World Health Organization

WHO/DRAFT/NCE_Adjuvanted vaccines/8 April 2013
ENGLISH ONLY

Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines

Proposed guidelines

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed WHO *Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received by 10 May 2013 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Quality Safety and Standards (QSS). Comments may also be submitted electronically to the Responsible Officer: Dr TieQun Zhou at email: zhout@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).

city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

Recommendations and guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If a NRA so desires, these Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Guidelines made only on condition that modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the guidelines set out below.

Contents

Appendix 2. List of tissues (depending on the species) to be collected in a repeated dose toxicity study

Introduction

This document provides guidance to National Regulatory Authorities (NRAs) and manufacturers on the nonclinical and initial clinical evaluation of vaccine adjuvants and adjuvanted vaccines by outlining the international regulatory expectations in this area. It should be read in conjunction with the existing guidelines on nonclinical and clinical evaluation of vaccines published by the WHO (1, 2). There is substantial diversity among vaccine adjuvants and adjuvanted vaccines and their nonclinical and clinical testing programs will depend on product specific features and their clinical indications. Therefore, the following text is written in the form of guidelines instead of recommendations. "Guidelines" allow greater flexibility than "Recommendations" with respect to specific issues related to particular adjuvanted vaccines.

Over the past decades, strategies and approaches for the development and delivery of vaccine antigens have expanded. Some of these antigens are weakly immunogenic and require the presence of adjuvants for the induction or enhancement of an adequate immune response. Vaccines with aluminum-based adjuvants have been extensively used in immunization programs worldwide and a significant body of safety information has accumulated for them (3, 4). As knowledge of immunology and the mechanisms of vaccine adjuvant action have developed, the number of vaccines containing novel adjuvants being evaluated in clinical trials has increased. Vaccines containing adjuvants other than aluminum-containing compounds have been authorized for use in several countries, and a number of vaccines with novel adjuvants are currently under development, including, but not limited to, vaccines against human papillomavirus (HPV), human immunodeficiency virus (HIV), malaria, and tuberculosis, as well as new generation vaccines against influenza and other diseases. However, the development and evaluation of adjuvanted vaccines presents regulatory challenges. Vaccine manufacturers and regulators have questions about the type of information and extent of data that would be required to support proceeding to clinical trials with adjuvanted vaccines and to their eventual authorization. Existing WHO guidelines on nonclinical evaluation of vaccines (1) provide valuable general

guidance; however, they provide limited information specifically related to new adjuvants and adjuvanted vaccines. Additionally, some of the issues addressed here are also discussed in other international guidance documents (5, 6). Given the importance and the complexity of the issues, this up-dated and more extensive guidance on the nonclinical and preclinical testing of adjuvants and adjuvanted vaccines should allow manufacturers and regulators to proceed in an efficient manner on the critical path towards development and licensure of adjuvanted vaccines indicated for the control of diseases with important global public health impact.

Background

Over the past decades, there have been a number of international workshops and meetings that have discussed the issues covered by these Guidelines (7-12). To address the need for additional international guidance on nonclinical evaluation of adjuvanted vaccines, a consultation was organized by the World Health Organization (WHO) on 7-8 September 2011 in Rockville, Maryland, USA, to initiate the process of developing a new WHO guideline on the subject. The consultation was attended by experts from academia, National Regulatory Authorities (NRAs), National Control Laboratories (NCLs) and industry involved in the research, manufacture, and approval of adjuvanted vaccines from countries around the world. The purpose was to review the scientific information and available data and to discuss and identify the issues to be considered for the development of such international guidelines. On 27- 28 November 2012, the WHO organized an informal consultation in its headquarters, Geneva, Switzerland, attended by academia, researchers, vaccine manufacturers and regulators who are involved in the evaluation of adjuvanted vaccines, to review the draft guidelines prepared by the drafting group and to seek consensus on key regulatory issues. The approaches to nonclinical and initial clinical evaluation of vaccine adjuvants and adjuvanted vaccines discussed in this document are a result of the efforts of this and other international working groups.

Scope

This document addresses regulatory considerations related to the nonclinical and initial clinical evaluation of adjuvanted vaccines. The goal of this document is to provide consistent and harmonized guidance on nonclinical testing approaches to support the use of candidate

adjuvanted vaccines in all stages of clinical development and ultimately for marketing authorization of the product. However, each NRA may determine the regulatory requirements applicable for adjuvanted vaccines to be used and marketed in their country.

Vaccine adjuvants are substances or combination of substances that are used in conjunction with a vaccine antigen to enhance (e.g., increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine. For the purposes of this document, the term "adjuvant" includes formulations that contain one individual adjuvant as well as adjuvant combinations that contain multiple adjuvants. These Guidelines specifically address vaccine adjuvants that are either separate substances that are mixed with the vaccine antigens and administered at the same time and location as the vaccine antigens as well as immunostimulatory moieties that are engineered by recombinant DNA technology to be an inherent part of the antigen molecule (e.g., in fusion proteins). This document does not deal with the carrier proteins that are covalently linked to polysaccharide antigens in conjugate vaccines. Also, the immune enhancing properties that are intrinsic to certain vaccine antigen preparations, such as the naturally-occurring adjuvant activity of whole-cell pertussis vaccines, are not considered "adjuvants" within this document.

This document covers adjuvanted vaccines used in both prophylactic and therapeutic indications against infectious diseases. In this context, it should be noted that no vaccine adjuvant is authorized in its own right, but only as a component of a particular adjuvanted vaccine. Although these guidelines are primarily for preventive and therapeutic adjuvanted vaccines for infectious disease indications, some of the principles outlined below may be applicable to the nonclinical and initial clinical testing of adjuvanted therapeutic vaccines for other indications as well (e.g., cancer).

Nonclinical evaluation, within the context of this document, refers to all *in vivo* (in animal) and *in vitro* testing performed before and during the clinical development of adjuvanted vaccines and includes product characterization, proof of concept and immunogenicity studies, as well as safety testing in animals. Preclinical testing specifically refers to the nonclinical testing done prior to initiation of any human testing and is a prerequisite to movement of a candidate adjuvanted

vaccine from the laboratory to the clinic. Thus, for the remainder of this document, the term, preclinical, will be used only when referring specifically to the nonclinical evaluation done prior to the first-in-human clinical trials.

Many regulatory agencies, in addition to defining an adjuvant based on its immune-enhancing biological activity, provide a regulatory and/or legal classification for the adjuvant component (e.g., excipient, active ingredient, constituent material, or medicinal ingredient/product). It is possible that depending on the particular definition used by the regulatory authority, additional testing may be required. These regulatory and legal issues are specific for each regulatory authority and are beyond the scope of this document.

1. General considerations

Adjuvants have been used for decades to enhance the immune response to vaccine antigens (7). Possible benefits of administering antigens in conjunction with adjuvants include the induction of long term protection, better targeting of effector responses, induction of long-term memory, reduction of the antigen amount and/or the number of vaccine doses needed for a successful immunization, and optimization for populations with poor responsiveness. For certain complex diseases, stimulation of cell-mediated immune responses appears to be critical, and adjuvants can be employed to optimize a desired immune response, such as the induction of cytotoxic or helper T lymphocyte responses. In addition, certain adjuvants can be used to promote antibody responses in a relevant immunoglobulin class or at mucosal surfaces.

Successful preclinical evaluation of adjuvanted vaccines, including physicochemical characterization, proof of concept testing in animals, and toxicity testing, is an important step towards their clinical development. In addition, studies in animals are valuable tools to help select a safe dose, schedule, and route of administration, and to identify unexpected or potential adverse effects for specific monitoring in clinical trials. Safety concerns include potential inherent toxicities of the vaccine antigen and/or adjuvant, potential toxicities of any impurities and contaminants, and potential toxicities due to interactions of the components present in the final formulation. The regulatory considerations for adjuvanted vaccines are similar to those for vaccines in general, with additional issues being considered that are unique to novel adjuvants.

Throughout this document, guidance is provided related to the evaluation of new and novel antigens, adjuvants, and adjuvanted vaccines. In this document, the term, novel, will be used to designate vaccine antigens and adjuvants that have not been included in a licensed vaccine. New antigens, adjuvants, and vaccines may include those that have been included in licensed vaccines, but have undergone significant changes in the production process, previously licensed products that have undergone major formulation changes (e.g., a change in adjuvant or addition or removal of one of the components), or previously licensed products given by a new route of administration.

Established benefits and increased availability of adjuvants have stimulated an interest in transferring adjuvant production technology from one adjuvant or adjuvanted vaccine manufacturer to another. As stated above, adjuvants are not approved in their own right. In the context of vaccines against infectious diseases, adjuvants may only exist as components in licensed vaccines that consist of specific antigen/adjuvant combinations. Thus, each new adjuvanted vaccine is considered a new entity that will require appropriate physicochemical characterization and nonclinical and clinical evaluation. However, in cases of technology transfer, existing data from similar antigen and adjuvant components and/or adjuvanted vaccines held by the original manufacturer can provide important information to guide and potentially accelerate the nonclinical and clinical studies (e.g., data from adjuvant alone study arms). The need for and extent of nonclinical testing will depend on the adjuvanted vaccine under consideration; manufacturers are encouraged to consult with the NRA.

Vaccine adjuvants have been divided broadly into two main types – those known as vaccine delivery systems and those known as immunostimulators, although this division has become less clear since some delivery systems are now known to have direct immune stimulatory effects in addition to their ability to enhance the delivery of the antigen to the local lymph node. Immunostimulators in general include substances that enhance the immune response to vaccine antigens by activating the innate immune system, which usually sets off a cascade of events including, but not limited to, increased antigen uptake into antigen presenting cells, increased release of stimulatory molecules such as cytokines, and increased localization of the antigen in

the local lymph node. They may include cytokines or other substances that are generally described as "immune potentiators" because they exert direct effects on immune cells.

Adjuvants also can be classified according to their source (e.g., synthetic or microbial-derived), mechanism of action, and physical or chemical properties. A list of the most commonly described adjuvant classes, with specific examples, is provided in Appendix 1. It should be noted that a given vaccine adjuvant may be a combination adjuvant (see Definitions) that consists of multiple types of adjuvants and thus can fall into more than one of the listed categories.

2. Definitions

The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts.

Adjuvanted vaccine

The complete formulation that includes one or more antigens, an adjuvant(s), and any additives, (which may include, e.g., , excipients, or preservatives), the administration of which is intended to stimulate the immune system to result in an immune response that leads to the prevention or treatment of an infection or infectious disease.

