

# Immune correlates of protection against influenza: challenges for licensure of seasonal and pandemic influenza vaccines, Miami, FL, USA, March 1–3, 2010

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The emergence of a novel swine-origin pandemic influenza virus in 2009, together with the continuing circulation of highly pathogenic avian H5N1 viruses and the urgent global need to produce effective vaccines against such public health threats, has prompted a renewed interest in improving our understanding of the immune correlates of protection against influenza. As new influenza vaccine technologies, including non-HA based approaches and novel production platforms are developed and undergo clinical evaluation, it has become clear that existing immune correlates such as serum hemagglutination-inhibition

antibodies may be unsuitable to estimate vaccine immunogenicity and protective efficacy of such vaccines. This International Society for Influenza and other Respiratory Virus Diseases (ISIRV) sponsored international meeting held in Miami, Florida USA on March 1–3, 2010, brought together scientists from industry, academia, and government agencies that develop and evaluate seasonal and pandemic influenza vaccines and scientists from regulatory authorities that approve them, to identify approaches to develop expanded immune correlates of protection to aid in vaccine licensure.

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## Introduction

This workshop is built on two previous international meetings addressing correlates of protection against influenza. The first workshop was held in Bergen, Norway, in 2002 and was co-sponsored by the University of Bergen and the International Association for Biologicals and focused on a reassessment of laboratory methods to detect relevant immune responses to influenza.<sup>1</sup> In 2007, the US Food and Drug Administration (FDA) and National Institutes of Health (NIH) together with the World Health Organization sponsored a meeting in Bethesda, MD, USA with an emphasis on understanding immune correlates of protection against influenza viruses to support pandemic vaccine development.<sup>2</sup> This 2010 meeting emphasized the need for expanded immune correlates of protection for next generation influenza vaccine technologies and their regulatory issues. Over 110 participants from the US, Europe, and Asia-Pacific attended the meeting. The 2-day meeting was divided into eight sessions comprised primarily of invited presentations but also including speakers invited from abstract submissions. The slide presentations are available to Influenza and other Respiratory Virus Diseases (ISIRV) members at <https://www.isirv.org/events/correlates/programme>.

## Session 1. Defining concepts, regulatory challenges, and current status of clinical trials requirements

Session 1 included invited presentations by Arnold S. Monto, (University of Michigan School of Public Health, Ann Arbor, MI, USA), Li Qin, (Vaccine and Infectious Disease Institute, Fred Hutchinson Cancer Research Center, Seattle, WA, USA), Bettie Voordouw, (Medicines Evaluation Board, the Netherlands), Jerry Weir, [Center for Biologics Evaluation and Research (CBER)/FDA, USA] and Gary Grohmann, [Therapeutic Drugs Administration (TGA), Australia].

As the ability to conduct well-controlled vaccine efficacy trials becomes ever more challenging and expensive, the need for well-defined immune correlates of protection against influenza is increasingly apparent. Appropriate immune correlates could limit the need for extensive trials, reducing vaccine development costs, as well as guide regulatory decisions and immunization policy. From both historic and recent data, the serum hemagglutination-inhibition (HI) titer is a strong predictor for efficacy of inactivated influenza vaccines. An HI titer of  $\geq 40$  remains the only universal immune correlate for a 50% reduction or

more in the risk of influenza infection or disease.<sup>3,4</sup> However, many studies used serologic evidence of infection, rather than more robust virologic confirmation, and it may be beneficial to revisit the role of HI antibody using contemporary diagnostic methods such as RT-PCR. The role of the HI titer as a correlate of protection for non-traditional vaccines, including live attenuated influenza vaccines (LAIV), is far less clear and additional correlates are needed to provide better predictions of protective efficacy.<sup>5</sup> When efficacy trials are conducted, consideration should be given to measure a broader range of immunologic responses in an effort to correlate vaccine efficacy with immunological surrogate markers. However, correlates of protection may differ by vaccine type and formulation, as well as age and health status of the volunteer population. For example, appropriate correlates of protection for pediatric populations have not been formally identified.

