GUIDELINE ON ADJUVANTS IN VACCINES

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1. INTRODUCTION

Adjuvants (immune potentiators or immunomodulators) have been used for decades to improve the immune response to vaccine antigens. The incorporation of adjuvants into vaccine formulations is aimed at enhancing, accelerating and prolonging the specific immune response towards the desired response to vaccine antigens. Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunisation, the reduction of the frequency of booster immunisations needed and an improved immune response in elderly and immunocompromised vaccinees. Selectively, adjuvants can be employed to optimise a desired immune response, e.g. with respect to immunoglobulin classes and induction of cytotoxic or helper T lymphocyte responses. In addition, certain adjuvants can be used to promote antibody responses at mucosal surfaces.

Interest in vaccine adjuvants has been growing rapidly for several reasons. Vaccine manufacturers and public health authorities, e.g. WHO, have established ambitious goals for enhancing present vaccines and for developing new ones, and new vaccine candidates have emerged over the past years against infectious, allergic and autoimmune diseases and also for cancer and fertility treatment. In many cases, because of their low immunogenicity these vaccines require adjuvants. New technologies in the fields of analytical biochemistry, macromolecular purification, recombinant technology, and a better understanding of immunological mechanisms and disease pathogenesis have helped to improve the technical basis for adjuvant development and application.

Adjuvants can be classified according to their source (natural, synthetic or endogenous), mechanism of action, or physical or chemical properties. The current most common described adjuvant classes are listed in footnote 2.

Adjuvant activity is a result of multiple factors and an enhanced immune response obtained with one antigen cannot as a rule be extrapolated to another antigen. Individual antigens vary in their physical, biological and immunogenic properties and antigens may have different needs for help from an adjuvant. Adjuvants should be chosen based on the type of immune response desired and should be formulated with the antigen in such a way that the optimal type of response with the minimal side effects, is obtained.

The major means by which adjuvants may exert their activities are: (i) presentation of the antigen, defined by the physical appearance of the antigen in the vaccine; (ii) antigen/adjuvant uptake; (iii) distribution (targeting to specific cells); (iv) immune potentiation/modulation which includes activities that regulate both quantitative and qualitative aspects of the ensuing immune responses; (v) the protection of the antigen from degradation and elimination.

In general, the mode of action of adsorbants and particulate adjuvants involves presentation of the antigen to the immune system, whereas the microbial, synthetic and endogenous adjuvants act by direct stimulation or modulation of the immune system. In addition to their role in the presentation of the antigen to the immune system, the mode of action of emulsions is to promote slow antigen release and protection from rapid elimination. The use of repository adjuvants like mineral salts is accompanied by the formation of an inflammatory focus at the site of injection which may lead to the synthesis of pro-inflammatory cytokines and stimulation of innate immunity important for the initial steps of the immune response.

Quality evaluation of a vaccine/adjuvant formulation therefore covers aspects such as demonstration of the compatibility of the adjuvant(s) with the antigenic component(s) present in the vaccine, proof of an adequate and consistent association of the antigen with the adjuvant, demonstration that no significant de-association takes place in the course of the shelf-life, degree of association throughout the shelf life, effect of the adjuvant on the ability to assay components, biochemical purity and pyrogenicity. As an example of association, adsorption is specific for aluminium hydroxide gels, aluminium phosphate gels, calcium phosphate gels and ISCOMS, whilst ionic interaction occurs with charged dimethyl dioctadecyl ammonium (DDA) micelles. For emulsions or liposomes the mechanism is encapsulation. With saponin derivatives or other extracts, interactions with antigens are lipophilic/hydrophilic or ionic.

Many adjuvants have been developed in the past, but were never accepted for routine vaccination because of safety concerns, e.g. acute toxicity and the possibility of delayed side effects. Therefore, the benefits of an adjuvant in a vaccine must be weighed against the risk of any adverse reaction inherent to it. The current attitude regarding risk-benefit of vaccination favours safety over efficacy when a vaccine is given to a healthy population. However, in high-risk groups, including patients with cancer and AIDS, and for other 'therapeutic vaccines', an increased level of toxicity may be acceptable if the benefit of the vaccine is substantial. Therefore, non-clinical safety evaluation should be addressed when relevant.

Even if no serious adverse effects are observed in an extensive non-clinical toxicological and safety study, it cannot be guaranteed that the new vaccine/adjuvant formulation presents no risks to vaccinees and unexpected events may occur. Unpredictability of adjuvant effects in humans results from a complex interplay between such factors as route of administration, antigen dose and the nature of the antigen. For this reason, a final safety evaluation of the newly developed vaccine formulation can only be conducted on the basis of clinical trials.