First-in-human trial

For the purposes of this document, this refers to the first evaluation in human subjects. Most commonly, the first-in-human clinical trials are carried out in small numbers (e.g., 20-80) of healthy and immunocompetent adults to test the properties of a vaccine, its tolerability, and, if appropriate, clinical laboratory and pharmacological parameters. These trials are primarily concerned with safety.

Good laboratory practice (GLP)

A quality system concerned with the organizational process and the conditions under which nonclinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported. GLP principles may be considered as a set of criteria to be satisfied as a

basis for ensuring the quality, reliability and integrity of studies, the reporting of verifiable conclusions and the traceability of data (1, 12a).

Good manufacturing practice (GMP)

A part of the pharmaceutical quality assurance which ensures that products are consistently produced and controlled according to the quality standards appropriate to their intended use and as required in the marketing authorization. In these guidelines, GMP refers to the current GMP guidelines published by WHO (13, 14).

Immunogenicity

Capacity of a vaccine/adjuvanted vaccine to induce antibody-mediated immunity, cell-mediated immunity, and/or immunological memory.

In vitro studies

In vitro studies refer to studies that are conducted in a laboratory environment using components (e.g., serum, cells or tissues) that were originally obtained from a living organism.

In vivo studies

In vivo studies refer to studies that are conducted with living organisms.

Nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines

Nonclinical testing includes all *in vivo* and *in vitro* testing performed before and in parallel with clinical development of adjuvanted vaccines. Nonclinical testing includes product characterization, proof of concept studies and animal *in vivo*/*in vitro* toxicity testing. The potential toxicity of an adjuvanted vaccine should be defined not only prior to initiation of human trials, but throughout clinical development, if appropriate (see also the definition of preclinical evaluation of vaccine adjuvants and adjuvanted vaccines).

Potency

The measure of biological activity, using a suitably quantitative biological assay, based on an attribute of the product that is believed to be linked to the relevant biological properties (1).

Preclinical evaluation of vaccine adjuvants and adjuvanted vaccines

Preclinical testing refers specifically to the nonclinical testing (see definition of nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines) done prior to the first-in-human clinical trials. Preclinical evaluation is a prerequisite to the initiation of clinical trials.

Proof of concept studies

Proof of concept studies as discussed in this document include the *in vivo* and *in vitro* nonclinical testing conducted to evaluate the immune response to the adjuvanted vaccine, the enhancement of the immune response to the antigen by the adjuvant, and/or the demonstration of the resulting protection against challenge with the infectious agent targeted by the adjuvanted vaccine. For therapeutic vaccines, proof of concept studies would include, when possible, studies to evaluate the capacity to control or ameliorate disease and/or clear infection.

Protocol or study/trial plan

A document that states the background, rationale and objectives of the nonclinical study or clinical trial, and describes its design, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed (1).

Route of administration

The means by which the candidate adjuvanted vaccine is introduced to the recipient. Routes of administration for adjuvanted vaccines may include, for example, the intramuscular, subcutaneous, transcutaneous (with or without scarification), intradermal**,** oral, intranasal, inhaled (aerosol), intravenous, intranodal, intravaginal or intrarectal routes.

Safety

The relative freedom from harmful effect to animals or persons affected, directly or indirectly, by a product when appropriately administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.

Vaccine adjuvants

Substances or combination of substances that are used in conjunction with a vaccine antigen to enhance (e.g., increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine. It may be any of the types of substances identified as examples of adjuvants in Appendix 1. The term "adjuvant" is used throughout the document to include adjuvants that exist as one individual substance as well as combination adjuvants that consist of multiple adjuvants and sometimes other additives.

Vaccine antigen

The active ingredient in a vaccine against which a specific immune response is raised. It may be a live attenuated preparation of bacteria, viruses or parasites; inactivated (killed) whole organisms; crude cellular fractions or purified antigens, including recombinant proteins (i.e., those derived from recombinant DNA expressed in a host cell); polysaccharides and conjugates formed by covalent linkage of polysaccharides to components such as mutated or inactivated proteins and/or toxoids; synthetic antigens; polynucleotides (such as plasmid DNA vaccines); or living vectored cells expressing specific heterologous immunogens. It may also be a combination of the antigens or immunogens listed above.

3. Manufacturing and quality considerations for nonclinical and clinical evaluation of vaccine adjuvants and adjuvanted vaccines

Adjuvanted vaccine manufacturers are encouraged to discuss with the NRA the extent of the manufacturing and quality-related information necessary to support the intended use of the antigen, the adjuvant and the adjuvanted vaccine. The extent of information necessary to evaluate and assure the consistent safety and effectiveness of adjuvanted vaccines will vary with the phase of nonclinical and clinical investigation. Similarly, the nature and extent of manufacturing controls needed to achieve and demonstrate appropriate adjuvanted vaccine quality differ not only among various phases of product development (i.e., research, pilot, investigational and commercial manufacture), but also among the various phases of clinical evaluation.

3.1 Production, characterization and quality assurance of lots to be used in nonclinical pharmacology studies

It is generally accepted that nonclinical pharmacology studies (e.g., the proof-of-concept and mechanism of action studies) may be done as non-GLP studies and that they are often conducted with research or pilot scale lots of antigen, adjuvant and/or adjuvanted vaccine formulations that are not necessarily manufactured in compliance with GMP. Also, these studies are often dose optimization studies where the antigen and adjuvant components may be provided in two separate containers to allow for the mixing of different amounts of each prior to administration and the generation of data that support the proposed dose of antigen and adjuvant to be used in the adjuvanted vaccine. While the level of characterization of the lots of antigen and adjuvant used in these exploratory studies may be less extensive than those utilized in the nonclinical toxicology studies, the same raw materials should be used, where possible, in their preparation and they should be manufactured by the same methods as the lots to be tested in the nonclinical toxicology studies. Also, the source and any testing (e.g., purity) of the raw materials should be documented and the general quality of the lots of antigen and adjuvant used to prepare the adjuvanted vaccine to be used in the nonclinical pharmacology studies should be adequately characterized preliminarily by biological and/or chemical testing such as immunogenicity, chemical composition, and purity. See Table 1 for more information.

3.2. Production, characterization and quality assurance of lots to be used in nonclinical toxicology studies and first-in-human clinical trials

The lots of the antigen, the adjuvant, and the adjuvanted vaccine used in the nonclinical toxicology studies ideally should be the same lots as those proposed for use in the first-in-human trials. Additionally, the quality and stability of the antigen, adjuvant and final adjuvanted vaccine formulation should be evaluated prior to, if not in parallel with, their use in a toxicology study. See Table 1 and Section 3.2.1 below for more information.

If use of the same lots is not feasible, the lots used for the nonclinical toxicology studies should be comparable to those proposed for use in the first-in-human trials with respect to manufacturing process and GMP-compliance, physicochemical characteristics, purity, potency, and stability. Where there are significant differences in the manufacture of the antigen or the adjuvant (or in the formulation of the adjuvanted vaccine) to be used in the nonclinical toxicology studies and the first-in-human clinical trial, a detailed description of the differences

and additional safety studies conducted to compare safety profiles should be provided. This information is provided to allow the NRA to evaluate the potential impact of such changes on the safety of the adjuvanted vaccine and to determine whether or not the differences are sufficient to warrant additional toxicology studies to support the safety for the proposed clinical use.

With respect to the control and testing of adjuvanted vaccine lots manufactured for use in first-in-human clinical trials, emphasis should generally be placed on elements that assure the safety of subjects. This usually includes identification and control of the raw materials used to manufacture the antigen and the adjuvant. For this reason, Certificates of Analysis with test specifications and results indicated should be provided for all ingredients that are acquired from contract suppliers for use in manufacturing the adjuvanted vaccine. For some adjuvanted vaccines, additional considerations related to manufacturing and testing of the vaccine adjuvant and its individual components may be needed to provide assurance that the adjuvant is manufactured consistently and has a consistent composition. This may apply particularly when one or more of the components of the adjuvant is biological in nature or for vaccines containing complex adjuvant mixtures or antigens adsorbed to mineral salts or gels. Therefore, it is important to use established quality control procedures that ensure the consistent manufacture of adjuvants and antigens to be used in the preparation of adjuvanted vaccines. The antigen and adjuvant or formulated adjuvanted vaccine used in the first-in-human trial should be manufactured in compliance with appropriate GMP. Use of quality control procedures established for vaccines and compliance with the appropriate GMP will facilitate the consistent manufacture of equivalent or comparable lots of antigen, adjuvant and adjuvanted vaccine for future clinical trials as needed. See Table 1 for more information.

The clinical lot(s) of adjuvanted vaccine, or separate lots of antigen and adjuvant, if provided in separate final containers, should be demonstrated to be stable for the duration of the clinical trial. Additionally, if the adjuvant is provided in a separate container (e.g., vial or syringe) to be used to reconstitute or be added to the antigen prior to vaccine administration, a detailed description of the procedure for mixing the components should be provided, along with a clear statement (that is supported by data) of the appropriate time and conditions for storage of the individual

components as well as the final adjuvanted vaccine. Also, the appearance of the adjuvanted vaccine after mixing should be described.

3.2.1 Analytical testing of adjuvant and adjuvanted vaccine

A detailed description of the adjuvant and adjuvanted vaccine should be provided and include information regarding the characterization conducted to assure the quality (e.g., identity, purity, sterility) and quantity of the antigen and adjuvant as well as the potency of the adjuvanted vaccine. It should be demonstrated that the adjuvant does not adversely affect the potency of the antigen upon mixing. In addition, information on the methods of manufacture and testing for the intermediates and final product, together with their preliminary release specifications, should be provided. Although it is not necessary to have validated methods for testing lots of antigen and adjuvant or adjuvanted vaccine to be used in nonclinical toxicology studies and first-in-human clinical trials, the scientific background should justify the choice of the testing methods and selected preliminary specifications. The NRA should be consulted to define analytical protocols appropriate for the identification and quantification of the content of the antigen(s), adjuvant(s) and any additives. It is important to assess attributes of each of the antigen and the adjuvant that may be relevant for adjuvant activity and adjuvanted vaccine potency. Additionally, the properties of each of the antigen and adjuvant that are most indicative of stability, both when stored individually and as a formulated final adjuvanted vaccine, should be identified.