From a regulatory perspective, harmonized regulatory requirements [e.g. for European Medicines Agency (EMA), FDA] derived from clinical trials that employ validated and standardized assays and alternative functional assays are highly desirable. The lack of standardized clinical endpoints when a novel vaccine is first administered to a target population is a substantial difficulty for regulators, as is the extrapolation of seasonal data to pandemic influenza vaccines. Another unresolved question is whether similar correlates of protection can be assumed for adjuvanted and non-adjuvanted formulations. These unknowns underscore the risk of simply transposing current correlates of protection on new vaccines and formulations. Regulators are aware that for non-traditional vaccines, other more appropriate laboratory correlates of protection may exist, but currently there is a lack of data to support the use of other surrogate markers. As the landscape of influenza vaccine development and technologies is quickly evolving, and many novel vaccines are at phase II and III development, regulators need to be flexible and consider correlates of protection other than HI antibody, and encourage vaccine developers to provide data to support the use of alternative approaches.

## Session 2. Influenza immunology

This session included invited presentations by James Stevens, [Influenza Division, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA], John Treanor, (University of Rochester Medical Center, Rochester, NY, USA) and Iain Stephenson, (Leicester Royal Infirmary, Infectious Diseases Unit, Leicester, UK).

The hemagglutinin (HA) is the major viral antigen responsible for virus entry, while the neuraminidase (NA) is important in progeny virus release from host cells, and is

a secondary viral target for antibodies. Antibodies directed against the HA that neutralize virus infectivity do so by multiple mechanisms. Antibodies that bind around the receptor-binding site block the interaction of HA with the receptor on the host cell surface, inhibiting viral entry. Antibodies that bind to the stalk region of HA can block the fusion between viral and host membranes which is essential to initiate virus replication. The accumulation of amino acid substitutions in antigenic sites of the HA limit the ability of antibodies to successfully neutralize virus infectivity and is the basis of antigenic drift. Acquisition of glycosylation sites on the HA molecule also affects the ability of antibodies to recognize and bind to HA. Significant antigenic drift variants typically possess amino acid substitutions in multiple antigenic sites.

Primary infection with influenza in childhood leads to the development of a diverse set of responses that contribute to immunity and confer immunological memory.<sup>6</sup> These responses include serum and local antibodies directed against the highly variable HA and NA and also against less variable proteins including the nucleoprotein and M proteins, and CD4<sup>+</sup> and CD8<sup>+</sup> T cells directed against both variable and highly conserved viral epitopes. Subsequent reinfections with antigenically drifted viruses are generally less severe because of the more rapid development of antigen-specific B- and T-cell responses that can limit virus replication and speed recovery. Protection against antigenically shifted viruses after natural infection (heterosubtypic immunity) is less apparent but may occur more readily in adults than children.<sup>7</sup> LAIV mimics the immune response achieved by natural infection, eliciting good serum anti-HA antibody levels in seronegative children, but less so in seropositive children and adults. Immune markers that correlate with lower rates of infection or disease have been evaluated for LAIV in wild-type challenge, cold-adapted virus challenge or natural infection studies. Nasal IgA and serum anti-HA antibodies are independent predictors of protection from infection by LAIV.<sup>5,8</sup> However, protection against infection is also observed in the absence of either of these effectors, suggesting that additional immune mechanisms of protection exist. The presence of CD4<sup>+</sup> interferon-gamma (IFN- $\gamma$ ) producing T cells appeared to correlate with protection in children that received LAIV, also stimulating influenza-specific CD8<sup>+</sup> T cells, particularly in those aged 5–9.<sup>9</sup> Cytotoxic T-cell responses correlated with a reduction in nasal virus titers in individuals without detectable serum HI or neuraminidase inhibition (NAI) antibodies.<sup>10</sup> There is a need for simple, standardized assays that reliably measure immune effectors that can predict protective efficacy of live attenuated vaccines.