2. SCOPE

This Guideline addresses the quality, non-clinical and clinical issues arising from the use of new or established adjuvants in vaccines. The applicability of this guideline to established adjuvants (i.e. aluminium hydroxide and aluminium or calcium phosphate) will vary on a case-by-case basis.

2.1. VACCINES

The vaccines[1] covered by this document are those that provide immunity against infectious disease.

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[1] The vaccines may contain one or more of the following:

- organisms inactivated by chemical or physical means whilst retaining adequate immunogenic properties;

Antigens may be in their native state, truncated or modified following introduction of mutations, detoxified by chemical or physical means and/or aggregated, polymerised or conjugated to a carrier (see also Ph.Eur. 04/2005:0153). So far, adjuvants have not been used in live vaccines for human use but this cannot be excluded in the future.

The principles of this guideline should also be applicable to quality and non-clinical aspects of 'therapeutic vaccines' (e.g. 'anti-idiotypic vaccines' such as monoclonal antibodies used as immunogens, 'tumour vaccines', allergen specific immunotherapy and vaccines used to treat infected persons); however, clinical aspects of 'therapeutic vaccines' are not within the scope of this document.

2.2. ADJUVANTS

A vaccine adjuvant[2] is a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses.

An active ingredient of a combined vaccine that has an adjuvant effect on other active ingredients of the vaccine is excluded from the scope of this Guideline. Also excluded are carriers for haptens, antigens (e.g., CRM_{197}, meningococcal OMP, tetanus toxoid and diphtheria toxoid that are used to conjugate polysaccharides) and excipients such as HSA.

More than one adjuvant may be present in the final vaccine product. They may be combined together with a single antigen or all antigens present in the vaccine, or each adjuvant may be combined with one particular antigen. Whatever the case, the guidance contained within this Guideline is applicable to each adjuvant and each antigen-adjuvant combination, as appropriate.

- living organisms that are naturally avirulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties;
- antigens extracted from or secreted by the infectious agent;
- antigens produced by recombinant DNA technology;
- a live, recombinant vector producing antigens *in vivo* in the vaccinated host
- plasmid DNA
- antigens produced by chemical synthesis *in vitro*.

The term 'vaccines' is used as defined by Ph. Eur. Other vaccines are qualified by terms such as 'therapeutic vaccines'

[2] These adjuvants include for instance:

- Mineral salts, e.g., aluminium hydroxide and aluminium or calcium phosphate gels.
- Oil emulsions and surfactant based formulations, e.g., MF59 (microfluidised detergent stabilised oil-in-water emulsion), QS21 (purified saponin), AS02 [SBAS2] (oil-in-water emulsion + MPL + QS-21), Montanide ISA-51 and ISA-720 (stabilised water-in-oil emulsion).
- Particulate adjuvants, e.g., virosomes (unilamellar liposomal vehicles incorporating influenza haemagglutinin), AS04 ([SBAS4] Al salt with MPL), ISCOMS (structured complex of saponins and lipids), polylactide co-glycolide (PLG).
- 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 oligonucleotides containing immunostimulatory CpG motifs), modified LT and CT (genetically modified bacterial toxins to provide non-toxic adjuvant effects).
- Endogenous human immunomodulators, e.g., hGM-CSF or hIL-12 (cytokines that can be administered either as protein or plasmid encoded), Immudaptin (C3d tandem array)
- Inert vehicles, such as gold particles

Other novel types of adjuvants not listed above may be under development and this guideline applies to these also.

3. QUALITY[3]

The origin and nature of the adjuvants currently being used or developed is highly diverse. For example, aluminium based adjuvants consist of simple inorganic compounds, PLG is a polymeric carbohydrate, virosomes can be derived from disparate viral particles, MDP is derived from bacterial cell walls, saponins are of plant origin, squalene is derived from shark liver and recombinant endogenous immunomodulators are derived from recombinant bacterial, yeast or mammalian cells. Consequently, it is not appropriate to provide a comprehensive list of individual tests that should be performed for any particular adjuvant or adjuvant/antigen combination in this Guideline. This guideline should also be read in conjunction with the Guideline on pharmaceutical and biological aspects of combined vaccines (CPMP/BWP/477/97). The guidance provided below must be applied by adjuvant/vaccine manufacturers as is appropriate for their adjuvant on a case-by-case basis. Relevant Monographs of the Ph.Eur. should be adhered to. For manufacturers of recombinant protein adjuvants it is useful to consult relevant CHMP and ICH guidelines, for instance cell substrates (CPMP/ICH/294/95), viral safety (CPMP/ICH/295/95), rDNA proteins (CPMP/ICH/139/95). Where the adjuvant is a nucleic acid, reference should be made to the CPMP Note for Guidance on the quality, pre-clinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99).