Assays used for characterization of the adjuvant may or may not be related to its mode of action, but should be adequate to ensure consistency of adjuvant production and to evaluate adjuvant stability. These assays may include, for example, appearance, particle size, presence of aggregates, and pH for the adjuvant, and degree of antigen adsorption for a vaccine adsorbed to an aluminum-containing compound. Analytical methods to evaluate antigen/adjuvant compatibility and/or physical interactions between the antigen and adjuvant in an adjuvanted vaccine (and/or between the components of the adjuvant, if a combination adjuvant) should be developed and validated as adjuvanted vaccine product development and clinical evaluation proceeds.

A quality control test evaluating the potency of the final adjuvanted vaccine should be developed as one of the assays to assess consistency of manufacture. Depending on the type of potency assessment conducted on the adjuvanted vaccine and the requirements of the NRA, the assessment may or may not reflect the contribution of the adjuvant to the potency of the adjuvanted vaccine. If it does not, it will be important to conduct assessments of the identity and content of the adjuvant in the final adjuvanted vaccine. Also, the purity and sterility of the final adjuvanted vaccine will need to be assessed to ensure its safety. If the adjuvant or adjuvanted vaccine is tested for endotoxin via the Limulus Amoebocyte Lysate (LAL) test method, evidence that the adjuvant or adjuvanted vaccine does not interfere with the LAL test (e.g., data from lipopolysaccharide spiking experiments with and without adjuvant) should be provided, as certain adjuvants, such as cationic liposomes, may interfere with the LAL test method.

If the final adjuvanted vaccine consists of co-packaged antigen and adjuvant where each is provided in a separate container to be mixed prior to administration, both the antigen and the adjuvant should be evaluated for identification, purity and sterility. In addition, the potency of the antigen and the content of the adjuvant should be assessed.

Consultation with the NRA is recommended to discuss both the need for and design of the quality control test known as the innocuity, general safety, or abnormal toxicity test for the adjuvanted vaccine. Additionally, if a particular NRA requires such a test for a formulated adjuvanted vaccine, it should be clarified whether only the antigen or both the antigen and adjuvant are to be tested when provided in separate final containers. While some regulatory authorities and the WHO no longer require this test to be performed on a routine basis once consistency of production has been established, some have further questioned the relevance of this test (15-17). In some countries there is a legal requirement to conduct an innocuity test with the objective of assessing the potential introduction of extraneous impurities into the final adjuvanted vaccine; however, this is not considered a toxicity test. When the innocuity test is required, investigations may be necessary to define the appropriate dose and route of administration for the adjuvanted vaccine because some investigational adjuvants and adjuvanted vaccines may not pass the innocuity test when administered according to the prescribed protocol, which is typically volume based and administered by the intraperitoneal route. In the countries

where the innocuity test is still necessary, it may be possible to request an exemption from conduct of the innocuity test as part of routine lot release testing once test data from many lots have been accumulated and consistency of production has been well established to the satisfaction of the NRA.

3.3 Information required for later stage clinical trials

In general, in the course of adjuvanted vaccine product development, the analytical technology or methodology evolves in parallel with the clinical investigations. As adjuvanted vaccine product development and clinical evaluation proceeds, quality control and quality assurance of the antigen and adjuvant should be refined. By the time of later stage clinical trials, manufacturing processes should be consistent and validated and a description of analytical procedures with appropriate validation information should be provided for all analytical procedures except for those that are from an official pharmacopeial compendium (13, 14). If a national or international standard is not yet available for a particular antigen, adjuvant, or adjuvanted vaccine, the manufacturer should establish its own primary reference material during later stage clinical trials.

A minimum of three consecutive lots of each of the antigen and the adjuvant intermediates (or final containers if provided separately) and formulated adjuvanted vaccine should be manufactured and tested for purposes of demonstrating consistency of manufacture of each component and the formulated adjuvanted vaccine. Any changes in the manufacture or formulation should be carefully assessed to determine if such changes directly or indirectly affect the quality and safety of the adjuvanted vaccine. When analytical data from tests conducted on the adjuvanted vaccine demonstrate that the antigen, adjuvant or adjuvanted vaccine manufactured before and after such changes is not comparable, additional qualification and/or bridging studies should be undertaken to support the safety of the materials proposed for continued clinical evaluation.

To ensure that appropriate stability data are collected during later stage clinical trials of the adjuvanted vaccine, a stability protocol to be used for the formal stability studies should be developed for the antigen, the adjuvant, and the adjuvanted vaccine. Stability programs should

be designed to monitor the chemical, physical, biological, and microbiological (if applicable) stability of the antigen and the adjuvant as well as the adjuvanted vaccine throughout the clinical testing program. The properties of each of the antigen and adjuvant that are most indicative of stability, both when stored individually and as a mixed final adjuvanted vaccine, should be identified as stability evaluations proceed (as mentioned above in section 3.2.1). If it is determined that degradation products accumulate from the either the antigen or the adjuvant over the shelf-life of the adjuvanted vaccine, these should be considered during stability testing of the final product and the NRA should be consulted to determine whether additional suitable nonclinical toxicological testing should be undertaken to confirm their safety. Additional guidance on stability testing of vaccines can be found in the WHO Guidelines on the stability evaluation of vaccines (18).

4. Rationale for the use of the adjuvant

Adjuvant activity is a result of multiple factors and an adjuvant-mediated enhancement of the immune response to one vaccine antigen, as a rule, cannot be extrapolated to the enhancement of the immune response to another antigen. Individual antigens vary in their physical, biological and immunogenic properties and antigens may have different needs for immunological help from an adjuvant. Manufacturers should justify the choice of the adjuvant based on the immune response desired, which may include effects on the magnitude, the breadth and/or the type of immune response to specific antigens. In addition, adjuvants are also used in antigen dose-sparing strategies with the aim of increasing the availability and supply of vaccines; for example, under emergency situations of an influenza pandemic (19) or as a strategy to decrease the cost of the vaccine (e.g., use of IPV for polio eradication) (20).

Many advances in the understanding of innate immunity have begun to provide insights into the immunological mechanisms of adjuvant action. Many of the immunostimulatory adjuvants are recognized by various members of the toll-like receptor (TLR) family, a subclass of pathogen-recognition receptors, while other adjuvants may target other families of pathogen recognition receptors that could prove to be important in shaping the adaptive immune response. Furthermore, there are complex regulatory interactions between the many families of innate receptors and other signaling pathways. Within this framework, the activities exerted by

adjuvants include, but are not limited to, the facilitation of (i) antigen uptake and presentation of the antigen(s) in the vaccine by antigen-presenting cells; (ii) secretion of proteins by antigen-presenting cells; (iii) recruitment, targeting and activation of specific cells; (iv) modulation of activities that regulate the ensuing immune responses; (v) protection of the antigen from degradation and elimination; and/or (vi) mobilization of antigen-presenting and/or polymorphonuclear cells.

The scientific rationale supporting the benefit of adding the adjuvant and the choice of specific adjuvant(s) should be provided by the adjuvanted vaccine manufacturer. Before evaluating a particular adjuvant in combination with an antigen in a clinical trial, it is recommended that data from *in vitro* and/or *in vivo* studies be generated to support the rationale for including the specific adjuvant in the vaccine formulation and for selecting the dose range of adjuvant to be tested. In the ideal case, the mode of action of the selected adjuvant as well as the mechanism of the enhanced immune response would be well understood before late clinical development. When the mode of adjuvant action is not well defined, supplemental *in vivo* or *in vitro* data (as discussed in sections 4.1 and 4.2, respectively) may be provided, in addition to the pivotal toxicity study, to establish the added benefit of the adjuvant to the immune response induced by the adjuvanted vaccine as well as the safety of the adjuvanted vaccine.

4.1 *In vivo* proof of concept studies

Data from proof of concept studies, including data from early studies conducted to evaluate optimal antigen/adjuvant formulations, can provide important information with regard to the characteristics of the adjuvanted vaccine. This includes evidence for the need for the adjuvant, the type and magnitude of the immune responses induced (i.e., innate immunity, humoral and cellular immunity), and their functional capacity to either protect against disease (i.e., prophylactic vaccine) or ameliorate an existing infectious disease (i.e., therapeutic vaccine). These ‘pilot’ or exploratory studies designed to identify and screen adjuvanted vaccine formulations may be non-GLP-compliant; however, they may identify unknown or potential adverse effects and provide crucial information for the design of GLP-compliant toxicity studies. In addition, such studies may provide the scientific justification for manufacturing changes and

for optimization of adjuvanted vaccine formulation, dose, and route of administration during the clinical development of the adjuvanted vaccine product.

It is recommended that proof of concept studies conducted to support the use of an adjuvant be carried out to evaluate vaccine formulations with and without the adjuvant. Depending on the specific antigen and/or adjuvant being considered, possible examples of these types of studies are as follows:

1) Evaluation of humoral immune responses with regard to magnitude (e.g., mean titer or concentration), quality (e.g., affinity or avidity), and functional activity (e.g., neutralizing activity).
2) Evaluation of cellular immune responses including assessment of the induction of specific types of cellular responses (e.g., examining Th1 or Th2 cytokine profiles or testing for the induction of cytotoxic T cells).
3) Evaluation of protective or therapeutic responses against the relevant pathogen using appropriate animal or *in vitro* disease models and/or evaluation of functional immune responses (e.g., neutralizing activity, serum bactericidal or opsonophagocytic antibody titers).
4) Evaluation of duration and breadth of the immune response induced (21).

These studies will contribute to the elucidation of the adjuvant mode of action and may provide indication of the adjuvant-specific immune modulatory effects. In addition, these studies may assist in the interpretation of nonclinical safety studies and the identification of potential adverse effects to be monitored during clinical development. The development of *in vitro* model systems, particularly those using human cells, is recommended also as they may provide additional relevant information to elucidate the mechanism of action of the adjuvant (see section 4.2 below).

4.2 *In vitro* supporting studies

Functional *in vitro* bioassays may also provide helpful insight in understanding the mode of action of a particular adjuvant and may provide valuable supplemental and complimentary data to the animal studies. This is important in particular when there are limitations of the animal

models such as species-specific differences (e.g., TLR receptors). Antigen-presenting cells or other immune cells are widely used to assess and monitor the direct or indirect effects of adjuvants by measuring activation parameters (such as changes in the expression of cell surface molecules and the pattern of cytokine secretion), and more recently such human cells have been used to develop *in vitro* assays that may be predictive of adjuvant safety *in vivo* (22). More complex tissue culture systems containing a mixture of human immune cells mimicking lymphoid tissue are being explored with the aim of evaluating human immune responses *in vitro* (23).