Currently licensed inactivated vaccines are based on the stimulation of serum antibody responses to HA. Although licensure criteria are based, in part, on achievement of

seroprotective HI titers  $\geq 40$ , the suitability of this threshold titer to correlate with at least 50% protection against influenza at the population level, is dependent on age, as well as virus strain, subtype, or type. Development of improved inactivated vaccines that elicit broader and more durable responses to HA, and/or immune responses to other viral proteins is the focus of current research. Higher doses of HA in inactivated vaccines improve the proportion of responders and geometric mean titres among older adults (>65 years), and such vaccines are now licensed for older adults in the US.<sup>11</sup> Other strategies include targeting the generally conserved M2e protein either alone or in combination with other conserved viral targets NP or M1 proteins.<sup>12</sup> Combination vaccines that target HA and one or more viral proteins including NA are in clinical development, using a variety of delivery strategies (DNA, protein or viral vector based) and a number of different adjuvant strategies. The challenges for the successful licensure of such novel vaccines include a lack of knowledge on immune mediators of protection in humans, the lack of standardized assays that measure non anti-HA based immune responses, and the lack of reagents and procedures that can be used to measure vaccine potency for standardization of novel target vaccines.

### Session 3. Regulatory challenges for live attenuated and non-traditional vaccines and case studies of regulatory issues associated with non-traditional vaccines

Invited presentations were by Rick Bright, (Vaccine Development Global Program, PATH, Washington, DC, USA) and James S. Robertson, [Division of Virology, National Institute for Biological Standards and Control (NIBSC), Hertfordshire, UK].

There is a need for low-cost, rapid-response technologies to address the gap in global vaccine supply and demand and to respond quickly to a pandemic. Many innovative technologies are in development, but significant hurdles are yet to be overcome including demonstration of safety, immunogenicity, scalability and regulatory scrutiny. Regulatory agencies are also facing hurdles in licensing non-HA based vaccines or vaccines produced by non-traditional manufacturing processes such as DNA vaccines, or recombinant proteins made in bacteria, insect cells or plants, including virus-like particle (VLP) vaccines. The safety aspects regarding adventitious agents and unexpected immunological vaccine responses were discussed in relation to autoimmune reactions and co-expression of cytokines or co-stimulatory molecules. It is highly unlikely that plant viruses can infect mammals, but there is an absence of clear data. Viruses with broader host ranges, such as the nodamura virus, which can be found in insects, animals, plants,

and yeasts, are also a cause for concern with respect to vaccine safety. A European Union (EU) guideline for live recombinant vector vaccines has been developed [EMA/Committee for Medicinal Products for Human Use (CHMP)/VWP/141697/2009],<sup>13</sup> and final guidelines are expected by late 2010. Reassuringly, regulatory agencies will be proactive in assessing criteria for and reactive to changing events associated with licensure of new vaccine technologies.

De-chu Tang, (Vaxin Inc., Birmingham, AL, USA) presented preclinical data from a Phase I clinical trial of an adenovirus-vectored nasal avian influenza vaccine. No safety issues have been observed in animals and humans after immunization with a replication-competent adenovirus-free Ad5-vectored nasal influenza vaccine.<sup>14</sup> Ad5-vectored nasal influenza vaccine can protect animals against a lethal influenza challenge in the absence of detectable serum HI antibody titers. Ad5-vectored nasal influenza vaccine can induce seroconversion in animals and humans in the presence of pre-existing Ad5 immunity. Human challenge studies are essential to demonstrate efficacy and to find immune correlates of protection against influenza.

Catherine J. Luke, [National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA] summarized four clinical trials in adults performed with LAIV targeting avian subtypes.<sup>15–17</sup> After two doses of H7 or H9 vaccines, nearly all vaccinees responded by a fourfold antibody rise in at least one of the following tests: serum HI, – neutralization antibodies, ELISA IgA or – IgG titers. Data from two H5 studies showed a poor serum HI response. There is therefore a need for well-defined quantitative correlates of immunity for LAIV.

