Immunological Assay Standardisation and Development for Use in Assessment of Correlates of Protection for Human Influenza Vaccines

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Need for public-private collaboration research

- Research on standardisation of immunological assays to measure the response to seasonal human influenza vaccines and, as needed, of their development, represents an ideal ground for public-private collaboration. Several levels of benefits would be derived for both environments:
    - Common agreement on the way to perform assays such as HAI which are currently utilised for influenza vaccine registration
    - Common assay used by all groups, private &public, when testing influenza vaccines in humans
    - Rigorous and unequivocal assays when establishing correlates of protection
    - Consensus on the way(s) to measure neuraminidase inhibiting (NI) antibodies to be possibly applied to future clinical trials
    - Possibility to apply this knowledge to pandemic influenza vaccines
    - Advanced understanding of the different immunological parameters (cellular, transcriptomic, etc.) which could be tested in trials with influenza vaccine
    - Ultimately, the evolution of the scientific and technical knowledge will also mould the necessary evolution of the regulatory guidance and ultimately the practice of the pharmaceutical industry

Objectives of the full project

- Improve and standardise the existing immunological assays and to develop new assays to better evaluate seasonal human influenza vaccines

- Generate validated standardised serological assays and applicable supportive immunological assays that can be used in studies aimed at developing clinically relevant surrogate markers of protection for influenza vaccines

- These ultimate goals would be achieved through 3 intermediate objectives:
    1. achieving standardisation of HAI and VN assays, as a primary goal
    2. advancing the understanding and application of CMI and NA assays as tools for evaluating influenza vaccine performance, as a secondary goal
    3. consideration of new technology yet to be applied to population based evaluations of influenza vaccines, as an exploratory goal

Pre-competitive nature

- Human influenza vaccines are evaluated serologically essentially using HAI (correlates exist for adults and elderly only, although controversial) and VN (no correlates) → strong variability among laboratories → need for standardisation and harmonisation

  - HAI: cheap, easy, variable, it may not detect all functionally active antibodies

  - VN: more relevant, more sensitive than HAI; more variable and more complex;

- Role of NA Ab unclear (likely some protection against disease)

  - NA content in vaccines not required for licensure

  - NA antibody assays not standardised nor required by RA

  - Their measurement complicated in the presence of anti-HA Ab

- Role of CMI in protection still unclear

  - inherent complexity of CMI assays (including practical barriers)

  - No agreement on which assays to run as best correlates and how to run them

Problem Statement
1. Measurement of antibodies

- International collaborative studies have concluded that[1]:
  - Strong inter-laboratory variability of GMT of Ab measured by HAI (up to 6 fold) and by VN (up to 7 fold)
  - Qualified serum standards often reduces this variability
  - Open questions to be answered:
    - Same standard for both HAI and VN?
    - Strain-specific? Type-specific? And for new strains?
    - Need for consensus on calculation of standard

- Other recent studies[2] have stressed the need for for more exploratory studies, especially for VN (which cell lines? Same for all virus strains? Which best way to measure?)

- Lack of agreement on ways to measure anti-NA Ab, standardisation of reagents, ways to make measurements, etc → need for substantial experimental work
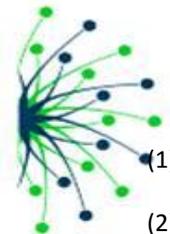
(1) Wagner R et al (2012) Vaccine 30: 4113; Stephenson I et al (2007) Vaccine 25: 4056; Stephenson I et al (2009) Emerg Infect Dis 15: 1250; Wood J et al (2012) Vaccine 30: 210.
(2) Laurie KL et al (2012) Influenza Other Respir Viruses 8: 1750

Problem Statement
2. CMI and other biomarkers

- The role of CMI in protection against influenza remains controversial

- Ag-specific CMI can participate in protection against influenza following experimental infections in mice and, more recently, in humans. However, unlike Ab-mediated protection, CMI alone is unable to protect against infection, but only against severe disease and against some clinical endpoints[1]

- These cellular responses are directed not only against proteins present in inactivated vaccines but also against internal proteins, like M1, NP, which are not present or present as traces in inactivated vaccines

- Unlike serology, the evaluation of CMI faces serious hurdles on the significance of the different assays existing and on the best way to have them performed in different centres (in addition to practical issues)

- New analytical tools are being developed that could give insights in the protection induced by influenza vaccines. Research devoted to new tools, which include systems biology, definition of biomarkers, etc. may uncover still unknown parameters that could/should be tested as potential CoP
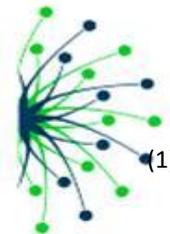
(1)   Wilkinson TM et al (2012) Nature Med 18: 274

Expected impact on the R&D process

- The availability of standardised, validated serological assays for human infuenza vaccines, agreed and used by everybody in the private and in the public sector, would have tremendous impact on the R&D process globally:
  - Results of clinical trials would be more stringently comparable even if run not concomitantly and in different geographic areas and using different influenza vaccines
  - The interpretation of the data would be unequivocal
  - A stronger scientific understanding of biomarkers other than antibodies may help design novel vaccine approaches
  - Last, but not least, the evolution of the scientific and technical knowledge will also mould the necessary evolution of the regulatory guidance and ultimately the practice of the pharmaceutical industry