3.1. THE ADJUVANT

3.1.1. Description

The nature or chemical composition of the adjuvant should be described in detail. When more than one adjuvant is used and/or when an adjuvant has more than one component, the function of each adjuvant and/or each component should be described to the extent that it is known.

3.1.2. Manufacture

The manufacture of the adjuvant should be described in detail. Special attention should be given to the source material for the adjuvant especially if this is biological in nature and any special considerations that may apply. Parameters that are critical in conferring the correct physical, biochemical, biological or adsorptive properties of the adjuvant should be defined. Attention should be paid to the use of any material of ruminant origin and if so, compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01) is required.

3.1.3. Characterisation

The results an assessment of a number of parameters used to characterise the adjuvant should be described. Critical parameters should be identified and described. Such parameters are likely to be part of the routine testing of batches of the adjuvant. Other parameters will also be analysed to characterise the adjuvant and some of these may also form part of routine testing. The

[3] The Quality-related data on the adjuvant should be presented in a self-standing section in 3.2.S of the CTD dossier with the same relevant subsections as for the active substance.

parameters which define an adjuvant will depend on the nature of the adjuvant and may include, but will not necessarily be limited to:

- chemical composition (qualitative and quantitative)
- physical characteristics (e.g., visual appearance, density, viscosity, pH, size and size distribution, surface charge)
- biochemical characteristics
- purity (e.g., endotoxin content, bioburden, manufacturing residuals)

3.1.4. **Routine testing**

A list of tests to be applied routinely to the adjuvant should be defined as appropriate for the adjuvant in question and should be based on the parameters used to characterise the adjuvant as detailed above. Specifications should be set.

3.1.5. **Stability**

Relevant physico-chemical and/or biological properties, based on the characteristics of the adjuvant, should be employed in assessing the stability of the adjuvant during storage. Stability-indicating parameters may include structure and antigen adsorption/binding characteristics.

3.2. ADJUVANT/ANTIGEN COMBINATION

3.2.1. **Development and manufacture of the combination**

Combining the antigen with the adjuvant is a crucial aspect of the final adjuvant-antigen combination. The mechanism of association and association efficiency between antigen and adjuvant should be defined and described. Aspects that are critical for the biological properties of the adjuvant-antigen combination (e.g. adsorption, binding characteristics) should be identified and monitored. If more than one adjuvant is to be incorporated, appropriate information for each adjuvant should be supplied and compatibility studies should be performed on the intended combination of adjuvant(s) and antigen(s).

The entire manufacturing process of the adjuvant-antigen combination should be described in detail.

An intermediate bulk may be formed during antigen and adjuvant combination, prior to formulation. In other cases, formulation will take place simultaneously with the adjuvant and antigen combination (final bulk). Alternatively combining the antigen with the adjuvant, formulation and filling (final product) may be a single process.

Any excipient or diluent added to the adjuvant-antigen combination during the preparation of the final bulk (formulation) should not adversely affect the potency of the vaccine or the association of the antigen(s) with the adjuvant(s).

In each case, the characterisation, routine testing and stability testing of the intermediate bulk, final bulk and the final product, where relevant, must be performed as detailed below. The vaccine manufacturer should clearly delineate and justify the tests that are being performed at each stage.

3.2.2. Characterisation

The adjuvant-antigen combination should be characterised as appropriate. This may include the level and consistency of association of the antigen with the adjuvant, the integrity of the antigen in association with the adjuvant, the effect of adjuvant on the ability to assay the antigen and the extent of release of the antigen from the adjuvant (stability). Other parameters may include chemical and physical characteristics (e.g., particle size, viscosity).

3.2.3. Routine testing

Tests for routine verification of the adjuvant-antigen combination should be identified, described and validated. Such tests should be based on the parameters assessed during full characterisation of the adjuvant-antigen combination.

3.2.4. Stability

The long-term stability of the adjuvant-antigen combination should be assessed investigating relevant physical and biochemical properties. The extent of dissociation of antigen from the adjuvant and its integrity may be important parameters.

3.2.5. Multiple antigen/adjuvant combinations

If the final vaccine product comprises an antigen(s) in addition to the antigen present in the adjuvant-vaccine combination, then the effect of the adjuvant on this additional antigen(s) must be assessed using relevant tests for that antigen. Similarly, any effects of an additional antigen(s) on the adjuvant-antigen complex must be assessed.

If the final vaccine product comprises more than one adjuvant-antigen combination, testing appropriate to the nature of the adjuvants (whether identical or not) will be required including any adverse effects occurring between the different adjuvant-antigen combinations.

3.3. Final product

The final vaccine product should be subjected to tests for potency, identity and stability. Relevant requirements from existing CHMP guidelines and Ph. Eur. Monographs should be adhered to.

Specific considerations for testing and stability studies should be defined and validated.

Interference by an adjuvant(s) on an antigen(s) may have an impact on the performance of certain standard tests at the level of the final product or formulated final bulk. Whereas alternative methods should be investigated, it may be necessary to extrapolate from tests performed at earlier stages of the production where interference is absent.

4. NON-CLINICAL

4.1. PROOF OF CONCEPT

There are major areas in which adjuvants may exert their activities:

- Physical presentation of the antigen in the vaccine

- Optimisation of antigen uptake

- Targeting to specific cells (dendritic cells, Langerhans cells, macrophages, and others), e.g. stimulating Toll-like receptors by LipidA analogues or by oligodeoxynucleotides (ODNs) with CpG motives.

- Immune potentiation and modulation, e.g. through intracellular transport and processing of antigens, association with MHC class I or II molecules and the expansion of T cells, with different profiles of cytokine production.

The rationale for the proposed effects of an adjuvant should be given. The increased immunological response to the adjuvant/antigen combination should be shown in a relevant animal model. It should be considered whether the adjuvant triggers the cells of the innate immune system. Furthermore, it should be shown to what extent the humoral and cellular immune response is activated by the adjuvant given together with the antigen. Data from combinations with other antigens could be used as supportive evidence for understanding the mechanism of action of the adjuvant. Ideally the relevant animal model should demonstrate protection against a lethal challenge of the pathogenic organism (infectious disease). If such a model is not present an animal species in which an immunological response can be induced that resembles the expected human immune response in character should be chosen. Public literature could be used as supportive information for the proof of concept.

4.2. PHARMACOKINETICS

Pharmacokinetic studies (e.g. determining serum concentrations of antigens) are not required. (see CPMP/SWP/465/95 Note for Guidance on preclinical pharmacological and toxicological testing of vaccines; WHO Guideline on non-clinical testing of vaccines). In some cases, distribution studies may be of value in understanding the mode of action of the adjuvant.

4.3. TOXICITY OF ADJUVANT ALONE

The methodology used to study the toxicity of adjuvants should follow the pattern of use of the vaccine. Adjuvanted vaccines might be administered repeatedly at intervals of a few weeks, up to several years. Generally, adjuvants will consist of a small amount of material, which will be given only a few times in a lifetime.

The adjuvant should be tested alone taking into consideration its use as an adjuvant in vaccines and the testing strategy should reflect this use.

Adjuvants can be used in relation with different antigens and are often not very species-specific. They should be tested in two species unless otherwise justified (rodent and non-rodent)

Adjuvants belonging to different biological classes might exert a high level of species specificity (e.g. some cytokines), that makes this discussion only theoretical as testing in more than one animal species does not make sense. However, other adjuvants (e.g. oil emulsions) exert less species specificity and based on toxicological principles testing in at least two appropriate species is the default option. The evidence found in the second species does support the evidence in the first one.

The choice of species depends primarily on the choice of antigen the adjuvant is intended to be combined with. Ideally the selected species should be the same as in which the proof-of-concept has been studied.

4.3.1. **Local tolerance**

The local irritation induced by an adjuvant should be studied depending on the route of administration. For example:

- For oral and intranasal administration local and regional tolerance need to assessed.

- For injectable vaccines, special consideration should be given to the possibility of induction of later granulomatous reactions as seen for example when using particles and also some mineral oils.

4.3.2. **Induction of hypersensitivity and anaphylaxis**

Adjuvants themselves might be immunogenic and testing should be considered with respect to the induction of hypersensitivity in appropriate models (e.g. passive cutaneous anaphylaxis assay [PCA], and the active systemic anaphylaxis assay [ASA]). An adjuvant-induced increase of IgE against the antigen should be considered as a possible concern for induction of hypersensitivity and anaphylaxis.