### Session 4. Laboratory correlates/technicalities and case studies in laboratory measurements of immunity

Invited presentations were by John Wood, (Division of Virology, NIBSC, Hertfordshire, UK); Emanuele Montomoli, (University of Siena, Siena, Italy); Guus F. Rimmelzwaan, (Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands); Maryna Eichelberger, (Division of Viral Products, CBER/FDA, Bethesda, MD, USA); Giuseppe Del Giudice, (Novartis Vaccines and Diagnostics Research Center, Siena, Italy).

This session examined the different serologic methods that are in use or could be used to evaluate immunogenicity of influenza vaccines and measure immune responses associated with protection from influenza. The HI assay remains the most widely used method to evaluate antibody responses to influenza infection or vaccination because of its technical simplicity, a correlate of protection is known for seasonal influenza viruses and its acceptance by regula-

tory authorities. However, it lacks sensitivity for detecting antibody to some influenza viruses (influenza B, H5 and H7). Virus neutralization assays (VN) are gaining popularity, because they detect a broader range of functional antibodies and show enhanced sensitivity for detecting antibody to some viruses. Limitations of VN assays include a requirement for live virus, defined titers that correlate with protection are unknown, and hence, some regulatory agencies are uncertain about using VN data for vaccine licensure. Both assays suffer from inter-laboratory variation, which may be overcome, in part, through the use of common protocols and international antibody standards. Such standards have been developed for H5N1 clade 1 viruses and the 2009 pandemic H1N1 virus.<sup>18,19</sup>

The single radial hemolysis (SRH) assay is an antibody gel diffusion method that relies on complement-mediated hemolysis to detect zones of antibody-antigen complexes, the size of which is proportional to the concentration of influenza specific IgG in sera.<sup>20</sup> EMA recognizes the SRH as an assay of choice for the assessment of vaccine immunogenicity and there is a known correlate of protection (zone area  $\geq 25$  mm<sup>2</sup>). The assay has been modified to use turkey instead of sheep RBC for the detection of antibody to H5N1 viruses. The SRH titers show good correlation with HI and VN (microneutralization) titers. However, use of SRH is limited to a few experienced European laboratories with validated assays.

Virus-specific T lymphocytes are critical components of the adaptive immune response to influenza virus. Methods that detect antigen-specific T cells include the use of carboxyfluorescein succinimidyl ester labeling, which allows flow cytometric identification of CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets proliferating in response to specific antigen stimulation, and can be applied to both human and animal systems. Newer approaches for measuring cytotoxic T lymphocytes (CTL) include the fluorescent-antigen-transfected target cell CTL assay that does not require isotope usage or autologous cell lines preparation but uses plasmid DNA vectors to get *de novo* synthesis of viral epitopes in culture.<sup>21</sup> Two other approaches, which detect the production of specific cytokines, are the ELISPOT assay and Intracellular Cytokine Staining (ICS). Standardized reagents are commercially available for the ELISPOT assay, which has high-throughput potential. ICS assays can be performed in a standardized approach with positive and negative control antigens and validation parameters can be established. The detection of epitope-specific human T cells remains challenging because of the highly polymorphic nature of the human leukocyte antigen (HLA) system and the need to know individual HLA backgrounds of study subjects, as well as variations that exist between influenza T-cell epitopes. A limitation to all cellular assays is that there are no established criteria to define a 'good' T-cell response to vaccination.

Antibodies to the NA inhibit release of virus from infected cells, reducing levels of virus replication and ameliorating disease. A standardized and validated functional assay that detects inhibition of enzymatic activity is needed to determine the NA-inhibiting antibody titer that correlates with disease protection. Two assays for the measurement of NA-inhibiting antibody responses have been optimized: a miniaturized version of the classical thiobarbituric acid method (Warren-Aminoff) and an enzyme-linked lectin assay (ELLA) first developed by Lambré and colleagues in 1990.<sup>22</sup> For optimal accuracy, such assays need to use a substrate that mimics the size of the natural substrate and virus that contains a mismatched HA so that anti-HA antibodies do not interfere in the quantification of anti-NA antibodies. Interlaboratory validation of the ELLA is planned. A third cell based assay, the Accelerated Viral Inhibition using NA to quantify virus can detect either antibodies to HA or NA, depending on the use of reassortant viruses possessing the relevant surface glycoprotein of interest and a mismatched second glycoprotein.<sup>23</sup>