Suggested architecture of the project
WP1. Standardisation of HAI & VN

- HAI
  - Critical aspects to be evaluated include (but are not limited to) all reagents, assay conditions and execution, standardisation of calculation, statistical evaluation

- VN
  - Critical aspects to be evaluated include (but are not limited to) all reagents, assay conditions and execution of both short-term and long-term VN, including the cell types to be used, standardisation of calculation, statistical evaluation

- Harmonisation of assay validation procedures
  - ICH and FDA guidelines designed for analytical assays not necessarily fully apply to bioassays
  - Then, need for agreement on common definitions, procedures, and acceptance criteria. Involvement of biostatisticians for all aspects of assay validation

Suggested architecture of the project
WP2. CMI and NA antibodies

- Research-oriented activities on anti-NA Ab
  - Activities will include definition of assays to be evaluated comparatively, definition of critical reagents, identification of potential clinical standards, suitability of the assay format, reach a consensus on the assay & standard(s) to be used in future clinical studies

- Research-oriented activities on CMI
  - Activities will be focused on definition of critical issues and possible solutions: procedures of separation, storage, thawing of PBMC, comparison of fresh vs. frozen cells, clear definition of assays for T- and/or B-cell immunity, and why's and how's to perform them, and their standardisation from the simplest and less sensitive (e.g. proliferation, ELISPOT, etc) to the most complex and sensitive (e.g. cell sorting, CyTOF, etc)

Suggested architecture of the project
WP3. Novel technologies

- New analytical tools are being developed that could give insights to antibody or cellular arms of the immune system in association with vaccine responses and relationship to protection from influenza disease: e.g. systems biology, microarrays, multiplex EIAs with different HA subunits, etc. or measurements of immune quality

- The applicant consortium is encouraged to propose novel methods and assays that could supplement the above technologies in the tool-box for future studies to develop correlates of protection

- These new methods must be suitable for population studies, must not impinge upon IP restrictions, must be reasonable in cost, must be easily transferable to multiple laboratories, and must be capable of being validated

Expected contributions of the applicants

- The applicant consortium is expected to consist of small- and medium-sized enterprises (SMEs), academic centres (both clinical and experimental), centres from national and/or supranational public health bodies, regulators

- The consortium should combine partners with an established and well recognised experience in the field of human influenza, of developing and validating immunological assays for detection of functionally active antibodies against influenza viruses following natural infection or vaccination, of cell-mediated immunity applied to influenza infection and vaccination, and of novel technologies for definition of biomarkers following vaccination

Expected (in kind) contributions of EFPIA members

- EFPIA members will be: Novartis (coordinator), Sanofi-Pasteur (deputy coordinator), GSK, Abbott, Crucell (Johnson & Johnson) and MedImmune (Astra Zeneca)

- EFPIA partners are experts in development, optimisation, standardisation and validation of serology assays (especially HAI and VN), and in development and application of assays for dissecting cellular immune responses at both T- and B-cell level

- EFPIA partners will contribute where appropriate to the development and review of experimental designs, and as required supply materials, reagents, protocols and laboratory efforts that will support the success of each of the 3 Work Packages.

What's in it for you?

- All stakeholders would benefit from this project:
  - Academic researchers would have the opportunity to access to information, material, protocols from influenza vaccine manufacturers; thanks to the in-kind contribution from EFPIA members they will actively contribute to the advancement of the scientific knowledge in various fields of influenza vaccination
  - SMEs will have the opportunity to share protocols, information material with vaccine manufacturers and academic institutions and to expand their future internal capacities
  - Regulatory Agencies will actively and even proactively interact with both industry and academy for the definition of key parameters essential for vaccine registration
  - An open and scientifically driven collaboration between academy and vaccine manufacturers will contribute to increase the transparency of all activities behind vaccine research, release and licensure, with a consequent increase in the awareness of and in the trust by patients' organisations

Key deliverables of the full project

- Reach an agreement in both the private and the public sectors on the way to perform assays for seasonal human influenza vaccines such as HAI which are currently utilised for influenza vaccine registration
- Establish common protocols for all assays that will be used by all groups, private &public, when testing influenza vaccines in humans
- Apply rigorous and unequivocal assays when establishing correlates of protection
- Understand and agree upon the relative or absolute benefit of different assays, such as HAI and VN (and the different ways the latter can be performed), in measuring functionally active anti-influenza antibodies
- Reach a consensus on the way(s) to measure neuraminidase inhibiting (NI) antibodies to be possibly applied to future clinical trials
- Advance the understanding of the different immunological parameters (cellular, transcriptomic, etc) which could be tested in a clinical trial with influenza vaccine to dissect the quality of the immune response induced by the different existing or novel influenza vaccines

Questions?

Contact the **IMI Executive Office**

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