4.3.3. **Pyrogenicity**

Adjuvants should be tested with respect to their possible pyrogenic effects. Alternative *in vitro* tests for fever-inducing substances are under development and should be used if validated.

4.3.4. **Systemic toxicity**

Adjuvants of various classes may be distributed systemically and may induce toxicity in various organs. Protocols should be designed to establish dose-relationships and include repeated administration at intervals reflecting the proposed clinical use. Full necropsy and tissue collection should be conducted. Histopathology should always include

- pivotal organs: heart, lung, brain, liver, kidney, reproductive organs etc.

- skin (if the site of administration),
- primary and secondary immune organs: spleen, thymus, bone marrow, lymph nodes (local and distant to the application site)

Full tissue examination is recommended in case of novel adjuvants with no prior nonclinical and clinical experience.

Toxicity would mainly result from the immunostimulating effect of the adjuvant, but direct toxicity on non-targeted organs cannot be excluded. The range of doses may remain relatively low reflecting its clinical use rather than reaching necessarily a maximum tolerated dose. With respect to the endpoints, refer to the Note for Guidance on Repeated Dose Toxicity.

4.3.5. **Reproduction toxicity**

As vaccination programs may include women of childbearing potential, it is of importance to consider the need for reproduction toxicity studies. Furthermore, vaccines might be intended to be given during pregnancy in order to prevent infectious disease in the young infant through passive immunization. Reproduction toxicity studies with adjuvant intended to be used in this type of vaccines should be performed. The protocol should reflect the intended schedule of administration. As the immunological response to the booster might be different from the first response, it should also be considered to give the first dose before mating, while giving the booster during the pregnancy.

4.3.6. **Genotoxicity**

Adjuvants might be derived from biological as well as from synthetic origin. In line with requirements published for biotechnological products (ICH S6) genotoxicity studies for biologically derived adjuvants might not be regarded as relevant. For synthetic adjuvants the standard battery (ICH S2B) can be seen as the default position and any deviations should be scientifically justified.

4.3.7. **Carcinogenicity**

As adjuvants are intended to be used only a few times with low dosages the risk of induction of tumours by these compounds in a direct way is negligable. Furthermore, the action of the adjuvant is to stimulate the immune system, and not to act as a general immunosuppressant, reducing the risk on the spontaneous formation of lymphoid tumours. Therefore, carcinogenicity studies are not needed.

4.3.8. **Combination of adjuvants**

Administration of substances with immunomodulatory properties, along with adjuvants improving the presentation of the antigen may further increase adjuvant activity. An appropriate set of toxicity studies should be provided to support its safety of the combination in addition of data on each individual component. Toxicity studies with the separate constituents might be seen as pilot studies. A study with the final combination should be done under GLP.

4.4. TOXICITY OF ADJUVANT IN COMBINATION WITH THE PROPOSED ANTIGEN

The pre-clinical safety aspects of the combination of adjuvant with the proposed antigen should be considered in line with the existing Note for Guidance Preclinical Pharmacological and Toxicological testing of vaccines CPMP/SWP/465/95. Specific attention should be given to:

4.4.1. **Local tolerance**

Injection of antigens in combination with adjuvants might induce more severe local reactions than after administration of the adjuvant alone. The optimal dose-ratio of adjuvant and antigen with respect to benefit and risk should be explored.

4.4.2. **Repeated dose toxicity studies**

A dosing schedule should be used in accordance with the proposed clinical schedule. In order to ascertain the safety of the repeated schedule (where an increase in the severity of the immune response might occur) the number of administrations should be higher than the number planned for human administration.

4.4.3. **Characterization of the immune response**

As a minimal requirement the following non-clinical immunogenicity data are expected:

- Dose-response studies investigating the effect of different doses of adjuvant combined with different doses of vaccine antigen.

- Comparative studies to assess the effect of a new adjuvant with reference to a vaccine antigen alone or adjuvanted with a well-established adjuvant

The nature and extent of an immune response (humoral and cellular) determines the efficacy of a vaccine. The type of an immune response against the same vaccine antigen might be different in animals and in man. Thus, these data should be extrapolated only very carefully. On the other hand a proof-of-concept needs to be provided from non-clinical investigations before clinical trials can be started.

If feasible, further studies in relevant animal models should focus on the more detailed investigation of the immunological mode of action of the new adjuvant (see Proof of Concept, § 4.1).

If a combination of adjuvants is proposed, the rationale for this choice should be provided based on experimental data.

5. CLINICAL

5.1. INTRODUCTION

The inclusion of an adjuvant in a vaccine must always be justified. There must be evidence to demonstrate that the benefit in terms of improvement of the immune response has been achieved without an undue increase in local and systemic adverse reactions.

It is critical that the clinical data demonstrate that the amount of adjuvant used in the vaccine is appropriate to enhance the immune response to the antigen(s), to further direct the immune response towards the intended effect, or to improve the safety profile. In a combination vaccine, the adjuvant should improve the response to at least one of the relevant antigen(s) without exerting a clinically significant detrimental effect on immune responses to any other antigen in the vaccine. Any increase in the rates and/or severity of adverse reactions as a consequence of the presence of an adjuvant in a vaccine is of concern. Therefore, the risk associated with the adjuvant must be outweighed by the potential benefit conferred by enhancement of the immune response.

This section addresses:

- The clinical assessment of a novel adjuvant when it is to be incorporated either into a novel (*i.e.* as yet unlicensed) or licensed prophylactic vaccine and

- The clinical data that would be required to support any change (removal, addition and/or replacement) in the adjuvant content of a licensed vaccine.

The general principles covered in this section are applicable to both single antigen and combination vaccines and to any of the possible routes of vaccine administration. There are special considerations for the characterisation of the immune response as part of the assessment of safety and efficacy. The various scenarios to be considered include the following (note that the term *established adjuvant* refers to any such compound that is already included in at least one licensed vaccine to enhance the immunogenicity of one or more antigens):

1. Novel vaccine

- Inclusion of one or more novel or established adjuvant(s) in a novel vaccine in order to enhance the immune response to one or more antigens or further direct the immune response towards the intended effect.

2. Changes to an already licensed vaccine

Changes to already licensed vaccines may be made to enhance or modulate the immune response and/or to improve the safety profile. In special circumstances (e.g. pandemic influenza vaccine) inclusion of an adjuvant may be used in order to reduce the amount of antigen needed. Changes may include:

- Addition of one or more novel or established adjuvant(s).

- Increase in the amount of an adjuvant.

- Decrease or removal of one or more adjuvant(s) (without replacement).

- Replacement of one or more adjuvant(s) with one or more novel or established adjuvant(s).

5.2. PRELIMINARY STUDIES

Whether the adjuvant is a novel or an established compound, the preliminary studies should establish the effect of the adjuvant on the nature of the immunological responses to the antigen(s) with which it is to be combined. If more than one adjuvant is to be used in a vaccine, then the studies should evaluate the effect(s) of the combination of adjuvants on responses to the antigens. In addition, for a vaccine intended to contain more than one adjuvant/antigen combination, the action of each adjuvant on its intended antigen should be documented.

5.2.1. **Effect of the adjuvant on the immunological response**

In general, the characterisation of the immune response should involve the administration of each antigen that is anticipated in the final product alone and with the adjuvant(s). In the development of combination vaccines, it may be sufficient to compare the combination without adjuvant with the combination plus each adjuvant. These early studies should also provide important, although limited, data on safety.

It is likely that these studies will be performed mainly in healthy adults and in relatively small numbers of individuals. If the vaccine is wholly or predominantly intended for use in infants or young children or is very likely to be administered to the elderly, subsequent to studies in healthy adults some data should be obtained from these age groups if possible.

The studies should involve a comprehensive assessment of the potential effects of the adjuvant on the immune response to all antigens that are to be included in the final product. In addition, the potential that the adjuvant itself might be immunogenic should be explored. The range of tests that will be appropriate will depend on the nature of the antigen and of the adjuvant and cannot be pre-specified in detail in this Note for Guidance. In addition, it is recognised that advances in the assessment of immunological responses may mean that experimental methodologies are used in describing the effects of the adjuvant.

Whenever possible, the assessment of the humoral immune response should include the detection and titration of functional antibodies (neutralising, opsonophagocytic or bactericidal, antibodies) against an international standard (WHO or equivalent). Immunoglobulin subclass responses should be investigated. Circulatory and/or secretory IgA may be measured if relevant. It may also be appropriate to estimate other properties of the antibody response such as avidity.

Assessment of the cell-mediated component of the immune response is considered important. It is recommended that studies should monitor antigen specific T-cell responses (including Th1, Th2 and T regulator cells, and/or relevant cytokines). The range of tests performed, with an explanation of the rational for each investigation, should be justified in the application dossier.

