Innovative Medicines Initiative

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Annex 1

10th Call for Proposals 2013

Innovative Medicines Initiative

Version 2

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GENERAL PRINCIPLES

The Innovative Medicines Initiative Joint Undertaking (IMI JU) is a unique pan-European public private partnership between the European Commission and EFPIA[i] driving collaboration between all relevant stakeholders including large and small biopharmaceutical and healthcare companies, regulators, academia, and patients.

The aim of IMI is to propose a coordinated approach to overcome identified research bottlenecks in the drug development process, in order to accelerate the development of safe and more effective medicines for patients, by fostering collaboration between all stakeholders such as industry, public authorities (including regulators), organisations of patients, academia and clinical centres, and enhancing Europe's competitiveness.

The revised IMI Scientific Research Agenda http://www.imi.europa.eu/content/research-agenda describes the research bottlenecks in the drug development process and identifies new and established research priorities correlated to at least one of the seven IMI Areas of Research Interest.

The IMI 10th Call 2013 for proposals includes a topic covering the following key research priority:

- Infectious diseases (correlated to the area of interest: Disease Drug Efficacy)

The 10th Call topic is:

- Immunological Assay Standardisation and Development for use in Assessments of Correlates Of Protection for Influenza Vaccines

Applicant Consortia are invited to submit expressions of interest to this topic.

The expressions of interest should address all aspects of the topic.

The size of each consortium should be adapted to the scientific goals and the expected key deliverables.

Further information can be found under the section 'Synopsis of Call and evaluation processes'.

Before submitting an expression of interest, the various Call documents, such as *IMI JU Rules for submission, evaluation and selection of Expressions of Interest and Full Project Proposals*, *Rules for Participation*, *the IMI Intellectual Property Policy*, etc., shall be considered carefully. These documents are published on the IMI website www.imi.europa.eu at the time of the 10th Call 2013 launch.

Synergies and complementarities with other IMI and EU funded projects should be explored in order to avoid overlaps and duplications and to maximise European added value in health research.

DURATION OF THE PROJECT

The indicative duration of the project is 5 years. Please note that the Council Regulation 73/2007 set up the IMI JU as a body responsible for implementing the 7th Framework Programme for Research and Development for a period up to 31 December 2017. In

[i] European Federation of Pharmaceutical Industries and Associations – www.efpia.eu

accordance with the statutes of the IMI JU an ad hoc procedure will be set up to ensure appropriate management of the concerned Grant Agreement(s) after the termination of the IMI JU. The Programme Office will work with the Commission services and the concerned project coordinator(s) to ensure a sound and smooth transfer of the grant agreement(s), the associated commitments and payments, the project file(s), the IT tools access and the audits rights.

FUNDING OF THE PROJECT

For this Call, the total available financial contribution from the IMI JU to participants eligible for funding will be maximum EUR 6 100 000.

The indicative EFPIA 'in kind'[ii] contribution will be EUR 6 100 000.

The Applicant Consortia shall keep in mind that the budget of each expression of interest is to be adapted to the scientific goals and the expected key deliverables of the project.

SYNOPSIS OF CALL AND EVALUATION PROCESS

The IMI JU supports research activities following open and competitive Calls for proposals, independent evaluation and the conclusion of Project and Grant Agreements.

The topic included in the 10th Call is associated with a group of pharmaceutical companies that are members of EFPIA (hereafter called the 'EFPIA Consortium') and which are committed to collaborate with public and private organisations eligible for funding by the IMI JU. The EFPIA members will provide 'in kind' contributions to support their activities within the research project.

The IMI JU applies a two stage Call process. In the first stage, 'Applicant Consortia' (i.e. formed by academia, small- and medium-sized enterprises (SMEs), patient organisations, non EFPIA companies, etc.) are invited to submit, to the IMI JU, an expression of interest (EoI) in response to a Call topic.

