biological standards: an example
Collaborative study to establish the WHO 2nd International Standard for antibody to influenza A(H1N1)pdm09

Antibody titres to H1N1pdm09

Antibody titres relative to 10/202 (2nd IS)
Biological standards: example 2

European re-testing exercise: included WHO 1\textsuperscript{st} International Standard for antibody to influenza A(H1N1)pdm09

1 Wagner et al, Vaccine, 2012, 30:4113-4122
Use of biological standards for vaccines that induce broad immune response?

• Where antibody response is targeted, antibody/serum standards may be feasible
  • May have to be product specific if particular epitopes are targeted
• Where specific biological analytes (e.g. cytokines) are measured, standards may be available or feasible
• Cell standards for CMI assays have been made and more could be prepared
  • Freeze-dried cells for intracellular cytokine staining
  • Freeze-dried cells for ELIspot
Proficiency testing

• Testing a laboratory’s quality system with samples of known but undisclosed content
• Provides a snapshot of a lab’s performance
• Provides a comparison of a lab’s performance with that of other labs
• Can highlight problems
  • But does not solve them
• Can drive move to standardisation

Examples of EQA schemes:
• WHO EQAP for PCR detection of influenza A and B viruses
• UK NEQAS EQA schemes for detection of various pathogens
  Includes a few serological EQA schemes (e.g. measles and mumps, HIV)
Alternative approach: Use of centralised testing lab

- Is not standardisation, but can help to make results comparable
- When standardisation has not been achieved yet
- When standardisation is difficult
- For ‘niche’ methods or products – incentive to standardise may be low

Considerations:
- Central lab should be expert
- Assay should be well established, qualified or validated
- Central lab should have capacity to take on work now and in future (from competitors?)
Efforts towards standardisation of immunoassays for evaluation of influenza vaccines

- Value of standardisation recognised by many in the field
- Even well-established, old assays not fully standardised
- Primary focus so far on classical assays (HI and VN)
- Several initiatives
CONSISE

• ‘Consortium for the Standardisation of Influenza Seroepidemiology’
• Mission statement: CONSISE is a global partnership aiming to standardise the seroepidemiology of influenza and other respiratory pathogens, and to develop comprehensive investigation protocols for use in optimising the control of influenza and other respiratory pathogens.
• Comprised of two interactive working groups: Epidemiology and Laboratory; and a Steering Committee
• More than 100 members from over 40 countries
• CONSISE shares study protocols and laboratory assay protocols and other information on the internet with free access. Membership is free and open to all individuals whose activities may contribute to those of CONSISE
• http://consise.tghn.org
CONSISE: Development of consensus assay protocols

- To improve assay standardisation, CONSISE developed consensus protocols for the HI and VN assays.
  - Parameters were identified within each assay with potential variables listed.
  - Laboratories entered the variable they preferred, adding a new variable if not specified.
  - All details were collated, de-identified and the data summarised.
  - Consensus protocols were developed; parameters classified as ‘required’ or ‘recommended’.
- Consensus protocols for HAI and MN assays are published on the CONSISE website.
- Many member laboratories have aligned their assay parameters to the consensus protocols
- Data analysis of comparative study to evaluate the use of consensus protocols vs in-house protocols is on-going
Other initiatives (1)

- **UNISEC**: European, FP7 funded consortium to identify, develop and clinically test the most promising leads for a universal influenza vaccine

- One of the objectives: ‘To establish experimental conditions including standardized assays which allow the comparative evaluation of vaccine concepts in animal models and in clinical trials’

- Standardisation of CMI assays challenging

- Work-around: testing in a centralised lab
Other initiatives (2)

- **FLUCOP**: European, IMI funded project
- Long term goal:
  - ‘to improve and standardise the existing immunological assays applicable for the definition of correlates of protection in future efficacy trials’
  - ‘to develop new assays’
- Standardisation of HI and VN assays
- Understanding of other assays
Other initiatives (3)

- **EDUFLUVAC**: European, FP7 funded project
- With NIAID, co-organised a workshop last year at NIBSC:
  - Workshop on immunoassay standardisation for universal flu vaccines
- Conclusions:
  - Many assays used
  - Efforts on harmonisation and standardisation need to concentrate on primary evaluation criteria
  - Standardisation is an important goal
  - There is a long way to go

http://www.edufluvac.eu/node/1117
Conclusions

• Established inactivated vaccines have been assessed using established serologic assays: HI, VN, SRH
• Correlates of protection do not exist for all assays and all age/risk groups
• Existing correlates of protection are being questioned even where they exist
• New EU guidelines focus on better characterisation of the vaccine and the immune response and vaccine effectiveness/efficacy data
• Novel vaccines employing different mechanisms of action require new or modified assays
• Standardisation of immunoassays is challenging but important and may aid the establishment of new CoPs
Acknowledgements

Philip Minor
Stacey Efstathiou
Diane Major
John Wood
Jim Robertson
Joanna Waldock
Richard Stebbings

Maria Van Kerkhove
Karen Laurie
Angus Nicoll

• Odile Leroy
• Flavia D'Alessio
• Sophie Houard
• Ed Remarque
• Norbert Stockhofe
• Ed Schmidt
• David Spiro
• Rachelle Salomon
• Stephen Norley