Immunological priming is the activation and expansion of antigen-specific T cells that confer memory and exert effector functions, providing more rapid development of protective immune responses upon repeat exposure to a related antigen. The 2009 H1N1 pandemic highlighted the need to evaluate immunological priming to understand better what proportion of the population and age groups may need a two-dose versus a one-dose vaccination regimen. Serum antibody responses are not appropriate markers of priming as the absence of detectable strain-specific responses is not indicative of absence of priming. In contrast, detection of antibody secreting cells in peripheral blood mononuclear cells (PBMC) can provide information on whether a primary response has occurred and the relative size of the memory B cell pool. Antigen-specific cytokine-producing T cells can be detected by multiparameter flow cytometry, detecting both cell surface markers and intracellular cytokines. In H5N1 vaccine trials, the detection of CD4<sup>+</sup> T cells producing one or more cytokines including IL-2 after the first vaccine dose, was the earliest and most accurate predictor of whether a subsequent (third) vaccine dose resulted in high and durable neutralizing antibody titers. Although such cellular responses can be readily measured using current technologies, the timing and volume of whole blood collection, methods of PBMC preparation, form of antigen for restimulation *in vitro*, and reproducibility of assays are all critical factors to the success of this approach.

Steven Pincus, (Novavax, Inc., Rockville, MD, USA) described the development of VLP recombinant influenza vaccines using the baculovirus expression system in insect cells to produce the HA, NA, and M1 vaccine components. A trivalent VLP vaccine (15 or 60  $\mu$ g doses) based on the

2008–2009 season's vaccine antigens was well tolerated and elicited serum HI titers in adults aged 18–49 that generally met licensure criteria. A VLP-based NA inhibition assay was developed and used to detect anti-NA antibody seroconversions against the H3N2 and influenza B antigen in >50% of subjects, highlighting the potential for VLP vaccines to induce immunity to non-HA viral components. Surender Khurana, (CBER/FDA, Bethesda, MD, USA) reported on the use of whole-Genome-Fragment Phage Display Libraries expressing all open reading frames of avian H5N1 virus to investigate the repertoire of antibody responses in subjects vaccinated with H5N1 non-adjuvanted versus alum- or MF-59- adjuvanted vaccines.<sup>24</sup> While anti-HA epitope specificity spanned both HA1 and HA2, antibodies to conformational epitopes in HA1 were more frequent in sera from MF59 adjuvanted vaccine groups. Antibody epitopes were also detected in the NA catalytic site, the M2 ectodomain and interestingly, in the PB1-F2 protein. Laurent Coudeville, (Sanofi Pasteur, Inc., Lyon, France) described the use of a statistical model to re-evaluate the relationship between HI antibody titers and clinical protection using data from 15 influenza vaccine studies. A strong positive relationship was found between HI titers and 50% clinical protection against influenza.<sup>25</sup> No significant differences were detected between vaccinated and unvaccinated subjects, or between influenza A and B viruses. Interestingly, additional protection was marginal with HI titers above 80.

## Session 5. New adjuvants, rational design, and regulatory approval

Invited presentations were from Steven Reed, (Infectious Disease Research Institute and Immune Design Corporation, Seattle, WA, USA), David Wood on behalf of Martin Friede, (Initiative for Vaccine Research, WHO, Geneva, Switzerland) and Hana Golding (CBER/FDA, Bethesda, MD, USA).