In preparing their EoIs, the Applicant Consortia should carefully read the Guidance Notes for Submission and Preparation of Expression of Interest published on the IMI website www.imi.europa.eu at the time of the 10th Call 2013 launch, in addition to the specific Applicant Consortium expectations/requirements outlined within the description of the topic.

The Applicant Consortium shall consider the research contribution that an EFPIA Consortium will make to a given project.

Each EoI submitted will be reviewed by independent experts according to predefined evaluation criteria.

Each Applicant Consortium with the highest ranked EoI will be invited to develop a full project proposal together with the EFPIA Consortium.

For each topic, the full project proposal will then be subject to a final review by independent experts according to predefined evaluation criteria.

[ii] In kind contribution is e.g. personnel, clinical research, equipment, consumables.

Only a full project proposal that has been favourably reviewed in the evaluation process can be selected for funding. This project will then be invited by the IMI JU to conclude a Grant Agreement governing the relationship between the selected project consortium and the IMI JU. Consortia also must conclude a Project Agreement before the Grant Agreement can be signed.

For full details, applicants should refer to the *IMI JU Rules for submission, evaluation and selection of Expressions of Interest and Full Project Proposals* published on the IMI JU website www.imi.europa.eu at the time of the launch of the 10th Call.

ELIGIBILITY TO PARTICIPATE IN PROJECTS AND TO RECEIVE FUNDING FROM THE IMI JU

Criteria of eligibility to participate in IMI projects and the criteria to receive funding from the IMI JU are specified under the *Rules for participation* in the IMI JU collaborative projects published on the IMI JU website www.imi.europa.eu.

The IMI JU financial contribution will be based on the reimbursement of the eligible costs. The following funding rates apply to the legal entities eligible for funding: For research and technological development activities, up to 75% of the eligible costs and for other activities (including management and training activities) up to 100% of the eligible costs charged to the project are eligible for funding. For the indirect costs (overheads), the legal entities eligible for funding may opt for one of the following indirect costs methods: the actual indirect costs; or the simplified method which is a modality of the actual indirect costs for organisations which do not aggregate their indirect costs at a detailed level, but can aggregate them at the level of the legal entity; or a flat rate of 20% of total eligible direct costs (excluding subcontracting costs and the costs of resources made available by third parties which are not used on the premises of the beneficiary).

For full details, Applicant Consortia are invited to refer to the *Rules for Participation* in the IMI JU collaborative projects (www.imi.europa.eu).

The research-based companies that are members of EFPIA shall not be eligible to receive financial contributions from the IMI JU.

IMI INTELLECTUAL PROPERTY POLICY

The IMI Intellectual Property Policy (IMI IP policy, www.imi.europa.eu) has been developed to be aligned with the objectives of the IMI JU to ensure knowledge creation, together with the swift dissemination and exploitation of knowledge, and fair reward for innovation.

The IMI IP Policy sets out *inter alia* basic principles regarding ownership of Background and Foreground, access rights depending on the entity and the purpose, and dissemination.

In submitting an EoI, the Applicant Consortia fully understand the principles laid out in the IMI IP policy that will apply to all research projects conducted under the IMI JU.

The IP policy does not foresee all details and does not aim to answer to all possible practical situations participants may be faced with. Flexibility is provided for participants to establish the most appropriate agreements (e.g. the Project Agreement) serving each individual project's objectives, and considering the wider IMI objectives.

Applicant Consortia are invited to read carefully the Guidance Note on the IMI IP Policy (www.imi.europa.eu), whose purpose is to explore ways to handle related issues and pitfalls that participants may encounter during the preparation, negotiation and completion phases of the Grant Agreement and Project Agreement.

PROJECT AGREEMENT

The Project Agreement is a private agreement which the participants of an IMI project conclude amongst themselves to implement the provisions of the Grant Agreement and to regulate internal issues related to work organisation and objectives for each participant, consortium governance, IP, financial and other matters.