5. Considerations for selection of the animal species for nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines

Investigations of the properties that influence the safety and pharmacological activity of the adjuvant and the adjuvanted vaccine require the use of appropriate animal species. The animal species used for pharmacological and safety evaluations should be chosen carefully and justified. For ethical reasons, it is desirable to apply the 3R principles (reduction, replacement, refinement) to the use of animals where scientifically appropriate (24). Both manufacturers and NCL/NRA staff are encouraged to further develop *in vitro* assays and to evaluate their suitability for the control of vaccines (25).

5.1 Selection of animal species for nonclinical pharmacology studies

For the purpose of this document the nonclinical pharmacological activity of an adjuvanted vaccine is defined as the ability of the adjuvanted vaccine to induce and/or modify an immune response in an animal species. Factors influencing the selection of a particular animal species include the vaccine antigen, the adjuvant chosen, the type of immunity (i.e., cell-mediated or humoral) to be induced and the route of administration. It is recommended that proof-of-concept studies be undertaken using an animal species in which (i) an immune response to the vaccine antigen is developed and, (ii) the immune response to the antigen is enhanced by the adjuvant through a mechanism similar to that expected in humans (e.g., TLRs known to be targeted by the adjuvant are present in the species, and enhanced humoral and/or cellular immunity is observed). However, it is acknowledged that species-specific differences in the immune responses induced in the animal species compared to the human are likely. Proof- of concept studies most

commonly are conducted in several animal species, including both naïve and pre-exposed animals. In addition to evaluating the immune response induced by the vaccine antigen alone and in the presence of adjuvant, the mechanism of action of the adjuvant in the absence of the vaccine antigen should also be evaluated.

If the adjuvanted vaccine is a therapeutic vaccine for an infectious disease indication, where feasible, disease animal models may need to be developed to study the pharmacologic activity of the adjuvanted vaccine and its effect on the disease. For preventive adjuvanted vaccines, the use, when available, of an animal species sensitive to the human pathogen, may provide important insight into the mechanism of protection from the disease, e.g., the ferret model for human influenza.

Nonclinical pharmacology studies may be conducted under non-GLP compliant conditions. It is advisable to incorporate into the study design toxicological endpoints to guide the design of GLP-compliant nonclinical safety studies. It is sufficient to conduct these studies in small animal species if it can be demonstrated that the animal species chosen is relevant and responsive to the vaccine antigen and the adjuvant. Therefore, non-human primates should only be used if no other relevant animal species is available.

5.2 Selection of animal species for nonclinical safety studies

When selecting the animal species in for the nonclinical safety studies, it is important to document the pharmacological activity of the vaccine in the presence and absence of adjuvant in that species. It is recommended that manufacturers conduct nonclinical safety studies in compliance with GLP (see Table 1 and section 6.2) and using an animal species in which an immune response to the vaccine antigen is developed and, ideally, the immune response to the antigen is enhanced by the adjuvant through a similar mechanism as expected in humans. It is not necessary, however, to conduct the nonclinical safety study in the same animal species used for proof of concept or nonclinical pharmacology studies (see sections 4.1 and 5.1). Non-human primates should only be used if no other relevant animal species is available. In situations where no animal species is available that is responsive to the adjuvanted vaccine, the choice of the animal species should be justified. In some circumstances, use of *in vitro* model systems,

particularly those using human cells, to evaluate the toxicity of the adjuvanted vaccine may provide additional supplementary information to assist in interpreting toxicity data (22).

It is highly recommended that the animal species chosen is one for which relevant and sufficient historical control data exist. Analysis and interpretation of data from the toxicity studies commonly includes a comparison with the inactive control (e.g., saline control) in the same study. However, historical control data from the same laboratory in which the study was conducted and for animals of comparable age and from the same species and/or strain may provide additional information. Where historical control data is used, the data should be provided.

The route of administration used in the toxicity study should correspond to that intended for use in the clinic. Also, when the adjuvanted vaccine is to be administered in the clinic using a particular device, the same device should be used in the animal study, where feasible. For example, a small rodent species may not be an appropriate choice for nonclinical evaluation of a vaccine that is to be delivered intranasally because some of the inoculum could be delivered to the lungs. In this case, a larger animal or one with nasal surface area, anatomy, and physiology similar to that of humans would be more appropriate (26).

The use of only one animal species has commonly been accepted based primarily on pragmatic considerations, for example, the ability to predict the human immune response may be limited due to species-specificity of the response in animals to the antigen, the adjuvant, or both. Although use of a single species is generally acceptable (see section 6.2), consultation with the relevant NRA is encouraged to verify the suitability of the proposed approach.

5.3 Limitations of animal studies

The limitations of using animals to characterize the pharmacologic and safety profile of an adjuvant or adjuvanted vaccine are acknowledged. The ability to predict the human immune response based on pharmacologic studies in an animal may be limited due to the species-specificity of the response to the antigen, the adjuvant or both. Similarly, local and systemic adverse effects observed in a nonclinical safety study may not be directly translatable to the

clinic. In addition, rare and/or late onset adverse events that may occur in human subjects as a result of adjuvanted vaccine administration may not be observed in animal studies. Nevertheless, these studies offer the best currently available tools to evaluate the preclinical safety and pharmacology of adjuvanted vaccines.

6. Nonclinical safety assessment in animals

6.1 General remarks

Safety concerns for drug products such as vaccines include the potential inherent toxicities of the antigen and other vaccine components, as well as potential toxicities due to interactions of the components present in the final formulation. For adjuvanted vaccines, these concerns include the possibility that the immune modulatory and/or inflammatory response induced may lead to undesired toxic side effects. Additionally, some adjuvants may elicit elevated levels of pro-inflammatory cytokines and other mediators of toxicity irrespective of the immune response against the antigen.

Safety assessments in animals studies are valuable tools to help define an acceptable adjuvant/antigen ratio and a safe dose, as well as to identify unknown or potential adverse effects that should be taken into consideration for further product development or to be monitored in future clinical trials. The type of studies and the timing in relation to the clinical program are presented in below in section 6.2.

6.2 Toxicity studies of vaccine adjuvants and final adjuvanted vaccine formulations

The preclinical toxicity studies of the final adjuvanted vaccine formulation should be adequate to identify and characterize potential adverse effects of the vaccine in order to conclude that it is reasonably safe to proceed to first-in-human clinical investigation. As the mechanism of action of the adjuvant and/or adjuvanted vaccine formulation is often not fully understood, the toxicity studies should be designed to evaluate a broad spectrum of parameters due to the uncertainty of the *in vivo* effects and associated outcomes. Toxicity studies should be designed to mimic the intended route of administration in the clinic and to evaluate local reactogenicity (e.g., injection site inflammation) and systemic toxicity (i.e., toxicity that is occurring at sites distant from the

site of initial application). Pivotal toxicity studies should use the intended final formulation and dose of the adjuvanted vaccine (see section 3.2) and should be conducted in compliance with GLP.

When properly designed, conducted, and interpreted, and when no major safety signals are revealed in the study results, one repeated dose toxicity study in one relevant species should be sufficient. However, if there are significant manufacturing or formulation changes during product development, additional animal toxicity studies may be recommended to confirm that the safety profile of the product has not been changed. Also, throughout the clinical program, additional animal toxicity studies may be necessary to investigate any adverse events observed in clinical trials or to support the use of the vaccine in a special population (e.g. developmental and reproductive toxicity studies).

While comprehensive toxicity evaluations of the final adjuvanted vaccine formulation are considered essential, the advantages and limitations of toxicity studies with adjuvant alone have been discussed extensively in previous meetings and workshops (7-11). A comprehensive toxicity assessment of the adjuvant alone in animals (or of individual evaluations of its multiple components, if a combination adjuvant), may not be needed as a separate program. However, to enable the interpretation of immunogenicity and safety studies of the adjuvanted vaccine, a study arm receiving adjuvant alone may be included in the repeated dose toxicity studies (see sections below) that are part of the comprehensive toxicity evaluations of the final adjuvanted vaccine formulation.

6.2.1 Safety pharmacology studies

The purpose of safety pharmacology is to investigate the effects of the candidate vaccine on vital functions. Although not usually required, safety pharmacology studies may be recommended by the NRA in some cases. For example, if data from nonclinical and/or human clinical studies suggest that the adjuvanted vaccine may affect physiological functions (e.g., central nervous system, respiratory, cardiovascular, renal functions and body temperature) other than the immune system, safety pharmacology studies should be incorporated into the safety assessment program.

6.2.2. Repeated dose toxicity studies

This section highlights important considerations regarding the study design for pivotal toxicity studies that should be conducted with the same vaccine formulation intended to be used in clinical trials (see section 3.2). If more than one formulation is to be evaluated in the clinical study, the highest dose (i.e., "worst case") formulation should be included in the pivotal toxicity studies. Single dose toxicity studies on the final formulated vaccine product, which are applicable to small molecule chemical drugs, are usually not needed in the accordance with ICH M3(R2) (27). Acute effects of administering the vaccine can also be monitored in repeated dose toxicity studies if they are adequately designed (e.g., evaluation is conducted after the first administration). Alternatively, acute effects can be assessed in a single dose design as part of a local tolerance study. For a study intended to support a first-in-human clinical trial, the number of animals studied per sex, group, and time interval should be sufficient to allow meaningful scientific interpretation of the data generated. The size of the treatment group will depend on the animal species chosen, i.e., the number of animals included in studies using non-rodents (e.g., miniature pigs) would be expected to be less than the number included in studies using rodents. For mice and rats, it is recommended that at least ten animals/sex/group/time interval be used. For rabbits, it is recommended that at least five animals/sex/group/time interval be used. In general, the approximate age for rodents should be six to eight weeks, and for rabbits, 3 to 4 months, at the start of the study.

6.2.2.1 Dose, dosing regimen, and controls

Dose response evaluation for the adjuvanted vaccine is generally not required as part of the basic toxicity assessment. For adjuvanted vaccines, the toxicity study should be performed using the highest anticipated human dose (in absolute terms) of the final adjuvanted vaccine to be used in the proposed clinical trial, where feasible. Ideally this dose provides optimal exposure of the animal to the candidate vaccine and the immune response induced. However, in the case of a novel adjuvant, it may be advisable to include additional (lower and especially higher) doses of the adjuvanted vaccine formulation or adjuvant alone in order to identify a safe dose that could be used in a first-in-human clinical trial.

If the dose to be administered is limited by the total volume that can be administered in a single injection, guidelines for animal welfare should be followed (28). In such cases, the total volume may need to be administered at multiple sites using the same route of administration; however, it should be noted that the evaluation of local reactogenicity might be less reliable in such cases.

