For a combination vaccine consisting of several strains or serotypes, the primary end-point for clinical efficacy should be the prevention of disease caused by the different vaccine-type strains, or the ability of the vaccine to modify the course of such disease.

The study should have sufficient power to enable meaningful separate analyses to be made of the prevalent strains or serotypes identified as being of major significance to public health in the target area. The appropriateness of the coverage provided by the individual vaccine components in the target population should be justified e.g., in the case of multivalent vaccine that does not cover all serotypes of the disease such as pneumococcal conjugate vaccine, epidemiological data should be provided to justify the selection of strains for this vaccine. The feasibility of extrapolation from limited numbers of strains or serotypes to other strains or serotypes should be substantiated.

B.10.2 ***Safety analysis of combination vaccines***

For the safety evaluation of combination vaccines, as much information as possible should be obtained from randomized, controlled trials. Such studies are usually designed and analysed as non-inferiority trials that aim to demonstrate that the safety of the combination is not inferior to that of the individual components. Where applicable, the controls for the study should be the already marketed vaccines with the same antigen composition. The size chosen for the study groups should take into account differences in rates of common and/or clinically important adverse events. For vaccines intended for infants and children, defining differences in rates of high fever may be especially relevant. Blinding is virtually essential for making valid comparisons and for the accurate determination of the rates of events causally related to vaccination. If blinding of a study is not feasible, the methods used to minimize bias should be described.

The safety and efficacy of new formulations in which reduced doses of some or all of the components of a combination vaccine are necessitated by the volume of the combination of components being too large for safe administration must be demonstrated.

Simultaneous administration of vaccines

For monovalent vaccines intended for simultaneous administration with other vaccines to the target population, any clinically relevant interference with the other vaccines should be ruled out. Immunological interference and adverse safety interactions after simultaneous administration should be compared with the results of separate administration of the (new) vaccine component(s) at different times.

Authors

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Appendix

Summary protocol for vaccine evaluations

Title and summary _____

Brief description of the study site(s) _____

Investigators _____

Background and rationale _____

Preclinical and laboratory evaluation of vaccines _____

Summary of product characteristics (details of methods for production and control of candidate vaccine) _____

Primary and secondary objectives _____

Study design _____

— hypothesis _____

— end-points _____

— study plan _____

— trial size _____

— duration of study _____

Study population _____

— inclusion and exclusion criteria _____

Methods and procedures _____

— recruitment of subjects _____

— allocation of subjects _____

— vaccine delivery _____

— follow-up _____

— laboratory methods _____

— statistical plan and analyses _____

Monitoring of the trial _____

— data monitoring _____

— quality assurance of data and laboratory methods _____

Timetable _____

— start and end of recruitment _____

— end of follow-up _____

— date of report _____

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GUIDELINE ON ADJUVANTS IN VACCINES FOR HUMAN USE

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GUIDELINE ON ADJUVANTS IN VACCINES

TABLE OF CONTENTS

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

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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.

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.