It would not be envisaged that the adjuvant would have to be administered alone in these studies. If the adjuvant is novel, there should usually be sufficient safety data from the pre-clinical studies to allow for it to be given with antigen(s) from the outset. The same situation should apply to an established adjuvant when it is to be given at a higher dose than usual or by a new route of administration. However, if there is suspicion that an adjuvant might accumulate, consideration could be given to a pharmacokinetic evaluation in humans. If it is considered that the administration of adjuvant alone in clinical studies might be necessary, it may be appropriate to obtain further scientific /regulatory advice from EU Regulators.

5.2.2. **Dose-finding studies**

It is essential that there should be sufficient data to demonstrate that the amounts of adjuvant and antigen that are chosen for further study represent an acceptable balance between immune responses and the risk of adverse effects. In most adjuvant-antigen combinations, the aim will be to use as little as possible of one or both of these so as to achieve the required immune response with the minimum of adverse reactions. A preliminary estimate of the relative amounts of each adjuvant and antigen to be combined should emerge from the investigations, described in 5.2.1,which may overlap with dose-finding studies.

The extent of the dose-finding studies that would be considered necessary will be at least partly influenced by the aim of the final product. For example, if it is proposed to incorporate an established adjuvant at a dose that is already in use in at least one licensed product, then it may be more important to focus on different amounts of antigen. Alternatively, if it is proposed to add the adjuvant to the same dose of an antigen or combination of antigens that is already approved in one or more products, then it may be more important to focus on the amount of adjuvant. However, in the case of a novel adjuvant and/or a novel antigen, alone or in combination, more extensive dose-finding studies would likely be necessary.

Whenever possible, these studies should be performed in the target population for the vaccine. However, this may, on occasion, prove difficult so that a dose may have to be chosen on the basis of studies in a population that may differ from the target population. Also, when the dose-finding studies fail to point to a single antigen-adjuvant dose, more than one product may have to be evaluated in confirmatory trials. In these cases, it is desirable to characterise the immune response to the chosen antigen-adjuvant combinations in at least a subset of subjects who are enrolled into the confirmatory trials.

5.3. CONFIRMATORY TRIALS

5.3.1. **General considerations**

In general, clinical trials should be randomised, double blind, controlled trials. The design of the trials should depend on the characteristics of the antigen /adjuvant formulation. It is anticipated that the majority of trials, especially if a modification is being made to the antigen content of a licensed vaccine, will likely involve only an assessment of immune responses against validated immunological correlates for protection. If there is no validated immunological correlate for protection, but a formal assessment of efficacy is feasible, the provision of immunogenicity data alone should be justified.

These trials should be performed in the final target population. If this spans a wide range of age groups, studies may need to pre-stratify by age group or more than one study may need to be performed. For example, in some instances, the demonstration of enhancement of the immune response to at least one antigen may be reasonably expected to apply in only one or some of the possible age groups.

5.3.2. **Possible scenarios**

5.3.2.1. New vaccines with a new or established adjuvant

The *Note for Guidance on Clinical Evaluation of New Vaccines* (EWP 463/97) applies whenever the product in question meets the definition for a new vaccine[4] as described in the guidance. Therefore, further details will not be discussed herein.

5.3.2.2. Changes to the adjuvant content of a licensed vaccine

At least one confirmatory study is needed to support a change(s). The study design will be determined by the primary objective, as follows:

Changes for efficacy reasons

If the primary objective is to enhance the immune response to one or more antigens, or further direct the immune response towards the intended effect, in general the trial should be designed to demonstrate the superiority of the modified over the existing product. For combination products, superiority should be shown with respect to the immune response to at least one of the antigens and the study should have the secondary aim of demonstrating non-inferiority with respect to responses to any other antigen(s) that may be present. The demonstration of non-inferiority with respect to other antigens is relevant only if superiority is demonstrated with respect to the designated primary efficacy variable(s). The definition of what constitutes superiority and non-inferiority of immune responses must be justified according to the antigen(s) in question.

If an adjuvant is to be given at a higher dose than used previously and/or via a different route of administration a specific safety study may need to be considered Whether or not such a study should be wholly performed before first licensure of the modified product should be discussed with EU Regulators before submitting an application for a marketing authorisation.

Changes for safety reasons

If the primary objective is driven by (a) safety reason(s), the clinical program should aim to show non-inferiority of the amended vaccine with respect to the existing vaccine in terms of immune responses to each antigen. The definition of what constitutes non-inferiority must be

[4] New vaccines are those containing antigens not yet described in the Ph.Eur. monographs or WHO requirements, or using a new conjugate for a known antigen, or any new combination of known and/or new vaccines.

justified according to the antigen(s) in question. The safety data from this study would be expected to show an improvement in the safety profile.

The safety data should allow for estimation of, with a reasonable degree of precision, the likely rates of reactions that may be expected based on the known properties of the adjuvant(s) and antigen(s). In some cases, it may be appropriate that the data focus on immune mediated reactions. In all instances, the risk-benefit relationship for the modified product should be at least as favourable as for the existing product.

A post-marketing surveillance program should be considered whenever there has been a change in the adjuvant content of an already licensed vaccine.

5.3.3. **Statistical considerations**

The hypotheses to be tested and the statistical methods to analyse them have to be clearly stated in the trial protocol. The sample size should ensure that the trial has sufficient power to answer its scientific question. For any test on non-inferiority, the non-inferiority margin has to be defined and justified in advance. In planning the analysis and sample size of the clinical trial possible multiplicity issues have to be accounted for appropriately. For further details please refer to the relevant methodological guidelines[5].

[5] e.g. ICH Note for Guidance on Statistical Principles for Clinical Trials (ICH topic E9)
Points to Consider on Multiplicity Issues in Clinical Trials (CPMP/EWP/908/99)

資料（4）

ワクチンフォーラム2010
「日本発のワクチン開発をめざしてⅣ」

（平成22年9月14日）

【講演スライド】

① 感染症予防ワクチンの非臨床試験ガイドラインについて
国立感染症研究所　血液・安全性研究部長　濱口　功

② 感染症予防ワクチンの臨床試験ガイドラインについて
（独）国立病院機構本部総合研究センター臨床研究統括部長　伊藤 澄信

《アジュバントワークショップ》

③ アジュバント開発研究とその審査行政の現状と未来
（独）医薬基盤研究所アジュバント開発プロジェクトリーダー　石井　健

④ Cタイプレクチンを介する結核菌アジュバント作用機序
九州大学生体防御医学研究所分子免疫学分野教授　山崎　晶

⑤ アラムアジュバントをふくむ粒子状物質の新規免疫学的メカニズム
産業医科大学医学部免疫学寄生虫学教室講師　黒田 悦史

⑥ α-Galcer アジュバントの免疫制御メカニズムと臨床応用
（独）理化学研究所免疫・アレルギー科学総合研究センターワクチンデザイン研究チームリーダー　石井 保之

⑦ 粘膜アジュバントとDDS研究の新展開
東京大学医科学研究所炎症免疫学分野教授　清野　宏

⑧ 核酸アジュバントによる樹状細胞活性化の分子メカニズム
（独）理化学研究所免疫・アレルギー科学総合研究センター生体防御研究チームリーダー　改正 恒康

ワクチンフォーラム2010

感染予防ワクチンの非臨床試験ガイドラインについて

浜口　功

国立感染症研究所

本日の内容

- ワクチン開発、導入の状況と問題点
- 欧米におけるワクチン開発、安全性システム
- 非臨床試験ガイドランの内容

日米のワクチン導入の状況

	日本	米国
1985	B型肝炎米国は1982導入	
1987 1988	水痘生 肺炎球菌米国は1977導入 遺伝子組換えB型肝炎 MMR米国は1971導入	Hib、不活化ポリオ(IPV)
1991		aP(無細胞百日咳、日本は1981導入)
1992		DTaP、日本脳炎(日本は1976導入)、DTaP-Hib
1994		ペスト
1995	不活化A型肝炎	水痘生(日本から技術導入)
1996		Hib-B型肝炎、不活化A型肝炎
2000		7価肺炎球菌
2001		A型-B型肝炎
2002 2003 2005	MR	DTaP-IPV-B型肝炎 経鼻インフル生、DPT成人用 MMR-水痘、髄膜炎菌
2006		ロタウイルス、HPV
2007	Hib、沈降新型インフル(H5N1)	プレパンデミックインフル
2008		DTaP-IPV-Hib、DTaP-IPV
2009	7価肺炎球菌、HPV	

(ワクチン産業化ビジョン より)

日本における感染症予防ワクチン開発の問題点

国内での開発状況は決して活発でなく、国産の新しいワクチンがなかなか市場に出てこない。

多くの国でもちいられている海外で開発されたワクチンが未承認で使用出来ない状況「ワクチンギャップ」の存在。