For adjuvanted vaccines intended to be given repeatedly, the number of doses administered to the animals in repeated dose toxicity studies should equal or exceed the number of doses proposed in humans. However, in many cases, the studies are designed to include one dose more than planned for the clinical trial to allow for the possible inclusion of an additional dose in the clinical trial. To simulate the proposed clinical usage, vaccine doses should be given as episodic doses, but the dosing interval used in the toxicity study may be reduced (e.g., 2 to 3 weeks interval) compared to the proposed clinical dosing interval (which usually is greater than 2 to 3 weeks). The nonclinical dosing interval should primarily be based on the kinetics of the primary and secondary antibody response observed in the animal study.

In general, the study design should include a negative control group that receives an inert placebo such as saline to evaluate a baseline level of treatment and an adjuvant alone arm to aid in the interpretation of safety data from the adjuvanted vaccine. Also, the study should include additional treatment groups to be sacrificed and evaluated as described below at later time points after treatment, to evaluate reversibility of adverse effects observed during the treatment period and to detect potential delayed adverse effects.

6.2.2.2 Route of administration

The route of administration should correspond to that intended for use in the clinical trials. When the vaccine will be administered in human clinical trials using a particular device, the same device should be used in the animal study, where feasible.

6.2.2.3. End-points in toxicity studies

The following section discusses endpoints which are especially relevant and important in the evaluation of adjuvanted vaccines in repeated dose toxicity studies using the final vaccine formulation. In general, potential adverse effects of the adjuvanted vaccine should be evaluated

in repeated dose studies with regard to target organs (see appendix 2), dose, route(s) of exposure, duration and frequency of exposure, and potential reversibility of observed toxic effects.

6.2.2.3.1 Parameters for monitoring of systemic toxicity

Toxicity studies, repeated dose toxicity studies in particular, should address the potential for systemic toxicity, including, but not limited to, the systemic effects on the immune system. A broad spectrum of information should be obtained from the toxicity study, and both in-life and post-mortem data should be collected. This routinely includes careful monitoring of body weight and food consumption, body temperature, coagulation parameters and acute phase reactants. In addition, the immune response should be evaluated in a group of treated animals to confirm that the anticipated immune response occurred during the toxicity study. A detailed description of the assay(s) used should be provided with the toxicity study results.

While the standard in-life parameters routinely assessed for general pharmaceuticals (e.g., clinical observations, body weights, and food consumption) are appropriate, it is important to note that for adjuvanted vaccines, daily measurements of body weight and food consumption are recommended, especially during the first week after the administration of each dose, as these parameters are very sensitive in detecting systemic toxicity effects. After the first week, body weights may be collected less frequently, e.g., 2-3 times a week. Body temperature should also be evaluated prior to, and 6 and 24 hours after each dose. If there is an increase in temperature, additional measurements should be taken every 24 hours until the values return to baseline. Interim analysis of haematology and serum chemistry should be considered within approximately 1 to 3 days following the first and last dose administration and at the end of the recovery period; in addition, the collection of a pre-dosing sample is recommended. In some cases, evaluation of coagulation parameters, urine samples, and serum immunoglobulin classes may be of value. The measurement of acute phase reactants (e.g., C-reactive protein) in the toxicity study prior to immunization, 24 to 48 hours after the administration of the adjuvant or adjuvanted vaccine, and after a recovery phase of 7 days, can help to predict possible systemic toxicities in humans (29). When measuring acute phase reactants, the choice of the animal species may determine which proteins can be measured as these reactants vary among species (30). The data discussed above should be collected not only prior to and during the treatment

phase, but also following the treatment free (recovery) phase (i.e., 2 or more weeks following the last dose) to determine persistence, exacerbation and/or reversibility of potential adverse effects.

Post-mortem data including complete gross necropsy (with tissue collection and preservation, including gross lesions and organ weights) should be collected within two days following the last dose and following the above-mentioned recovery period (e.g., two or more weeks following the last dose) (1). At study termination, final body weights (following overnight fasting) should be obtained. Terminal blood collection and analysis should include serum chemistry and haematology as well as an immune response evaluation; where appropriate, the type of immune response should be evaluated. Histopathological examinations should always include pivotal organs (brain, lung, heart, kidneys, liver, reproductive organs) and the site of adjuvant or adjuvanted vaccine administration. Special attention should be paid to the immune organs, i.e., lymph nodes (draining and distant to application site), thymus, spleen, bone marrow and Peyer's patches or bronchus-associated lymphoid tissue, as well as organs that may be primarily affected due to the particular route of administration. The extent of the list of tissues to be examined (i.e., the full tissue list as provided in Appendix 2 vs. the reduced list mentioned above limited to immune and pivotal organs) will depend on the adjuvant or adjuvanted vaccine in question, as well as on the experience and knowledge obtained through previous nonclinical and clinical testing of the vaccine components. Additionally, any known target organs of the adjuvant/adjuvanted vaccine should be evaluated. For novel adjuvanted vaccines, it is recommended that the full tissue list be evaluated.

6.2.2.3.2. Parameters for monitoring of local reactogenicity

Local toxicities should be determined at the site(s) of adjuvant or adjuvanted vaccine administration and any other sites that come into contact with the adjuvant or adjuvanted vaccine components as a result of the method of administration. Local toxicity with intramuscularly administered vaccines should preferably be conducted in animals with sufficient muscle mass to test the full human dose of the final vaccine formulation.

Injection site reaction after inoculation should be scored using a prospectively defined system, e.g., the modified Draize test (31), along with an assessment of any vesiculation, ulceration, severe eschar formation, and other manifestations of significant toxicity, e.g., limb impairment.

The site of administration and any other site that comes in contact with the adjuvant or adjuvanted vaccine (e.g., eye exposure during aerosol administration or digestive track after oral administration) should also be evaluated histopathologically; the type and extent of infiltrating immune cells as well as any manifestation of tissue damage at the site of injection and surrounding anatomic structures (e.g., sciatic nerves, nasal cavities, or olfactory bulb) should be evaluated and reported.

6.2.3. Developmental and reproductive toxicity

As vaccination programs may include women of childbearing potential, it is important to consider the need for developmental and reproductive toxicity studies. As is the case for general toxicity, the use of a novel adjuvant may require adding an adjuvant-alone arm in the reproductive toxicity studies. However, the study design is also dependent on the intended clinical use of the vaccine. For example, vaccination may be given early in pregnancy to protect the mother at risk, or might be given later in pregnancy to induce passive immunization to protect the infant directly from birth. Depending on the adjuvant, there may be concern about an adjuvant-induced systemic inflammatory response, e.g., fever, which may adversely affect early pregnancy (e.g., implantation or placental growth) (32). In these cases, it is recommended to include in the study design an additional treatment group to evaluate the effect of adjuvant on early pregnancy parameters. Rather than dosing this treatment arm prior to mating, it is recommended to dose animals post-mating and prior to implantation, e.g., post-mating day one.

In general, the administration of one or several additional doses during organogenesis (i.e., implantation to closure of the hard palate) is recommended in order to evaluate potential direct embryotoxic effects of the components of the vaccine formulation, and, depending on the animal model, to allow maternal antibody transfer to the fetus during pregnancy or the lactation period. Endpoints in the embryofetal/peri-postnatal toxicity studies include, but are not limited to, viability, abortions, number of resorptions, fetal body weight, morphology, pre-weaning

development and growth as well as survival incidence and developmental landmarks. For details on such studies, please see the *FDA Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications* (33) and the *WHO Guidelines on Nonclinical Evaluation of Vaccines* (1).

In most cases, the developmental and reproductive toxicity studies can be performed in parallel to the clinical trials. However, some NRAs require that women of child-bearing potential be excluded from large scale, late stage clinical trials that are conducted prior to the completion of developmental and reproductive toxicity studies; other NRAs require the use of appropriate birth control methods for women of child bearing potential that are included in clinical trials. Further considerations can be found in ICH M3(R2) (27).

6.2.4 Biodistribution studies

Adjuvants are expected to exert their action locally in close connection to the antigen. Biodistribution studies can be helpful in understanding the distribution of the adjuvant following injection. The feasibility of and need for such biodistribution studies should be evaluated on a case by case basis.

6.2.5 Genotoxicity/carcinogenicity studies

Genotoxicity studies are normally not needed for the final vaccine formulation (1). However, a standard battery of genotoxicity studies is generally recommended for most novel adjuvants that are (or contain) new chemical entities (27, 34). This standard battery might not be adequate, however, for detecting the genotoxicity risk, especially for an oligodeoxynucleotide-type of adjuvant that can participate in triplex formation (35). Based on previous experience, carcinogenicity studies are generally not needed for adjuvants or adjuvanted vaccines.

6.2.6. Toxicity studies of adjuvant alone

As noted above (section 6.1), comprehensive toxicity assessment of the adjuvant alone in animals may be included as part of the study design with the adjuvanted vaccine (see 6.2). However, evaluation of the adjuvant alone can be important for novel adjuvants that have not been studied previously or will be used in multiple different vaccine formulations. In the case of

a novel adjuvant or combination adjuvant, it may be advisable to include additional (lower and especially higher) doses of the adjuvant component(s) in order to identify a safe dose that could be used in a first-in-human clinical trial and, as well as safety signals that should be monitored in the proposed clinical trial.

Although not usually required, safety pharmacology studies may be recommended in some cases to demonstrate that a novel adjuvant has no adverse effects on physiological functions (e.g., central nervous system, respiratory, cardiovascular, renal functions, and body temperature) other than the immune system. If needed, such studies could also be included as a specific arm with the adjuvant alone in the repeated-dose toxicity study of the intended final vaccine formulation (1, 36). It is expected that these studies would be conducted before initiating first-in-human clinical trials.

6.2.7 Summary of recommendations regarding timing of studies

In general, the guidance provided in this document regarding the timing of studies in relation to clinical trials is consistent with that of other guidance documents (e.g., reference 27). A repeated dose toxicology study (including safety pharmacology endpoints, if needed) should be conducted before the first-in-human clinical trial. It may be important to conduct some studies with adjuvant alone (e.g., systemic toxicity and genotoxicity, where needed) prior to initiation of clinical trials (27). Developmental toxicology studies should be performed prior to initiation of any clinical study to be conducted in pregnant women, i.e., for those vaccines specifically developed for use in pregnancy. For vaccines indicated for females of childbearing potential, subjects can be enrolled in clinical trials provided that appropriate precautions are taken to avoid vaccination during pregnancy, such as pregnancy testing and use of birth control. For these products, developmental toxicity studies (section 6.2.3) may be performed in parallel with the clinical study. All nonclinical safety studies should be completed by the time a license application is submitted to the NRA.

