The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 25 March 2004
EMEA/CPMP/VEG/17/03/2004v 5/Consultation

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

GUIDELINE ON ADJUVANTS IN VACCINES

DISCUSSION IN THE VEG, BWP, SWP and EWP	January 2003 - March 2004
TRANSMISSION TO CPMP	March 2004
RELEASE FOR CONSULTATION	March 2004
DEADLINE FOR COMMENTS	September 2004
DISCUSSION IN THE WORKING PARTIES	
TRANSMISSION TO CPMP	
ADOPTION BY CPMP	
DATE FOR COMING INTO OPERATION	

Note:

Further to the recommendation in the Concept paper on the development of a CPMP Note for Guidance on requirements for the evaluation of new adjuvants in vaccines (CPMP/BWP/6622/02), this Guideline is published for consultation.

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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 to vaccine antigens. Advantages of adjuvants include the enhancement of the immunogenicity of weaker antigens, 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 also 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.

Despite proposals to introduce numerous new adjuvants and despite the fact that there are a number of adjuvants in phase 1, 2 or 3 human trials, essentially only aluminium hydroxide and aluminium or calcium phosphate have been used routinely in human vaccines. More recently, antigens incorporated into IRIV's (immunostimulating reconstituted influenza virosomes) and vaccines containing the emulsion-based adjuvant MF59 have been licensed in most EU countries. In large scale clinical trials of vaccines, newly developed adjuvants such as monophosphoryl lipid A derivatives, purified endotoxin obtained from Salmonella strains and cell wall skeleton isolated from Mycobacteria strains have been investigated.

There are several adjuvants licensed for veterinary vaccines, such as mineral oil emulsions that are too reactogenic for human use. Similarly, complete Freund's adjuvant, although being one of the most powerful adjuvants known, is not suitable for human use.

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 will 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, mechanism of action and physical or chemical properties. The most commonly described adjuvant classes are gel-type, microbial, oil-emulsion and emulsifier-based, particulate, synthetic and cytokines.

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 both are optimally distributed and presented to the relevant lymphatic tissues.

The major ways 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.

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 adsorption of antigenic components present in a vaccine, demonstration that no significant desorption takes place in the course of the shelf-life period, degree of adsorption throughout the shelf life, effect of the adjuvant on the ability to assay components, biochemical purity and pyrogenicity.

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 aspects should be covered, if relevant, in a safety evaluation.

Even when 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 for the human population to be vaccinated and unexpected events may occur. The 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.

2.1. VACCINES

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

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

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

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. 2001:0153). So far, adjuvants have not been used in live vaccines but this cannot be excluded in the future.

In principle, the 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, allergy vaccines and vaccines used to treat infected persons); however, clinical aspects of therapeutic vaccines are not considered in this document.

2.2. ADJUVANTS

A vaccine adjuvant[2] is a component that increases specific immune responses to an antigen.

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.

In general, the mode of action of adsorbants and particulate adjuvants involves presentation of the antigen to the immune system, whereas the microbial, emulsion, synthetic and endogenous immunomodulator adjuvants act by direct stimulation or modulation of the immune system.

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.

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, e.g. the endogenous immunomodulators, relevant CPMP and ICH guidelines should be applied. 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. The function of each component of the adjuvant 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 parameters, which define an adjuvant will depend on the nature of the adjuvant and may include, but will not necessarily be limited to:

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[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.

- chemical composition (qualitative and quantitative)
- physical characteristics (e.g., visual appearance, density, viscosity, pH, size and size distribution, surface charge)
- biochemical characteristics (e.g., adsorption, binding or coupling of an antigen)
- 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/coupling 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 or coupling 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.

3.2.2. **Characterisation**

The adjuvant-antigen combination should be characterised as appropriate. This will include the level and consistency of adsorption of the antigen by the adjuvant, the integrity of the antigen after adsorption, the effect of adjuvant on the ability to assay the antigen and the extent of release or desorption 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 will be important parameters.

3.2.5. **Formulation**

It should be demonstrated that any excipient or diluent added to the adjuvant-antigen combination during the preparation of the final bulk does not adversely affect the potency of the vaccine or the association of the antigen(s) with the adjuvant(s).

3.3. FINAL PRODUCT

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

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

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. These tests may alternatively be performed on the final bulk.

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. These tests may alternatively be performed on the final bulk.

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).

- Immune potentiation and modulation, including 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 introduced 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 normally not needed. (see CPMP/SWP/465/95 Note for Guidance on preclinical pharmacological and toxicological testing of vaccines). However, if there is a reason, based on the nature of the vaccine, indicating that an adjuvant might be distributed over the body, pharmacokinetic studies with respect to the adjuvant (alone and in combination with the antigen) should be considered.

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 lifetime.

The mode of action of adjuvant should be explored in order to differentiate between stimulating non-specific resistance to infections, and increasing immune responses to specific antigens.

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

Furthermore, as the proposed action is directed to the immunological system, the intended action is to induce long-lasting changes in the immune system by influencing the sensitivity to defined or unknown antigens (e.g. by changing the Th1/Th2 balance).

In general adjuvants should be tested in two species unless justified.

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 e.g. inflammation**

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

- For oral administration, the effects on the intestinal mucosa should be studied.

- If the intended route is intranasal administration the choice of species to test local tolerance is important.

- 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 (passive cutaneous anaphylaxis assay [PCA], and the active systemic anaphylaxis assay [ASA]). Demonstration of drug-specific IgE should be taken as an indication of hazard.

4.3.3. **Pyrogenicity**

Adjuvants should be tested with respect to their possible pyrogenic effects. Tests should be performed in a sensitive animal model e.g. intravenous administration in rabbits. Alternative *in vitro* tests for fever-inducing substances are under development and might 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 histopathology is required with the emphasis on primary and secondary immune organs. This toxicity would be mainly indirect as the consequence of the immunological response to the adjuvant. 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 child-bearing potential, who may become pregnant shortly after the time of vaccination, 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. Local, i.e. intramuscular application of body-incompatible material, such as metal salts, might be seen as factors at risk to induce local tumours. The applicant should make sure that adjuvants meant to control the release of antigen should be devoid of this type of properties.

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.

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 e.g. inflammation

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 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.

- Those studies to be conducted by using the vaccine antigen alone or in combination with a well-established adjuvant to show the synergistic effect of the 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

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):

- 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.

- Removal of one or more adjuvant(s) from a licensed vaccine (without replacement) in order to improve the safety profile.

- Replacement of one or more adjuvant(s) in a licensed vaccine with one or more novel or established adjuvant(s). This may be done primarily for enhancement of the immune response or for safety reasons, or may be prompted by both factors.

- Addition of one or more novel or established adjuvant(s) to a licensed vaccine in order to enhance the immune response.

- Increase or decrease in the amount of an adjuvant in a licensed vaccine.

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.

Thus, it is critical that the clinical data should demonstrate that the amount of each adjuvant used in each vaccine is sufficient to improve the immune response (whether this is in terms of total response and/or rapidity of onset of protection) to one or more antigen(s). For a combination vaccine, the adjuvant should enhance the response to at least one of the antigens without exerting a potentially clinically significant detrimental effect on immune responses to any other antigen that may be present. 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.

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 single product, then the studies should evaluate the effects of each adjuvant alone and together on responses to the antigen(s) and the optimal amount of adjuvant(s) and antigen(s) for further study should be identified.

5.2.1. Effect of the Adjuvant on the Immunological Response

In general, the characterisation of the immunological response should involve the administration of each antigen that is anticipated in the final product alone and with the adjuvant (or each adjuvant). 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, 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 neutralising antibodies against an international standard (WHO or equivalent). Immunoglobulin subclass responses should be investigated. Also 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, 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 man. If it is considered that the administration of adjuvant alone in man might be necessary, the decision to perform such a study needs to be considered on a case-by-case basis and it may be appropriate to obtain scientific 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 above investigations, 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, the data should be supplemented by characterisation of the immune responses to the chosen antigen-adjuvant combination in at least a subset of subjects who are enrolled into the confirmatory trials.

5.3. CONFIRMATORY TRIALS

5.3.1. General considerations

The design of the trials that would be required depends upon the entire antigen-adjuvant content of the vaccine. In particular, with regard to features such as whether it includes one or more novel adjuvant(s) or antigen(s), the amounts of adjuvant or antigen (novel or established) and to what extent the combination of adjuvant(s) and antigen(s) differs from that in any licensed product.

The primary efficacy parameters will depend on the antigen(s) in the vaccine. It is anticipated that the majority of studies, 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 studies 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 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.

[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.

5.3.2.2. Changes to licensed vaccines

At least one confirmatory study would be needed to support the change(s). The study design will be determined by the primary aim of the modification as follows:

Removal of one or more adjuvant(s) from a licensed vaccine (without replacement) or reduction in the adjuvant content in order to improve the safety profile.

The study 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 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.

Addition of one or more novel or established adjuvant(s) to a licensed vaccine or increase in the amount of adjuvant(s) in order to enhance the immune response.

The addition of, or increase in, the content of an adjuvant(s) should be supported by a confirmatory trial in which the primary objective should be to demonstrate the superiority of the modified over the existing product with respect to the immune response to the antigen. 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 definitions of what constitutes superiority and non-inferiority of immune responses must each be justified according to the antigen(s) in question.

Replacement of one or more adjuvant(s) in a licensed vaccine with one or more novel or established adjuvant(s).

This may be done primarily for enhancement of the immune response or for safety reasons, or may be prompted by both factors.

The study design will depend on whether the primary aim is to enhance immunogenicity (in which case as for addition or increase in adjuvant content) or safety (in which case as for removal or reduction in adjuvant content).

Statistical Considerations

In addition to any requirement to demonstrate superiority with respect to at least one antigen, one or more statistical tests for non-inferiority may be necessary. For these tests of non-inferiority, no alpha-adjustment will usually be required because non-inferiority must be demonstrated for each relevant antigen. Whenever more than one statistical test for non-inferiority is conducted, the Type II error will be inflated and the power of the study should be carefully considered.

5.4. SAFETY

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 as described in the guidance. Therefore, further details will not be discussed herein.

In all the scenarios mentioned in 5.3.2.2, the risk-benefit relationship for the modified product should be at least as favourable as for the existing product.

A specific safety study may need to be considered if an adjuvant is to be given at a higher dose than used previously and/or via a different route of administration. 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. In these and all other instances, the safety data should allow for estimation of, with a reasonable degree of precision, the likely rates of those 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.

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