Adjuvants are used to enhance and broaden immune responses and to allow for dose sparing of influenza vaccines.<sup>26</sup> A number of adjuvanted seasonal or pandemic influenza vaccines are now licensed including those formulated with proprietary squalene-based oil-in-water adjuvants (AS03 and MF-59) or aluminum hydroxide or aluminum phosphate (alum). Adjuvants in clinical trials for influenza vaccines include additional squalene oil-in-water products, adjuvants containing Toll-like receptor (TLR) agonists (TLR4, 5 and 9), cationic lipid-based or complement activator (inulin) components. Because all adjuvants have unique formulations, each must be investigated and reviewed independently for safety and efficacy. Furthermore, the exact mechanisms of action of many adjuvants remains incompletely understood, although likely

mechanisms include improved antigen delivery and immune potentiation, including greater antigen-presenting cell recruitment and activation. Future challenges for the clinical development of adjuvants include optimization for different delivery routes such as intradermal or mucosal routes, and targeting the boosting of CD8<sup>+</sup> T-cell responses, in addition to antibody responses.

Following licensure in Europe, general use of 2009 pandemic vaccines formulated with either AS03 or MF-59 began in October 2009. Assessing the risk of adverse events associated with adjuvanted vaccine use versus the protective benefit of such vaccines remains an important public health objective that can be addressed in continuing post-marketing surveillance. Public perception of adjuvanted vaccine safety will influence public acceptability. For other adjuvanted vaccines still in clinical trial, further knowledge of the frequency and causes of severe adverse events is needed as well as establishment of reproducible formulation methods. Each adjuvanted vaccine, and not the adjuvant alone, must be evaluated independently. An important preclinical consideration is the choice of animal model, because it is important that receptors involved in adjuvant action are expressed and function similarly to those in man, so ensuring that both enhancing effect and adverse events can be evaluated with relevance for human vaccination. However, innate receptors are often species specific, and therefore, traditional animal models may not *a priori* be suitable for preclinical safety evaluation of adjuvants. The use of human monocytic cell lines to assess the ability of candidate adjuvants to induce proinflammatory cytokine responses or pyrogenic substances such as prostaglandin-E2 may therefore provide data to supplement reactivity studies in rabbits and assist in the selection and development of safer adjuvants. Challenges for clinical evaluation of safety include detection of age and special population-specific differences in response to adjuvants, and implementing appropriate monitoring for long-term safety.

## Session 6. Measurements of protection

Invited presentations were by Jacqueline M. Katz, (Influenza Division, National Center for Immunization and Respiratory Diseases, CDC, Atlanta, GA, USA), Joost Kreijtz, (Erasmus University Medical Center, Rotterdam, The Netherlands) and Kanta Subbarao (Laboratory of Infectious Diseases, NIAID/NIH, Bethesda, MD, USA).

Although a number of small animal models exist for preclinical evaluation of influenza vaccines, mice and ferrets remain those most routinely used. Inbred mice are typically used as a first tier model for proof of concept and dose ranging studies, and an understanding of immune correlates of protection, but are optimal only for challenge

studies with non-human influenza viruses or mouse-adapted viruses that replicate optimally in the murine respiratory tract that expresses non-human virus preferred receptor glycans. Ferrets have become a popular second tier model owing to their susceptibility to seasonal and pandemic human viruses and animal-origin viruses with pandemic potential and clinical signs that reflect human disease.<sup>27</sup> They provide a useful bridging model to assess protective efficacy, particularly when clinical efficacy studies are not feasible; however, a broader range of immunological reagents is urgently needed.

Non-human primates (NHP) offer a large animal model that most closely resembles humans. Demonstrating immunogenicity and protective efficacy in this model establishes a clear path to clinical studies.<sup>28</sup> The similarity in host genome and the use of NHP as models for other pathogens has resulted in a broad array of immunologic reagents to assess both early virus–host interactions and adaptive immune responses. However, ethical and practical constraints, as well as cost limit their widespread use.