6.3 Additional considerations

Additional studies for safety assessment have been considered for the specific situation in which the target population for a vaccine containing a novel adjuvant includes very young subjects,

e.g., neonates, and newborns. Although vaccination of newborn children is a well-known practice (e.g., BCG, Hepatitis B), the use of adjuvants is uncommon at this early age. At this time, however, there is insufficient knowledge about suitable animal models to evaluate whether neonates and newborns with an immature immune system would adequately respond to adjuvanted vaccines or whether the adjuvant could modify the neonatal immune system in an undesirable way. Modified immune responses to vaccination also have been observed in elderly populations, however, there also is insufficient knowledge about animal models to evaluate the response to adjuvants and adjuvanted vaccines in the aging population. Further research to improve methods that can be used for nonclinical evaluation of adjuvanted vaccines that are targeted for neonatal and elderly populations is encouraged.

Thus far, there is no compelling clinical evidence that adjuvants are causally related to the induction of autoimmune phenomena (or autoimmune disease) or hypersensitivity in humans (4). Although there has been interest in developing animal models that could be used to screen adjuvants and adjuvanted vaccines for induction of autoimmunity or hypersensitivity, such models do not currently exist. Therefore, no recommendations can be made at this time regarding specific nonclinical studies that should be conducted. These are complex and multi-factorial conditions; further research is needed to identify additional biomarkers related to autoimmunity and hypersensitivity phenomena.

7. Considerations for first-in-human clinical trials

As with the nonclinical safety assessment considerations, the first-in-human trial considerations for novel adjuvanted vaccines are similar to those for unadjuvanted vaccines (2); however, some issues unique to clinical evaluation of vaccines with novel adjuvants may need to be considered. The initial clinical trials of adjuvanted vaccines are usually intended to 1) determine the subjects' tolerability to the range of doses of antigen and adjuvant and the dosing regimen that may be needed for later immunogenicity and clinical endpoint trials and 2) to aid in the collection of information on the nature of the adverse reactions that can be expected. This section provides guidance on the points to consider when transitioning from nonclinical to clinical testing of adjuvanted vaccines as signals observed in nonclinical studies can aid in the design of the first-

in-human clinical trials. This section is intended to supplement the information provided in the *WHO Guidelines on clinical evaluation of vaccines: regulatory expectations* (2).

Although there are limitations in the ability of animal and *in vitro* studies to predict safety in humans, all of the relevant nonclinical data, including the information on the pharmacologically active dose and the full toxicologic profile of the adjuvanted vaccine, should be considered when designing the first-in-human trials. These data may aid in the selection of a safe starting dose, schedule, and route of administration, and in the identification of potential adverse effects for specific monitoring in the first-in-human clinical trial. A summary of such data from the nonclinical studies with the adjuvanted vaccine and any available clinical data from similar or related adjuvanted vaccines should be provided in support of the proposed first-in-human clinical trial design. If, for example, dose limiting toxicity was observed with the adjuvanted vaccine in the animal studies and the studies were repeated with lower doses to identify a dose that was without adverse effect in animals, it would be important to point that out and to summarize the specific adverse effects observed in the nonclinical studies.

Manufacturers should provide a rationale and scientific support for the use of an adjuvant in their vaccine. This could include information supporting the "added benefit" of the adjuvant derived from nonclinical studies (e.g., *in vitro* assays and/or proof of concept studies in animal models including relevant challenge models when available) conducted prior to the initiation of clinical trials. In addition, it is recommended that the early clinical evaluations of an adjuvanted vaccine be designed to include the evaluation of both antigen alone and adjuvanted vaccine arms to demonstrate the added benefit of the adjuvant; such data may include, for example, evidence of enhanced immune responses or antigen sparing.

If the safety of the adjuvanted vaccine was evaluated in appropriately designed toxicology studies that were conducted in line with the recommendations outlined above and if there were no adverse effects observed in the toxicology studies conducted, the human dose tested in the toxicology studies may be acceptable as the starting dose in the first-in-human trials. However, such clinical trials are often designed as dose escalating studies where the antigen and/or the adjuvant are given at escalating doses. With this in mind, given the limitations of the animal

studies, it may be prudent to consider using a safety factor (for example, a safety factor of 10 has been used historically) and to divide the human dose tested in the toxicology studies by the safety factor to find the recommended starting dose and escalate the dose from there. While it is anticipated that the adjuvant may have an antigen sparing effect, the first-in-human trials should be designed to attempt to establish whether the adjuvant is needed and if so, the minimum dose of adjuvant that is necessary to achieve adequate immunogenicity.

Although an inactive control (placebo) group may not be required in the first-in-human trial of an adjuvanted vaccine, the inclusion of a group receiving inactive control, such as inert saline placebo, in early phase clinical trials will enhance interpretation of the initial safety data through control for placebo effects and circulating community acquired illnesses.

As with first-in-human trials of non-adjuvanted vaccines, those for adjuvanted vaccines are usually conducted in a limited number (e.g., 20-80) of healthy, adult volunteers (e.g., 18-50 years of age) with safety as the primary objective. To aid in the overall risk/benefit evaluation of the adjuvanted vaccine, the subject population should be clearly defined by inclusion and exclusion criteria, and the subjects should be closely monitored for safety. The clinical protocol should contain a safety monitoring plan with details regarding active post-vaccination monitoring and pre-defined toxicity criteria for assessing the severity of clinical and laboratory parameters (37). In addition, the plan for increasing the dose of antigen and adjuvant, with pre-defined step-wise criteria for doing so, should be included in the clinical protocol. Also, it is recommended, especially when a novel adjuvant is used, that safety monitoring be extended through 12 months following the last vaccination (where the last follow-up may be accomplished by a telephone call). In this regard, it is recommended that serum specimens be banked where possible for potential future assessment in the event of a serious adverse event (SAE), a new-onset medical condition, or an adverse event of special interest that develops later on in the course of the first-in-human clinical trial.

Any safety experience with the same adjuvant formulated with other vaccine antigens, if available, may assist in developing the safety monitoring plan for the adjuvanted vaccine. However, since the mode of action in humans for the adjuvant in the specific adjuvanted vaccine

to be evaluated in the first-in-human trial is usually unknown and adjuvants may exhibit a range of properties that invoke complex immune responses, it is recommended that first-in-human trials for adjuvanted vaccines include inquiries regarding specific adverse events. This may include, for example, local reactions (e.g., pain, redness, swelling, granuloma formation, abscess, necrosis; and regional lymphadenopathy); systemic reactions (e.g., fever, nausea, diarrhea, and malaise); immune-mediated toxicity (e.g., cytokine release, immune suppression, and autoimmune disease); and teratology. Examples of adverse events of "special interest" may include neuroinflammatory disorders (e.g., optic neuritis and transverse myelitis), musculoskeletal and connective tissue diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus (SLE), and Wegener's granulomatosis), and gastrointestinal disorders (e.g., Crohn's disease and ulcerative colitis). Additionally, targeted laboratory assessments [e.g., C-reactive protein, fibrinogen, antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), and rheumatoid factor] may aid in the evaluation of adverse events and medical conditions.

Table 1. Summary of manufacturing and quality information needed for pharmacology studies, nonclinical toxicology studies[1] and first-in-human trials

Comment on Manufacturing and Quality Information	Pharmacology	Toxicology[1]	First-in-human trials
Raw Materials Ideally, they should be the same throughout nonclinical pharmacology studies, toxicology studies and first-in-human trials.	Purity and source important	Purity and source important	Purity, source and control of manufacture important
Production	Small scale	Small scale, but should be manufactured in compliance with appropriate GMP. Ideally, should be the same lots as those for first-in-human trials (or should be of similar process as lots for first-in-human trials)	Small scale, but should be manufactured in compliance with the appropriate GMP
Presentation	Often provided in separate containers to be mixed prior to use	May be provided as an already mixed formulation ("adjuvanted vaccine") or provided in separate containers to be mixed prior to administration	May be provided as an already mixed formulation ("adjuvanted vaccine") or provided in separate containers to be mixed prior to administration
Characterization	May be less extensive characterization. Usually general quality (e.g., composition, purity, immunogenicity)	Should be considerable characterization of material (e.g., purity, potency [2]). Stability should be assessed.	Should be considerable characterization of material (e.g., purity, potency [3]). Stability should be assessed.

[1] Should be compliant with Good Laboratory Practices (GLP).

[2] Some regulatory authorities do not require demonstration of potency of the adjuvant; in this case, testing of the adjuvant identity and content is recommended.

[3] If the adjuvanted vaccine is provided pre-mixed in one container, the potency of the adjuvanted vaccine formulation should be determined. For some adjuvanted vaccines (e.g., aluminum-adsorbed vaccines) depending on the assay, it may not be possible to evaluate the potency of the adsorbed vaccine. In this case, the determination of potency of the antigen alone prior to adsorption would may be recommended as well as the development of an *in vivo* method for potency assessment.

Authors

First draft

Taking into account comments received at the WHO Consultation on the Nonclinical and Preclinical Evaluation of Adjuvanted Vaccines, held on 7-8 September 2011 in Rockville, Maryland, U.S.A., a first draft was prepared by the WHO Drafting Group: Dr M. Baca-Estrada, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, Canada; Dr G Coleman, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, Canada; Dr G. Raychaudhuri, Center for Biologics Evaluation and Research, US FDA, Maryland, USA; Dr E. Sutkowski, Center for Biologics Evaluation and Research, US FDA, Maryland, USA; Dr M. Gruber, Center for Biologics Evaluation and Research, US FDA, Maryland, USA; Dr J. W. van der Laan, Medicines Evaluation Board, Section on Pharmacology, Utrecht, The Netherlands; L Slamet, National Agency of Drug and Food Control, Jakarta, Indonesia; Dr T.Q Zhou, Quality, Safety and Standards, Immunization, Vaccines and Biologicals, WHO, Geneva, Switzerland; and Dr B. Meade, Meade Biologics, North Carolina, USA. Dr. C Wrzesinski, Center for Biologics Evaluation and Research, US FDA, Maryland, USA and Dr C Alfonso, WHO, provided critical input.