All participants of a selected IMI project are requested to start negotiation on the Project Agreement between them in parallel to the preparation of the full project proposal.

The Full Consortium shall ensure that the negotiation of the Project Agreement is completed no later than the finalisation of the full project Description of Work and prior to signing the Grant Agreement.

IMMUNOLOGICAL ASSAY STANDARDISATION AND DEVELOPMENT FOR USE IN ASSESSMENTS OF CORRELATES OF PROTECTION FOR INFLUENZA VACCINES

SUMMARY AND OVERALL OBJECTIVES

Despite the development and licensure of influenza vaccines along with their use for decades, the potential correlates of protection induced by these vaccines are still a matter of debate. This is due to some key factors: (i) for some assays, such as the haemagglutination inhibition (HAI) assay, which is widely accepted for vaccine registration, a rigorous standardisation of the assay is still lacking; (ii) for some other assays, such as the virus neutralisation (VN) assay, a clear correlate of protection has never been established, nor has the assay been standardised; in addition, there is controversy over the most appropriate way to carry out these assays; (iii) finally, progress in basic science in the fields of virology, immunology and molecular biology are opening opportunities to have a deeper understanding of the mechanisms of protection against influenza. They also offer the possibility of developing new tools which may ultimately be applied to better define correlates of protection.

For these reasons, the long-term vision behind this Call for proposals is to improve and standardise the existing immunological assays and to develop new assays to better evaluate influenza vaccines. To this end, the overall objective of this proposed project is to deliver standardised and validated 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.

This ultimate goal can be reached through defined, specific objectives which are as follows:

- As a primary goal, achieve standardisation of HAI (haemagglutination inhibition) and VN (virus neutralisation) assays.
- As a secondary goal, advance the understanding and application of CMI (cell-mediated immunity) and NA (neuraminidase) assays as tools for evaluating influenza vaccine performance.
- Finally, as an exploratory goal, consideration of new technology yet to be applied to population-based evaluations of influenza vaccines.

BACKGROUND

Influenza vaccines are typically monitored by traditional serological assays such as HAI and VN. Other antibody assays may be used, however they are not the most common methods used by health authorities to evaluate the performance of influenza vaccines. Any effort to better define correlates of protection from influenza vaccines would benefit from a larger tool-box of relevant immunological assays. Such a tool-box would increase the likelihood of success to find the biomarkers best suited for predicting protection from vaccines.

Several past and on-going initiatives were/are aimed at improving standardisation and/or harmonisation of serological assays, but the variability among laboratories is preventing the establishment of a common correlate of protection.

It is acknowledged among influenza experts that the HAI assay, despite its limitations, is a well-known, simple and low-cost technique that can be readily performed in

laboratories worldwide [1]. However, the VN assay is seen as more relevant since a wider range of functionally active, infection-blocking antibodies are detected. The VN assay is also usually more sensitive compared to the HAI assay, but is usually more variable and complex to set up. The added value of the VN assay over HAI (e.g. the potential for also measuring anti-neuraminidase activity) still remains unclear and seems to depend on the strain and assay format, but also on the type of vaccine. Live-attenuated influenza vaccines (LAIV), split/subunit virus or recombinant vaccines can trigger different immune responses. To further investigate the appropriateness and added value of the VN response as a potential correlate for protection for influenza, harmonisation of the VN assay with the goal of a defined protocol (e.g. short vs long incubation time, cell type to be used, reading method) is needed.

Antibodies directed against NA may contribute to protection from clinical disease by reducing virus spreading [2, 3, 4]. The role of anti-NA antibodies may play an important role in the clinical efficacy of LAIV type of vaccines, however, its role for inactivated vaccines remains a matter of debate because (i) control and measurement of the amount of NA present in vaccines as an active pharmaceutical ingredient is not required by current pharmacopoeias; (ii) no standard assay to measure anti-NA antibodies is currently accepted by regulatory authorities; and (iii) since anti-NA antibodies do not block primary infection (i.e. are not neutralizing) the separate measurement of anti-NA specific antibodies, in the presence of anti-HA antibodies, is complicated.