Experimental challenge studies in humans can provide direct clinical evidence of vaccine efficacy and immune correlates of protection. However, ethical considerations, including the need for a strong scientific rationale, the minimization of risk and discomfort and protection of rights of volunteers, the limited availability of Good Manufacturing Practice grade virus pools, as well as overall costs of such studies, has restricted this approach at present.<sup>29</sup> On the other hand, the new antiviral drugs will add additional protection to volunteers when challenged under controlled quarantine conditions. Although experimental infection may not accurately replicate clinical illness in the field, past studies have nevertheless contributed substantially to our knowledge of immune correlates of protection against influenza. These include the correlation of pre-challenge serum neutralizing antibody with protection from influenza illness and earlier clearance of virus and the demonstration that CTL responses were associated with a lack of virus shedding in seronegative volunteers. Additionally, the inability to correlate nasal antibody, serum anti-HA or NAI antibody with protection from severe disease is noteworthy.

## Session 7. Current status of pandemic vaccines and case studies of pandemic vaccines

Session 7 included invited presentations by Wendy Keitel (Baylor College of Medicine, Houston, TX, USA), Frederick Cassels (NIAID/NIH, Bethesda, MD, USA) and David Wood (Department of Immunization, Vaccines and Biologicals, WHO, Geneva, Switzerland).

There is now over a decade of experience in clinical trials for vaccines against avian viruses, but several uncertainties

and hurdles remain. The overall poor immunogenicity of H5 and H7 virus vaccines remains poorly understood. Pre-clinical studies have demonstrated, in some cases, a lack of correlation between HI antibodies and protection against avian virus challenge. Some whole virion formulations performed better than split or subunit formulations. The use of alum adjuvant resulted in variable outcomes, whereas vaccines using oil-in-water adjuvants performed well and gave a better cross-reactive antibody profile.<sup>30</sup> Head-to-head comparisons between different vaccine formulations, with and without different adjuvants, are needed to better ascertain any differences in immunogenicity. The use of oil-in-water adjuvants provided antigen dose sparing and the possibility of expanding a limited vaccine supply. Pre-pandemic immunization can prime individuals for a more robust response to a pandemic antigen of the same subtype and is a potentially useful public health strategy.

For the current H1N1 pandemic, NIAID/NIH studies have facilitated availability of immunogenicity data in different populations, such as healthy adults, elderly, and children (6 months–17 years) to help inform policy decisions. A single 15- $\mu$ g dose of vaccine elicited a serum antibody response in most individuals aged  $\geq 10$  years. Reactogenicity and adverse events were comparable to that seen with seasonal influenza vaccination. Concurrent or sequential use of trivalent seasonal vaccine did not affect the response to pandemic vaccine.

David Wood gave initial reflections on the global pandemic vaccine response. All aspects of the pandemic response will be formally reported to the May 2011 World Health Assembly. Pledges for donating pandemic vaccine to developing countries were given by several manufacturers and some individual countries. Delivery in a timely manner was hampered by very tight time frames imposed by rapid spread of the pandemic virus as well as the changing expectations. It was initially (June 2009) assumed that an H1N1 seed virus would produce quantities of vaccine comparable to seasonal H1N1 virus, whereas in reality, pandemic virus vaccine yield was in early stages only about a third of anticipated output. Additionally, because most vaccine manufacturers' production capacity was until mid-2009 used for seasonal vaccine, the supply to developing countries was hampered. The complexities of improving global access to vaccines demonstrated the limitations of an *ad hoc* approach.

Bascom F. Anthony [Biomedical Advanced Research Development Authority (BARDA), Department of Health and Human Services, Washington, DC, USA] explained that Biomedical Advanced Research Development Authority supported development and manufacture of 2009 H1N1 vaccines in the US and prepared documentation for Emergency Use Authorization for adjuvanted vaccine. In all, BARDA supported 14 trials conducted by manufacturers, involving over 15 000 subjects that received non-adjuvant-

ed or adjuvanted inactivated vaccines or LAIV. Kawsar R. Talaat (Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA) reported on a 2009 pandemic H1N1 vaccine trial in different aged populations. A single 7.5- $\mu$ g dose was well tolerated and highly immunogenic in all groups, including the 'younger elderly' and 'very elderly', aged  $\geq 70$  years.<sup>31</sup> HI antibody responses were detected as early as 7 days post-immunization, and age and receipt of seasonal influenza vaccine in the previous year were predictive of baseline antibody titers. Nathalie Landry (Medicago, Inc., Québec, QC, Canada) described the production of recombinant proteins based on transient expression technology in plants for the formulation of a VLP vaccine that has undergone initial Phase I clinical trial evaluation.<sup>32</sup> Two doses of 20  $\mu$ g of alum-adjuvanted H5 VLP vaccine was well tolerated and elicited HI antibody responses that achieved two of three CHMP criteria for licensure of seasonal vaccines, as well as neutralizing antibodies. The H5 VLP induced cross-H5 clade protection against lethal challenge in ferrets, even when HI titers were modest against the challenge strain. Ted M. Ross (Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, USA) described a seroprevalence study to estimate the prevalence of antibodies (HI titer  $\geq 40$ ) to 2009 H1N1 virus.<sup>33</sup> Extrapolating from seroprevalence results, it was estimated that approximately 21% of Pittsburgh area residents, including more than 70 000 school-age children were positive for novel H1N1 influenza following the second pandemic wave.

## Session 8. Summary session and the way forward

The final session of the meeting consisted of an open discussion by all participants on current limitations and gaps in knowledge and possible next steps for identification of additional immune correlates of protection against influenza. Despite the many advances in virology, biotechnology, and immunology since the last pandemic of 1968/1969, our understanding of the immune response to influenza is still fragmented. Although serum HI antibody responses remain the primary criteria for evaluation of seasonal and pandemic vaccine immunogenicity and licensure, additional supplementary correlates of protection are lacking. The extent to which an HI titer of  $\geq 40$  represents a 50% protective titer against highly virulent H5 and H7 subtypes, or is valid for pediatric populations, remains unclear. Clinical testing of the 2009 H1N1 vaccine revealed a greater degree of population priming than previously anticipated. Immune markers to assess the level of priming in a population would help guide vaccine dosing and priorities. The many new vaccine platforms entering clinical trials add further complexity and urgency to identifying additional surrogate immune markers that can be corre-

lated with protection in vaccine efficacy or human challenge studies.

A central theme reiterated over the 2-day meeting was the need for harmonization of validated laboratory assays, including the use of unified laboratory protocols, standard reagents and the development and use of international standards that could be used to reduce inter-laboratory variation and provide a benchmark for comparison of immunogenicity data across laboratories and among different vaccine types. While progress has been made in this area for well-established methods such as the HI and VN assays, it is particularly important that laboratories working on newer methods to assess non-HA-based immune responses (e.g. anti-NA and anti-M2 antibody) begin a harmonization process before laboratories validate in-house methods. It was suggested that the WHO should play a role in encouraging and facilitating international harmonization in this area. A point to consider for regulatory agencies was to encourage manufacturers, – or others conducting influenza vaccine clinical trials, – to expand protocols to include measurements of other immune parameters than serum anti-HA antibody. The spectrum of responses tested would depend on the nature of the vaccine but may include measurement of anti- NA, M2e and/or NP antibody responses, or assessment of phenotypic and functional CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Standardized and validated methods to assess T-cell responses have recently been described.<sup>34</sup> Generation over time of a database consisting of a wider repertoire of humoral and cellular responses may allow regulators to formulate additional licensing criteria. This may best be approached by the development of a consortium, consisting of laboratories with expertise in a variety of laboratory methods and different international regulatory agencies. Harmonization of criteria used by different regulatory authorities in influenza vaccine licensure was also seen as an important next step for which the WHO may again be able to provide a forum for discussion toward global harmonization. In closing, meeting participants acknowledged the importance of regular meetings to address the immune correlates of protection, particularly for new-generation influenza vaccines, and called for a follow-up meeting in approximately 2 years.

## Conflict of Interest

Dr Jacqueline M. Katz has received funds from Nobilion-Merck Sharp & Dohme Corp., Glaxo Smith Kline, and Juvaris Inc. for research not related to the current report.

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