The above consultation was attended by: Dr K Ishii, National Institute of Biomedical Innovation, Osaka, Japan; Dr B Meade, Meade Biologics, North Carolina, USA; Dr M Pallardy, Université Paris-Sud, France; Dr S Reed, Research and Development, Infectious Disease Research Institute, Seattle, USA; Dr J W van der Laan, Toxicology and Biotechnology, Medicines Evaluation Board, Den Haag, Netherlands; Dr M Baca-Estrada, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa; Dr E Griffiths, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa; Dr M Gruber, Center for Biologics Evaluation and Research, Maryland, USA; Mr B.L Moraes Moreira, Agencia Nacional de Vigilancia Sanitaria; Dr G Raychaudhuri, Food and Drug Administration, Maryland, USA; Dr H.L Davis, Pfizer Vaccine Research, Ottawa, Canada; Dr N Garçon, Global center adjuvants and Delivery systems GSK Biologicals, Wavre, Belgium; Dr. D.L Novicki, NOVARTIS VACCINES, Cambridge USA; Dr F Verdier, Sanofi Pasteur, USA; Dr S Gairola, Serum Institute of India; Dr I Raw, Technology and Scientific Council Avenida Vital Brazil, Brazil; Dr L Slamet, National Agency of Drug and Food Control, Jakarta, Indonesia; Dr C Conrad, WHO, Geneva, Switzerland; Dr C.

Alfonso, WHO, Geneva, Switzerland; Dr M-L Pombo, World Health Organization, Washington DC, USA; Dr D Pfeiffer, World Health Organization, Copenhagen, Denmark.

Second Draft

The second draft was prepared by the WHO Drafting Group listed above based on comments submitted by the following reviewers during a consultation of the 1st draft among involved experts: Dr M Khaitov, Nanobiomedical Technologies Department, NRC Institute of Immunology FMBA, Russia; Dr. Y Sun, Section of Viral Vaccines , Paul-Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Germany; Dr N Garcon, Global Adjuvant & Delivery Systems Centre, GSK Biologicals, Belgium; Dr H.L Davis, Pfizer Vaccine Research, Ottawa, Canada; IFPMA combined comments from: Dr N Garcon (GSK), Dr D Novicki (Novartis), Dr D Clarke (Pfizer), Drs S Gould and F Verdier (Sanofi Pasteur); Dr M Matsumoto and Ms. M Iguchi, Office of Vaccines and Blood Products, Pharmaceutical and Medical Devices Agency (PMDA), Japan; Dr F Verdier, Regulatory Development, Sanofi Pasteur, France; Dr J Southern, Medicines Control Council of South Africa, Ministry of Health, South Africa; combined comments from Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), USA (Drs M Gruber, C Wrzesinski, H Golding, M Zaitseva, B Baldwin and E Sutkowski). The second draft document was reviewed by broad experts at the WHO informal consultation mentioned below.

Third Draft

The third draft was prepared by the WHO Drafting Group listed above taking into account comments received during the WHO Informal Consultation on Guidelines for Nonclinical Evaluation of Adjuvanted Vaccines, held on 27-28 November 2012, WHO/HQ, Geneva, Switzerland. The consultation was attended by: Dr M Baca-Estrada, Bacterial and Combination Vaccines Division, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, Canada; Dr D Carter, Adjuvant Technology, Infectious Disease Research Institute, Seattle, USA; Dr L.G Castanheira, Quality and Efficacy Evaluation, National Health Surveillance Agency (ANVISA), Brasilia, Brazil; Dr G Coleman, Clinical Evaluation Division, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, Canada; Prof I Feavers, Division of Bacteriology, National Institute for Biological Standards &

Control (NIBSC), Potters Bar, UK; Dr E Griffiths, The Farthings, Kingston upon Thames, Surrey, UK; Dr M Gruber, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Rockville, Maryland 20852, USA, Ms M Iguchi, Office of Vaccines and Blood Products, Pharmaceuticals and Medical Devices Agency (PMDA), Tokyo, Japan; Dr K Ishii, Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation, Osaka, Japan; Mrs T Jivapaisarnpong, Institute of Biological Products, Department of Medical Sciences, Ministry of Public Health, Thailand; Ms J Dahlan, Directorate of Drug and Biological Product Evaluation, The National Agency of Drug and Food Control, Jakarta, Indonesia; Dr M Khaitov, Nanobiomedical Technologies Department, NRC Institute of Immunology, Federal Medical and Biological Agency of the Russian Federation (FMBA), Moscow, Russia; Dr D Masset, Toxicological Investigation and Non-clinical Evaluation Centre, Evaluation Directorate, Agence nationale de sécurité du médicament et des produits de santé (ANSM), F-93285 Saint-Denis cedex, France; Dr M Matsumoto, Office of Vaccines and Blood Products, Pharmaceuticals and Medical Devices Agency (PMDA), Tokyo, Japan; Dr B Meade, Meade Biologics, LLC, Hillsborough, North Carolina 27278 USA; Dr L Martinez Munoz, Centro para el Control Estatal de la Calidad de los Medicamentos (CECMED), Ministerio de Salud Pública, Ciudad La Habana, Cuba; Dr S-R Pakzad, Vaccine Potency and Standardization Section, Food and Drug Control Laboratory, Tehran, Iran; Dr M Pallardy, Université Paris-Sud, Faculté de Pharmacie, Châtenay-Malabry, France; Dr V.G. Somani, Central Drugs Control Organization, Ministry of Health and Family Welfare, Government of India, New Delhi, India; Dr J Southern, Medicines Control Council of South Africa, Ministry of Health, Cape Town, South Africa; Dr. med. Y Sun, Section of Viral Vaccines, Paul-Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany; Dr E Sutkowski, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Rockville, MD 20852 USA; Dr J.W van der Laan, Section on Pharmacology, Toxicology and Biotechnology, Medicines Evaluation Board, Utrecht, The Netherlands; Dr M Xu, Institute for Biological Products Control, National Institutes for Food and Drug Control (NIFDC), Beijing, China; Dr M Bonelli, Scientific Support and Projects, Human Medicines Special Areas, European Medicines Agency, London, United Kingdom of Great Britain & Northern Ireland; Dr B Fritzell, Representative of International Alliance for Biological Standardization (IABS), 21 rue Foy , 33000 Bordeaux, France; Representatives of International

Federation of Pharmaceutical Manufacturers and Associations (IFPMA): Dr D Clarke, Vaccines Drug Safety R&D, Pfizer Inc., New York, USA; Dr N Garcon, Global Adjuvant & Delivery Systems Centre, GSK, Belgium; Dr S Gould, Non-Clinical Safety, Sanofi Pasteur, France; Dr D Novicki, Toxicology, Novartis Vaccines, USA; Dr R Zahn, Core Immunology, Viral Vaccines, Crucell Holland B.V., The Netherlands. Representatives of Developing Countries Vaccine Manufactures Network (DCVMN): Dr S Gairola, Quality Control, Serum Institute of India Ltd., India; Dr M.A Medeiros, Molecular Biology Laboratory, Biomanguinhos, Brazil; Dr R.I. Modi, Cadila Pharmaceuticals Ltd., India; Dr J Petre, BioNet-Asia, Co Ltd. Bangkok, Thailand; Dr Q Zhao, Xiamen Innovax , P.R. China; Dr M Reers, Technical Operations, Biological E, Hyderabad, India; Other industry: Dr S Dewasthaly, Clinical & Medical Affairs, Intercell AG, Vienna, Austria; and WHO Secretariat: Dr J Fournier-Caruana, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland; Dr M.H. Friede, IER/IEA, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland; Dr U Fruth, Initiative for Vaccine Research (IVR), Immunization, Vaccines and Biologicals (IVB), Family and Community Health Cluster (FWC), WHO, Geneva, Switzerland; Dr I Knezevic, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland; Dr S Nishioka, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland; Dr D.J Wood, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland; Dr T.Q Zhou, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva , Switzerland; Dr P Zuber, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva , Switzerland.

Fourth draft

The fourth draft was prepared by the WHO Drafting Group listed above taking into account comments submitted by the following reviewers during the circulation of the third draft

document among the meeting participants of the WHO Informal Consultation on Guidelines for Nonclinical Evaluation of Adjuvanted Vaccines, held on 27-28 November 2012: Dr E Griffiths, The Farthings, Kingston upon Thames, Surrey, UK; Ms M iguchi, Office of Vaccines and Blood Products, Pharmaceuticals and Medical Devices Agency (PMDA), Tokyo, Japan; Dr K Ishii, Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation, Osaka, Japan; Ms J Dahlan, Directorate of Drug and Biological Product Evaluation, The National Agency of Drug and Food Control, Jakarta, Indonesia; Dr M Khaitov, Nanobiomedical Technologies Department, NRC Institute of Immunology, Federal Medical and Biological Agency of the Russian Federation (FMBA), Moscow, Russia; Dr M Matsumoto, Office of Vaccines and Blood Products, Pharmaceuticals and Medical Devices Agency (PMDA), Tokyo, Japan; Dr L Martinez Munoz, Centro para el Control Estatal de la Calidad de los Medicamentos (CECMED), Ministerio de Salud Pública, Ciudad La Habana, Cuba; Dr J Southern, Medicines Control Council of South Africa, Ministry of Health, Cape Town, South Africa; Dr D Clarke, Vaccines Drug Safety R&D, Pfizer Inc., New York, USA; Dr N Garcon, Global Adjuvant & Delivery Systems Centre, GSK, Belgium; Dr S Gould, Non-Clinical Safety, Sanofi Pasteur, France; Dr D Novicki, Toxicology, Novartis Vaccines, USA; Dr S Gairola, Quality Control, Serum Institute of India Ltd., India; combined comments from Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), USA (Drs M Gruber, C Wrzesinski, H Golding, M Zaitseva, B Baldwin and E Sutkowski); Dr J Fournier-Caruana and Dr S Nishioka, Quality, Safety, and Standards (QSS) Team, Essential Medicines and Health Products (EMP) Department, Health Systems and Innovation (HIS) Cluster, WHO, Geneva, Switzerland.