In addition to antibody responses, influenza infection and vaccination also induce measurable cell-mediated immunity (CMI). Despite an extensive body of evidence for the role of CMI in viral clearance and disease resolution (mainly in the mouse), there is no agreement on which assays may best correlate with efficacy of influenza vaccines.

Due to the biological complexity of CMI assays, their current stage of analytical development is at a research level with significant uncertainty that any of these assays can be translated into meaningful accepted measurements of protection. Significant analytical development and clinical sample evaluation must be invested to determine the usefulness of CMI assays and the limitations and practical barriers to implementation of these assays as potential tools to measure correlates of protection against influenza.

PROBLEM STATEMENT

Recent international collaborative studies involving several laboratories were conducted to evaluate assay reproducibility, using candidate standard serum preparations or sera panels from clinical vaccine trials [5, 6, 7, 8, 9]. The conclusions were: (i) there is a marked inter-laboratory variation of geometric titers determined by HAI (up to 6 fold) and VN (up to 7 fold); (ii) the implementation of qualified serum standards (from immune animals or humans) for the relative adjustment of original titers often effectively reduces inter-laboratory assay variability. However, several parameters remain to be evaluated: Should the same reference standards be used for both HAI and VN assays? Should strain-specific, or at least type-specific, qualified standards sera be used? If type- specific standard sera are used, how can results be translated to new viral strains for normalisation among different testing laboratories? How should the consensus value of the standard be calculated?, etc.

As per recent investigations at the Paul Ehrlich Institute (PEI) and the National Institute for Biological Standards and Control (NIBSC) (results presented at the May 2012 EMA serology workshop) and as also mentioned during the 2nd Global Influenza Seroepidemiology Expert meeting [10], exploratory experiments are still needed to determine the most suitable assay. This is particularly true for the VN assay, for which controversy remains about the most adequate cell line [11] , the best reading method

(HAU, ELISA, other), or the quality assessment/monitoring of cell preparations. In this context, it would be desirable to compare the classical "short-incubation" VN format (WHO/CDC method) and a "long-incubation" VN assay which, in theory, appears capable to capture a broader neutralising activity.
While there is general agreement on the assays to be used to evaluate antibody responses to HA, no agreement exists on the assay(s) to be utilised for anti-NA specific antibodies. Experimental work is needed in order to understand the minimum requirements necessary to have a rigorous and biologically meaningful assay able to measure functionally active antibodies against neuraminidase.

While it is well recognised that protection against influenza is mediated by antibodies, especially those directed against surface proteins and among those antibodies against HA, the role of CMI in protection is still a matter of debate. Antigen-specific cellular responses have been found to participate in protection against influenza following experimental infections in mice and, more recently, in humans [(12)]. However, unlike the protection mediated by antibodies, these cellular responses are unable to protect against infection, but only against severe disease and against some clinical endpoints. In addition, these cellular responses can be 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. This situation is different for LAIV due to the complexity of the antigenic repertoire present in the vaccine. For these reasons, unlike serology, the evaluation of CMI faces different hurdles on the significance of the different assays existing and on the best way to have them performed in different centres. All these aspects need to be approached comprehensively in order to achieve meaningful outcomes.

In addition to the analysis of antibody and cellular responses, there are now many new analytical tools 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. 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 correlate of protection.

NEED FOR PUBLIC-PRIVATE COLLABORATIVE RESEARCH

Fighting against influenza is a commitment that involves both the private and the public environments. Effective influenza vaccines are produced each year by several vaccine manufacturers who are committed to provide inactivated or live attenuated vaccines consisting of the strains of influenza virus recommended by WHO. In addition, laboratories in private industries are actively involved in developing novel influenza vaccines and/or in expanding the use of existing and novel vaccines to all age groups and categories. Public health and academic laboratories are constantly involved in investigating how the influenza vaccine performs in different groups of individuals, in monitoring their tolerability and are making recommendations on the ways the immunogenicity and efficacy of influenza vaccines could/should be evaluated.

A proposal focusing on research devoted to the standardisation of immunological assays and, as needed, of their development represents an ideal ground for public-private collaborative research. Several levels of benefits would derive to both environments from the present proposal:

- A common agreement on the way to perform assays such as HAI which are currently utilised for influenza vaccine registration. It is expected that all vaccine manufacturers would follow the protocols developed and agreed upon
- A common assay used by all groups, either private or public, when testing influenza vaccines in humans

- Availability of rigorous and unequivocal assays when establishing correlates of protection
- An understanding and agreement 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
- A consensus on the way(s) to measure neuraminidase inhibiting (NI) antibodies to be possibly applied to future clinical trials
- An advanced 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.
- 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

POTENTIAL SYNERGIES WITH EXISTING CONSORTIA

Care should be taken to maximise synergies with other initiatives in the field of influenza vaccines and to avoid duplication of efforts. For example, the FP-7 funded ADITEC and IMI-funded BIOVACSAFE projects should be considered. Potential learning from the work that has been undertaken or is currently done by ECDC, CONSISE, EDQM around vaccine evaluation should be taken into account.

OVERALL OBJECTIVES

The long-term goal of this Call is to improve and standardise the existing immunological assays and to develop new assays to better evaluate influenza vaccines. To this end, the overall objectives of this proposed project are to 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.

Several intermediate objectives need to be reached to achieve these overall objectives which are as follows: (i) achieving standardisation of HAI and VN assays, as a primary goal; (ii) advancing the understanding and application of CMI and NA assays as tools for evaluating influenza vaccine performance, as a secondary goal; (iii) consideration of new technology yet to be applied to population based evaluations of influenza vaccines, as an exploratory goal.

EXPECTED KEY DELIVERABLES

- Reach an agreement in both the private and the public sectors on the way to perform assays such as HAI which are currently utilised for influenza vaccine registration
- Establish common protocols for assays that will be used by all groups, either private or 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.

EFPIA PARTICIPANTS

Novartis (coordinator), Sanofi-Pasteur (deputy coordinator), GSK, Abbott, Crucell (Johnson & Johnson) and MedImmune (Astra Zeneca).

INDICATIVE DURATION OF THE PROJECT

The indicative duration of the project is 5 years. Please note that the Council Regulation 73/2007 set up the IMI JU as a body responsible for implementing the 7th Framework Programme for Research and Development for a period up to 31 December 2017. In accordance with the statutes of the IMI JU an ad hoc procedure will be set up to ensure appropriate management of the concerned Grant Agreement(s) after the termination of the IMI JU. The Programme Office will work with the Commission services and the concerned project coordinator(s) to ensure a sound and smooth transfer of the grant agreement(s), the associated commitments and payments, the project file(s), the IT tools access and the audits rights.

INDICATIVE BUDGET

The indicative total budget of the project is EUR 12 200 000.
The indicative total in kind budget from the EFPIA companies is EUR 6 100 000 over 5 years. The indicative IMI JU contribution will be up to EUR 6 100 000.

APPLICANT CONSORTIUM

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 influenza, of developing and validating immunological assays for detection of functionally active antibodies against influenza viruses following natural infection or vaccination, and of cell-mediated immunity applied to influenza infection and vaccination.

SUGGESTED ARCHITECTURE OF THE FULL PROJECT PROPOSAL

The applicant consortium is expected to address all the research objectives and make a key contribution on the defined deliverables in synergy with the EFPIA consortium.

The suggested architecture below for the full project is one proposed approach; different innovative designs are welcome, if properly justified.

Work Package 1: Standardisation of serological assays (HAI & VN)

To ensure assay performance and inter-laboratory comparability, it is critical that reagent preparation procedures complementary to the assay protocols be available to more accurately document the assay procedures. Indeed, the usefulness of the reference standards to properly control and reflect the assay performance is heavily dependent on the quality of these reagents (e.g. RBCs streaming pattern and speed of HAI reading).

To this end it is anticipated that both vaccine manufactures and reference/expert laboratories (under strict confidentiality) share current practices and procedures for

current assay protocols and supporting reagent production and qualification methods. A further requirement for success will be the availability of serum panels from clinical trials and controls to be used in the development of these standardised HAI and VN methods.

HAI assay
For the HAI assay, critical reagents that need in-depth evaluation are represented by virus source (egg- vs. cell-grown virus) and challenge dose, species and freshness of RBCs, or use of lectin-coated beads with coating density, RDE source, vendor, type, and dilution, serum controls or proficiency panel, setting acceptance range or specification for qualifying/bridging each reagent.

Critical aspects of the assay procedure are represented by temperature and duration for [serum+virus] reaction (4°C, 37°C, RT), confirmation of the 4HAU/25 ul virus or Ag input prior to each assay run – micro-adjustment on Ag dilution might be needed for each batch of RBCs, need for the application of RBC adsorption step – before or after the RDE treatment, inclusion of serum control (serum + RBCs) for each sample, reading technique for titre assignment.

Essential aspects of this work package are represented by the standardisation of the calculation of the antibody titres and the statistical evaluation. This includes the starting dilution, taking or not the volume of virus into account for determining the dilution factor of the serum.

VN assay
For the VN assay, critical reagents and their attributes requiring careful evaluation are represented by the virus source, strain, quality, challenge dose (this includes characterisation of the virus stock by various analytical methods e.g. virus growth kinetics, HA content, minimal dilution, qualification criteria for the virus stock, acceptable VN assay challenge dose), cell type, source, master and working cell bank qualification (this includes characterisation of the cell banks by growth kinetics, doubling time, cell susceptibility to the virus, optimal passages for VN use, and free of any contaminants), monoclonal antibodies (mAb) and secondary Ab qualification for ELISA-based readout method, serum controls or proficiency panel.

Critical aspects of the assay procedure that need in-depth analysis are essentially represented by a stringent comparison between the short vs. the long-term incubations.

For the short-term assay (overnight – WHO/CDC method, measuring the anti-HA neutralising activity): readout format: ELISA-based assay vs. CPE vs. HAU determination, common calculation method for neutralising titer assignment: 50% reduction in specific signal vs 100% end point; interpolation vs. discontinuous dilution, temperature of the assay, virus back titration, pre-formed cell monolayer vs. cell suspension.

For the long-term assay ($\geq$ 4 days assay – measuring the anti-HA and anti-NA neutralising activities): readout format, days post-infection, cell line, neutralisation condition, e.g. time and temperature, pre-formed cell monolayer vs. cell suspension, virus back titration.

For the VN assays, essential aspects of this work package are represented by the standardisation of the calculation of the antibody titres and the statistical evaluation. This includes titer calculation assignment based on different readout methods, starting dilution, and taking or not the volume of virus into account for determining the dilution factor of the serum.

Harmonisation of assay validation procedures

The validation of serological assays is often performed following the ICH and FDA guidelines; however, these guidelines are designed for analytical assays, which may not be 100% applicable to bioassays. Hence different interpretation or adaptation can occur. Agreement on common definitions and procedures for addressing assay characteristics that need to be validated (i.e. sample type, titer range, cut-off, LOB, LOQ, precision, sensitivity and specificity) as well as having agreeable acceptance criteria for each evaluated parameter could also be an important step towards assay standardisation. The involvement of biostatisticians will also be needed to help with modelling, defining the testing methods and setting the acceptance criteria for these validation parameters, or when laboratory bridging is needed to assess the level of agreement and to confirm that two laboratories can generate similar results. The agreed validation procedure should be applied to the prototype of HAI and VN assays ultimately recommended by the awarded consortium.

EFPIA Partner Contribution: EFPIA partners are experts in development, validation, performance of serological assays, and analysis/interpretation of data that support influenza vaccine immunogenicity assessments. This is especially true for HAI and VN assays. Additionally the partners are very experienced in standardisation of assays. 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 Work Package 1.

Work package 2: Advancing the understanding and application of CMI and NA assays as tools for evaluating influenza vaccine performance

Research-oriented activities on assays for detection and quantification of anti-NA antibodies

Some aspects for the optimisation, standardisation, and validation of anti-HA antibody assays also apply to anti-NA antibody assays. However, while there is general agreement on the assays to be used to evaluate antibody responses to HA, no agreement exists on the assay(s) to be utilised for anti-NA specific antibodies.

In addition, there is some consideration that extended incubation of VN assays may be useful for the detection of anti-NA antibodies in addition to the anti-HA antibodies. Because of this potential linkage, there is interest in determining the clinically relevant relationship between HAI, virus neutralisation (VN), and neuraminidase inhibition (NI) assays.

For these reasons, activities within this IMI project should be aimed at:

1. Defining which assay(s) to be included in comparative studies for the detection and measurement of anti-NA antibodies
2. Defining the source of critical reagents specifically needed for the assay(s) including the NA substrate (e.g. fetuin) and target virus (target virus HA needs to differ from the HA subtype used for vaccination in order to eliminate confounding anti-HA antibody influence in the NI assay)
3. Identifying potential standard clinical samples to be utilised in comparative studies. These samples could be from humans or animals immunised with inactivate/live attenuated vaccines. The vaccines, the number of injections, etc. should be well defined. The samples will require a rigorous characterisation for the presence of other families of antibodies (e.g. HAI, VN, antibodies to other viruses, etc.)
4. Considering the suitability of the assay format for testing large clinical trials should the work lead to a potential biomarker

5. Taking into account that there should be minimum IP restriction in making the assays broadly available
6. Reaching a consensus on which anti-NA antibody assay to be utilised in future clinical studies and on the protocol and the standard samples to be used

Research-oriented activities on cell-mediated immunity

Unlike serology, the evaluation of CMI faces different hurdles that need to be approached comprehensively in order to achieve meaningful outcomes. As a consequence, activities should be focused on defining issues and proposing research approaches to find analytical solutions:

(i) Procedures for separation of peripheral blood mononuclear cells (PBMC), their storage, and their thawing need to be uniform and rigorously standardised.

(ii) Some laboratories prefer to carry out their assays on fresh PBMC and/or on whole blood. Comparative analysis of fresh vs. frozen cells and on whole blood vs. PBMC needs to be performed to get comparable results.

(iii) For T-cell immunity, a clear definition of which assays to employ and why is needed. A panoply of assays exist, from the least sensitive proliferative assay to the very sensitive and sophisticated multiparametric assays such as the CyTOF [(13)], going through assays like intracellular staining for cytokines and FACS analysis, ELISpot for cytokine producing cells, multiplex assays for cytokine production in supernatants, and many others. Each one of these assays needs careful rigorous standardisation of protocols and calibration of equipment that in many instances are different from one laboratory to another, and in terms of antigenic stimuli in vitro which may lead to different results (for example whole virion, split or subunit vaccine antigens, peptide libraries, etc.). Also the presentation of the results needs to be uniform.

As a consequence, a large volume of blood is needed to (i) apply all assays in a single laboratory and (ii) apply each assay in a multicentre comparative study.

If this holds for adults and elderly, it becomes even more challenging for studies in young children for whom drawn blood volumes are often restricted to 5 ml or less by ethical committees. Some groups already carry out CMI analysis on these limited volumes of blood taken from young children. However, in most cases only a few (if not one single) parameters can be analysed.

For these reasons, activities within this project should be aimed at:

1. Under confidentiality, sharing protocols of separation, freezing, storage and thawing of PBMC samples, and ultimately reaching consensus of the minimum requirements indispensable to allow reliable and meaningful results. Vaccine manufacturers but also reference laboratories should share their procedures. One designated reference laboratory should organise and manage those operations. In these protocols, comprehensive and detailed information regarding the nature, quality, preparation, and source of essential materials needs to be shared.
2. Reaching a consensus on the assays to be used to answer specific questions, and on the protocols to be employed. This consensus should cover issues related to the antigenic stimuli used in vitro (whole virions, split or subunit vaccines, peptide libraries) and, whenever possible, the time points for CMI analysis. Most of these assays are likely to be applied to exploratory objectives of clinical trials, and very likely they will not need validation procedures similar to those needed for serological assays. This consensus should, however, define the minimum requirements of stringency for the qualification of each assay and for the ways results have to be presented (they currently differ significantly from one laboratory to another). With this respect, sharing expertise coming from the

different vaccine manufacturers and awardee laboratories is recommended to reach the most meaningful consensus with respect to the selected minimum requirements of the assay that will ultimately be used.

3. Given ethical and technical constraints (Informed consent form, remaining volume, storage conditions,…) it is unlikely that material from existing clinical trials may serve as source of adequate volumes of blood for applying the defined assays in the awardee laboratories. These samples could be derived from healthy blood donors that in most cases were not vaccinated.
4. Setting an agenda and, if possible, initiate activities on "miniaturisation" of CMI assays that may be reliably applied to paediatric studies.

EFPIA Partner Contribution: EFPIA partners are experts in development, optimisation, and performance of influenza immunological assays including various assays aimed at investigating CMI in subjects immunised with influenza vaccines. These assays may contribute the understanding how vaccine-related factors and host-related factors may influence the induction and the maintenance of the influenza-specific immune response at the level of T- and B-cells. 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 Work Package 2.

Work package 3: Consideration of new technologies yet to be applied to population based evaluations of influenza vaccines

This project provides an opportunity to bring forward new potentially useful technologies that will aide in defining biomarkers for correlates of protection from vaccines. There are many new analytical tools 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. For example these could be tools that help display immune profile responses (e.g. microarrays, multiplex EIAs with different HA subunits, etc.) or measurements of immune quality.

Under this work package, the topic encourages the applicant consortium to propose novel methods and assays that could supplement the above technologies in the tool-box for future studies to develop correlates of protection.

The awarded consortium should consider that 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. Please note that the new technologies proposed under this work package should not be the central aim of the consortium's proposal.

EFPIA Partner Contribution: EFPIA partners are experts in assays that support evaluation of a number of vaccines. The partners are experts in knowing the possible suitability and constraints new technologies may have in being applied to population based influenza vaccine evaluations. 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 Work Package 3.

GLOSSARY

CDC: Centers for Disease Control, USA
CMI: cell-mediated immunity

CONSISE: Consortium for the standardization of influenza seroepidemiology
CPE: cytopathic effect
EIA: enzyme immune assay
ECDC: European Center for Disease Prevention and Control
EDQM: European Directorate for the Quality of Medicines & Healthcare
ELISA: enzyme-linked immune sorbent assay
HA: haemagglutinin
HAI: haemagglutination inhibition
HAU: haemagglutination unit
IMI: Innovative Medicines Initiative
IP: intellectual property
LAIV: live attenuated influenza virus
LOB: limit of blank
LOQ: limit of quantitation
mAb: monoclonal antibody
NA: neuraminidase
NI: neuraminidase inhibition
NIBSC: National Institute of Biological Standardisation and Control, UK
PBMC: peripheral blood mononuclear cells
PEI: Paul Ehrlich Institute, DE
RBC: red blood cells
RT: room temperature
SME: small & medium enterprise
SN: supernatant
VN: virus neutralisation
WHO: World Health Organisation

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