References

1. WHO. WHO guidelines on nonclinical evaluation of vaccines. Annex 1. In: WHO Expert Committee on Biological Standardization. Fifty-fourth report. Geneva: World Health Organization (WHO Technical Report Series, No. 927); 2005.
2. WHO. Guidelines on clinical evaluation of vaccines: regulatory expectations. Annex 1. In: WHO Expert Committee on Biological Standardization. Fifty-second report. Geneva: World Health Organization (WHO Technical Report Series, No. 924); 2004.
3. Global Advisory Committee on Vaccine Safety, June 2012. Wkly Epidemiol Rec 2012;87(30):281-7.
4. Ahmed SS, Plotkin SA, Black S, Coffman RL. Assessing the safety of adjuvanted vaccines. Sci Transl Med 2011;3(93):93rv2.
5. European Medicines Agency. Committee for Human Medicinal Products Guideline on Adjuvants in Vaccines for Human Use. EMEA/CHMP/VEG/134716/2004 2005.
6. Japanese Guideline. Guideline for Nonclinical Studies of Preventive Vaccines for Infectious Diseases (Supplemental Information). 2010.
7. WHO. The Global Advisory Committee on Vaccine Safety. http://www.who.int/vaccine_safety/topics/adjuvants/en/.
8. Transcript. Workshop on: Non-clinical safety evaluation of preventive vaccines: Recent advances and regulatory considerations (http://www.fda.gov/downloads/biologicsbloodvaccines/newsevents/workshopsmeetingsconferences/transcriptsminutes/ucm054459.pdf). Society for Toxicology and Center for Biologics Evaluation and Research 2002.
9. van der Laan JW, Forster R, Ledwith B, Guber M, Gould S, Segal L, et al. Nonclinical Testing of Vaccines: Report From a Workshop (http://www.diahome.org/productfiles/8357/diaj_28131.pdf). Drug Information Journal 2009;43:97-107.
10. Transcript. Workshop on adjuvants and adjuvanted preventive and therapeutic vaccines for infectious disease indications (http://www.fda.gov/downloads/biologicsbloodvaccines/newsevents/workshopsmeetingsconf

erences/ucm095708.pdf). Center for Biologics Evaluation and Research and the National Institute of Allergy and Infectious Diseases 2008.

11. Mastelic B, Ahmed S, Egan WM, Del Giudice G, Golding H, Gust I, et al. Mode of action of adjuvants: implications for vaccine safety and design. Biologicals 2010;38(5):594-601.
12. Sun Y, Gruber M, Matsumoto M. Overview of global regulatory toxicology requirements for vaccines and adjuvants. J Pharmacol Toxicol Methods 2012;65(2):49-57.

12a. WHO. Handbook: good laboratory practice (GLP): quality practices for regulated non-clinical research and development. Second Edition. http://www.who.int/tdr/publications/documents/glp-handbook.pdf; 2009.

13. WHO. Good Manufacturing Practices for Biological Products. Annex 1. In: WHO Expert Committee on Biological Standardization. Forty-second report. Geneva: World Health Organization (WHO Technical Report Series, No. 822); 1992.
14. WHO. WHO good manufacturing practices: main principles for pharmaceutical products. Annex 3. In: WHO Expert Committee on Specifications for Pharmaceutical Products. Forty-fifth report. Geneva: World Health Organization (WHO Technical Report Series, No. 961); 2011.
15. Cussler K. A 4R concept for the safety testing of immunobiologicals. Dev Biol Stand 1999;101:121-6.
16. Gupta RK. Is the test for abnormal toxicity, general safety or innocuity necessary for vaccines? Vaccine 1996;14(17-18):1718.
17. Halder M. Three Rs potential in the development and quality control of immunobiologicals. ALTEX 2001;18 Suppl 1:13-47.
18. WHO. Guidelines on stability evaluation of vaccines. Annex 3. In: WHO Expert Committee on Biological Standardization. Fifty-seventh report. Geneva: World Health Organization (WHO Technical Report Series, No. 962); 2007.
19. Friede M, Palkonyay L, Alfonso C, Pervikov Y, Torelli G, Wood D, et al. WHO initiative to increase global and equitable access to influenza vaccine in the event of a pandemic: supporting developing country production capacity through technology transfer. Vaccine 2011;29 Suppl 1:A2-7.

20. Verdijk P, Rots NY, Bakker WA. Clinical development of a novel inactivated poliomyelitis vaccine based on attenuated Sabin poliovirus strains. Expert Rev Vaccines 2011;10(5):635-44.
21. Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. Vaccine 2006;24(33-34):5937-49.
22. Zaitseva M, Romantseva T, Blinova K, Beren J, Sirota L, Drane D, et al. Use of human MonoMac6 cells for development of in vitro assay predictive of adjuvant safety in vivo. Vaccine 2012;30(32):4859-65.
23. Ma Y, Poisson L, Sanchez-Schmitz G, Pawar S, Qu C, Randolph GJ, et al. Assessing the immunopotency of Toll-like receptor agonists in an in vitro tissue-engineered immunological model. Immunology 2010;130(3):374-87.
24. Shin J, Lei D, Conrad C, Knezevic I, Wood D. International regulatory requirements for vaccine safety and potency testing: a WHO perspective. Procedia in Vaccinology 2011;5:164-170.
25. WHO. Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities. In: WHO Expert Committee on Biological Standardization *(in press*). Geneva: World Health Organization (WHO Technical Report Series); 2010.
26. NIAID. Safety Evaluation of Toxin Adjuvants Delivered Intranasally: Summary of Workshop. http://www.niaid.nih.gov/topics/entericDiseases/Documents/intranasal.pdf. 2001.
27. ICH. Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, M3(R2). ICH Harmonized Tripartite Guideline 2009.
28. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 2001;21(1):15-23.
29. FDA. Internal Communications. 2013.
30. Kushner I, Mackiewicz A. The Acute Phase Response: an Overview. In: Mackiewicz A, Kushner I, Baumann H, editors. Acute Phase Proteins: Molecular Biology, Biochemistry, and Clinical Applications. Boca Raton: CRC Press; 1993. p. 3-19.

31. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Therapeut. 1944;82:377-390.
32. Herberts C, Melgert B, van der Laan JW, Faas M. New adjuvanted vaccines in pregnancy: what is known about their safety? Expert Rev Vaccines 2010;9(12):1411-22.
33. FDA. Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074827.htm). 2006.
34. ICH. Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, S2(R1). ICH Harmonized Tripartite Guideline 2011.
35. Committee for Human Medicinal Products. CHMP SWP Reflection paper on the assessment of the genotoxic potential of antisense oligodeoxynuccleotides, EMEA/CHMP/SWP/199726/2004. European Medicines Agency 2005.
36. ICH. Safety Pharmacology Studies for human pharmaceuticals. S7A. ICH Harmonized Tripartite Guideline 2000.
37. FDA. Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074775.htm 2007.

Appendix 1

Examples of adjuvant classes

The following list identifies the main classes of adjuvants either in current use in licensed vaccines or under evaluation. For each category, representative examples are provided for illustration.

Classification of adjuvants

- Mineral salts/gels, e.g., aluminium hydroxide and aluminium or calcium phosphate gels.
- Oil-in water and water-in-oil emulsions, amphiphilic molecules and surfactant based formulations, e.g., MF59 (microfluidised detergent stabilised oil-in-water emulsion), QS-21 (purified saponin, which is plant-derived), AS03 (consisting of an oil-in-water emulsion plus alpha-tocopherol), Montanide ISA-51, and Montanide ISA-720.
- Particulate adjuvants, e.g., liposomes, virosomes (unilamellar liposomal vehicles incorporating influenza haemagglutinin), ISCOMS (structured complex of saponins and lipids), and polylactide co-glycolide (PLG), PLG -Dimethylaminoethane-carbamoyl-Cholesterol (PLGA/DC-cholesterol) particles, and Iscomatrix.
- Microbial derivatives (natural and synthetic), e.g., monophosphoryl lipid A (MPL), Detox (MPL + M. Phlei cell wall skeleton), AGP [RC-529] (synthetic acylated monosaccharide), DC_Chol (lipoidal immunostimulators able to self-organise into liposomes), OM-174 (lipid A derivative), CpG motifs (synthetic oligodeoxynucleotides containing immunostimulatory CpG motifs), modified heat labile enterotoxin (LT) and cholera toxin (CT) (genetically modified bacterial toxins that have been genetically modified to provide non-toxic adjuvant effects); synthetic dsRNA, Poly IC:LC (Hiltonol) and Poly I: Poly $C_{12}U$ (Ampligen®).
- Endogenous human immunostimulators, e.g., hGM-CSF or hIL-12 (cytokines that can be administered either as protein or plasmid encoded), Immudaptin (C3d tandem array).
- Inert vehicles, e.g., gold particles.
- Inert polysaccharides, e.g., Advax (delta-inulin), derived from plants (dahlias).

- Combination adjuvants or adjuvant systems that usually consist of combinations of vaccine delivery systems and immunostimulatory agents and may result in more effective delivery of the immunostimulatory adjuvant as well as the antigen, e.g., AS01 consisting of liposomes, MPL, and QS-21; AS02 consisting of an oil-in-water emulsion plus MPL and QS-21; AS03 consisting of an oil-in-water emulsion plus alpha-tocopherol; AS04 consisting of MPL and aluminum hydroxide; AS15 consisting of liposomes, MPL, QS-21 and a CpG oligodeoxynucleotide; and GLA-SE consisting of a synthetic acylated monosaccharide in a stable oil in-water emulsion.

Appendix 2

List of tissues (depending on the species) to be collected in a repeated dose toxicity study

adrenal glands
aorta (thoracic)
bone (femur) with articulation
bone (sternum) with bone marrow
bone marrow smears[1]
brain
bronchi (main-stem)
caecum
colon
diaphragm
duodenum
epididymides
eyes
gall bladder
Harderian glands
heart
ileum
injection site(s) (a sample should be taken covering the area of injection)
jejunum
kidneys
lacrymal glands (main body and subconjunctival part)
larynx
liver
lungs
lymph nodes (draining the injection site(s))
lymph nodes (non-draining the injection site(s), e.g., mandibular, mesenteric)
nasal tissue (skull/nasal cavity)
mammary gland
oesophagus
optic nerves
ovaries
oviducts
pancreas
parathyroid glands
Peyer's patches
pituitary gland
prostate
rectum
salivary glands (mandibular, parotid, sublingual)
sciatic nerves
seminal vesicles
skeletal muscle
skin
spinal cord (cervical, thoracic, lumbar)
spleen
stomach
testes
thymus
thyroid glands
tongue
trachea
ureters
urinary bladder
uterus (body + horns + cervix)
vagina
tissues with macroscopic observations

[1] Bone marrow smears should be prepared at the scheduled necropsy for all animals including any moribund animals killed during the study. The smears should be fixed in methanol and then stained by the May-Grunwald-Giemsa method.

Reference

1. WHO guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization. Fifty-fourth report. Geneva: World Health Organization (WHO Technical Report Series, No. 927); 2005. Annex 1. http://www.who.int/entity/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf