Technical Guideline for Preventive Vaccines with Aluminum-containing Adjuvants

I. Preface

The Guideline is formulated in order to guide the research and development of the vaccines which contain the aluminum-containing adjuvants, strengthen the production and quality control of the aluminum-containing adjuvants and vaccines which contain the aluminum-containing adjuvants and further enhance the safety, efficacy and quality controllability of the vaccines.

Adjuvants refer to the substances which can assist the antigen response and mediate the immune responses and types. The effects of adjuvants include enhancing the immunogenicity of the antigen in the products, changing the nature of the immune response, reducing the amount of antigen and immunization times needs for successful immunization and enhancing the immune response in the inoculating population with immunodeficiency and so on [1, 2].

Aluminum-containing adjuvants are the most widely used adjuvants for human vaccines till now and acceptable safety and efficacy have been revealed in the marketed vaccines. The aluminum-containing adjuvants used for vaccines include the common aluminum hydroxide adjuvants and aluminum phosphate adjuvants. In addition,

the mixed system of the above aluminum-containing adjuvants and the adjuvant system composed of the aluminum-containing adjuvants and other adjuvant components such as AS04 adjuvant system are also included. The mechanism of action of the aluminum-containing adjuvants has not been completely determined and the available study results reflect that the potential modes of action mainly include[3]: store the antigens, enhance the innate immune response of the body, enhance the antigen presentation, enhance the Th2 cell-mediated adaptive immune response, activate the production of the B cell-induced antibody and activate the effect of the complements.

The vaccines with aluminum-containing adjuvants are a kind of relatively complex preparation system and its research and development and production control have many particularities. The necessity for addition of adjuvants in vaccines must be strictly demonstrated[2]. If adjuvants should be added, ensure that the added adjuvants will not cause unacceptable toxicity [2, 4,5] and the potential benefits for enhancing immune response caused by use of adjuvants must exceed the risks caused by it[2].

The safety limit criteria have been specified for the aluminum adjuvants in the form of international regulations or general requirements, and the limits are all calculated with aluminum ion content as the unit. The limit specified by World Health

Organization and Europe is not more than 1.25 mg/dose [6] and the limit specified by USA is not more than 0.85 mg/dose[7]. The limit is usually 0.3-0.5 mg/dose for most of the adjuvants of the currently marketed vaccines, and the limit of 0.85 mg/dose adopted for some vaccines is the highest dose for the aluminum adjuvant used for the marketed vaccines. In the monographs in Chinese Pharmacopoeia[8], the content of aluminum adjuvant is calculated on the content of aluminum hydroxide, i.e., 0.35 - 3.0 mg/ml for many vaccines, and this criterion has been proposed according to the range of the content of the aluminum adjutants of the marketed vaccines. If aluminum-containing adjuvants are needed for the vaccines, the international universal upper limit requirements for aluminum-containing adjuvants and the use experience in large scale population of the marketed vaccines (the limit not more than 0.5 mg/dose is usually selected) should be referred. The above candidate reference dose is only a recommended reference range, and the limits for the specific vaccines and the applicable optimal dose should also be determined by the screening conducted by necessary preclinical studies and clinical studies so as to achieve the minimum dose which can archive the intended purpose. The use information of the previous aluminum-containing adjuvants of the same type can be used as the basis for addition of the aluminum-containing adjuvants.

The special considerations involved in quality evaluation of the

aluminum adjuvant-containing Lead vaccines include the interactions and compatibility between the adjuvants and the antigen components of the vaccines, influences of the adjuvants on detection of the antigen component and stability during the whole shelf life.

Necessary preclinical studies and clinical studies must be conducted for all the vaccines with aluminum-containing adjuvants. Comprehensive and systematic study should usually be conducted first for the type of the aluminum-containing adjuvants or aluminum-containing adjuvant system, doses of the adjuvant and antigen and safety and efficacy of vaccines (including the combination of the aluminum-containing adjuvants in combined with other adjuvants) at the nonclinical study stage. Preliminarily determine the formulation of the preparations proposed to be used for the clinical trial. If reliable animal models or immune indexes for prediction of the immune effect are lack of, completion of related exploratory study in the early clinical trials should be considered. For development of the combined vaccines, sufficient evidences for presence or absence of aluminum-containing adjuvants should be provided for each kind of antigen component of the combined vaccines. The available study data of the similar products can provide important guiding information for the non-clinical and clinical studies [2,4,5].

II. Scope of Application

The Guideline proposes the technical requirements for the etc aspects such as pharmaceutical, preclinical studies , clinical studies and the post-marketing production quality control related to the vaccines with aluminum-containing adjuvants according to the study experiences and results of the common domestic and foreign aluminum-containing adjuvants and adjuvant-containing vaccines as well as the scientific consensus of related technical guidelines. It is applicable to the research and development and post-marketing changes of preventive vaccines for human use containing the aluminum salt of single type and the combination of the aluminum salts of different types, including the monovalent vaccines, combined vaccines, etc. The marketed vaccines with aluminum-containing adjuvants should also refer to the Guideline, i.e., the manufacturer of the marketed vaccines with aluminum-containing adjuvants should refer to this Guideline to strength the study and monitoring of the manufacturing process and vaccines with adjuvants, continuously improve and enhance the quality control level of the vaccines with aluminum-containing adjuvants, carry out the quality study and stability study of the adjuvants and enhance the quality level of the vaccine.

For the adjuvant systems formed by the aluminum-containing adjuvants and other adjuvant components (e.g., CpG, MPL, etc.) [9], corresponding studies should be conducted according to the product

characteristics and the nature of the specific adjuvants.

The research and development of the aluminum-containing adjuvants and vaccines which contain the aluminum-containing adjuvants usually should meet the current general requirements in the pharmacopoeia and requirements of the related guidelines. For the innovative vaccines or modified vaccines, the type and using quantity of aluminum-containing adjuvants should not simply understand and mechanically apply the monographs of *Chinese Pharmacopoeia*, and should be finally determined through sufficient study and risk-benefit assessment.

The production of the aluminum-containing adjuvants and vaccines which contain the aluminum-containing adjuvants should comply with the related requirements of the current GMP.

III. Pharmaceutical Studies

The current aluminum-containing adjuvants used in the vaccines include two sources such as purchasing and homemade by the vaccine enterprises. The corresponding parts of the Guideline should be Reference in the study process of the adjuvants and vaccines for the adjuvants of both sources and comprehensive study and analysis should be conducted for the adjuvants, adjuvant-antigen conjugates, finished products of vaccines in the aspects of raw materials, preparation process, quality control, efficacy and safety.

(I) Aluminum-containing Adjuvants

1. Description of the Aluminum-containing Adjuvants

The characteristics and chemical composition of the aluminum-containing adjuvants should be described in detail. The molecular formula/chemical formula of the adjuvants should be provided where appropriate. When one or many kinds of adjuvants are used and/or the adjuvant contains more than one kind of components, the functions of each kind of adjuvant and/or each kind of the component of the adjuvant should be described based on the available product knowledge[2].

The aluminum-containing adjuvants of different types are significant different in the aspects of structure morphology, antigen absorption selectivity and in vivo metabolism and elimination time. Meanwhile, the quality attributes of the aluminum-containing adjuvants highly depend on the manufacturing process and the quality attributes of the aluminum-containing adjuvants prepared by different processes are different in the aspects of particle size and distribution, isoelectric point, etc. Related basic information of the aluminum-containing adjuvants should be provided based on the adequate study results.

2. Source and Preparation

(1) Raw Materials for Production and Quality Control

The raw materials used for preparation of the aluminum-containing adjuvants are inorganic salts and should comply with the related

requirements for *Chinese Pharmacopoeia* "Requirements for Quality Control of Raw Materials and Excipients of Biological Products" in principle.

(2) Preparation and Manufacturing Process

The detailed information of the manufacturing process of the aluminum-containing adjuvants should be provided, including but not limited to the related data of the process study, in-process control and process validation data.

Strict quality attribute design and process parameter design are important premises for process development. The process development of the aluminum-containing adjuvants can refer to the international general requirements such as ICH Q8[10], etc. Determine the process route and process parameters based on the candidate critical quality attributes of the aluminum-containing adjuvants and/or vaccines and determine the process control strategy.

The common preparation methods of the aluminum hydroxide adjuvants include sodium hydroxide method and ammonia method. The common preparation method of the aluminum phosphate adjuvants is the reaction of the aluminum salt/phosphate mixed solution and sodium hydroxide solution or reaction of aluminum salt and phosphate. The preparation of the aluminum-containing adjuvants may include the steps such as precipitation reaction of the

aluminum salts, reaction termination, precipitate washing, concentration adjustment of the aluminum-containing adjuvants, aseptic processing, etc. [11] The study of the influences of the process parameters of each step on the quality attributes of the aluminum-containing adjuvants should be conducted at the process development stage.

The process parameters to be studied in the study of the aluminum salt production, for example, reaction temperature, pH, mixing/addition speed and method of the materials, stirring speed or air flow in the reaction, reaction time, volume of the reaction tank, yield of each batch, etc.; the process parameters to be studied in the study of the reaction endpoints include: pH of the reaction endpoint or other indexes, etc.; the process parameters to be studied for aluminum adjuvant precipitation and washing, for example, selection of the for precipitate washing solution, proportion of the precipitate and washing solution, washing times, etc.; the process parameters to be studied for concentration adjustment of the aluminum-containing adjuvants, for example, selection of the diluent (e.g., consider the compatibility with the finished products, etc.) and target concentration of the aluminum-containing adjuvants. In terms of the aseptic processing, attention should be paid to the influences of the sterilization conditions and sterilization times on quality attributes of the adjuvants if sterilization process is adopted.

Comprehensive risk assessment and validation should be conducted for the sterility assurance control of the preparation and use process of the adjuvants if the aseptic process is adopted for preparation. The manufacturing process of the aluminum-containing adjuvants should be determined through study and optimization of the above process parameters at the research and development stage.

Critical process parameters which affect the preparation of the aluminum-containing adjuvants should be made clear before the start of marketing production and sufficient manufacturing process control strategy should be established on this basis, including the control of the critical and main process operation parameters and test of the performance parameters of the intermediate products.

Sufficient process validation should be conducted through the evaluation of the indexes such as critical process parameters of the continuous production batches and critical quality attributes by referring to Chinese and foreign technical guidelines start of production before start of marketing production and prove that the manufacturing process of the aluminum-containing adjuvants is continuous, stable and controllable. Besides the release test items, study items of the important quality attributes of the aluminum-containing adjuvants such as point of zero charge should also be included for the process validation batches. Attention should be paid to the aseptic production validation of the

aluminum-containing adjuvants so as to prove that the proposed aseptic processing process can achieve the expected sterility assurance and will not cause adverse influences on quality attributes of the aluminum-containing adjuvants.

3. Quality Study

(1) Study of the Quality Characteristics

Comprehensive characteristics study should be conducted for the aluminum-containing adjuvants so as to determine the critical quality attributes (CQAs) and use them as the basis to establish the specification at the stages such as manufacturing process development of the aluminum-containing adjuvants and preliminary process verification stage. In addition, suitable testing items should be selected to carry out the comprehensive study of the quality attributes according to the product characteristics and manufacturing process if major pharmaceutical changes occur or the traceability study is conducted for the batches with deviations.

Quality study of the aluminum-containing adjuvants and can include but not limited to 1) chemical components, including the qualitative and quantitative determination; 2) physicochemical properties such as appearance, viscosity, pH, sedimentation rate, particle size and distribution and surface charge; 3) biological and chemical properties (e.g., adsorption ratio, etc.) and 4) purity such as endotoxin content, biological limit and test of the process impurities.

It is generally recognized that the quality characteristic indexes to be characterized for the aluminum hydroxide adjuvants and aluminum phosphate include: chemical composition, aluminum content, pH, isoelectric point, microscopic structure, particle size and distribution, adsorption rate, maximum adsorption capacity for the related antigen, process related impurities, sterility test and so on. Because the characteristics of the aluminum adjuvants of the two types are different, the study of the quality characteristics should also include the sedimentation rate, specific surface area and X-ray diffraction spectrum. The study of the quality characteristics also include the phosphorus/aluminum molar ratio. The comprehensive study of the quality characteristics does not limit to the above study items, and it is encouraged to carry out the study of the quality characteristics of the aluminum-containing adjuvants by the advanced methods.

(2) Specification

The establishment of the specifications of the aluminum-containing adjuvants can refer to the related requirements of ICH Q6A[12] and ICH Q6B[13].

The specifications of aluminum hydroxide adjuvants should comply with the related requirements of *Chinese Pharmacopoeia*. In addition, it is recommended to use isoelectric point, particle size and distribution as the testing items of process characterization and process validation batches and carry out the routine control.

The routine release testing items of the aluminum phosphate adjuvants usually include: appearance, aluminum content, sodium chloride content, pH, sterility test, endotoxin, identification (phosphate, aluminum salt), and consider whether tests of related heavy metals, sulfate, peroxide and arsenic should be included according to the process route and process control situations. It is recommended to use phosphorus/aluminum molar ratio, particle size and distribution and isoelectric point as the testing items of the process characterization and process validation batches and carry out the routine control.

4. Stability study

It is generally recognized that the aluminum-containing adjuvants can be stored in sterile tightly closed containers at 2-8℃ or temperature and cannot be frozen.

The storage time of the aluminum-containing adjuvants may affect the interactions of adjuvant-antigen and therefore the whole process of storage, transportation and use should be sufficiently considered for the stability study conditions of the aluminum-containing adjuvants.

Besides the routine release indexes, the study items of the stability study should include the indexes related to the adjuvant structure and antigen absorption/binding properties, for example, pH, particle size and distribution, antigen adsorption rate and immunological

activity. It is encouraged to introduce the new techniques such as X-ray diffraction spectrum to stability study of the aluminum-containing adjuvants.

The storage temperature, time and shelf life of the mother liquids of the adjuvants and diluents should be established based on the stability study in combination with the delivery/preparation time of the adjuvants, transfer and transportation situations, overall shelf life of the adjuvant-containing vaccines and quality requirements of the enterprise.

5. Special Considerations on the Purchased Aluminum-containing Adjuvants

The manufacturers of the purchased adjuvants should be specified and fixed and comprehensive quality control system should be established. The manufacturers of the vaccines should carry out the external audit of the suppliers of the aluminum-containing adjuvants according to the corresponding audit requirements.

Besides the external audit, the manufacturers of vaccines should also establish the in-house specifications of the aluminum-containing adjuvants according to the quality control items provided by the manufacturers of the adjuvants, related requirements of *Chinese Pharmacopoeia* and the Technical Guideline and batch release test should be conducted.

For the first selection of the adjuvants, change of the supplier and

change of production of the adjuvants (e.g., manufacturing process, production place, etc.), it is suggested to carry out the extended study of the quality characteristics of the aluminum-containing adjuvants by suitable methods based on the routine release test so as to accumulate the cognition of the characteristics of the aluminum adjuvants and ensure that the changes will not cause adverse influences on product quality, for example, the studies such as X-ray diffraction spectrum, determination of point of zero electric charge, and specific surface area[14].

(II) Adjuvant-Antigen Conjugates

1. Formulation Development and Adsorption Process Study of the Preparations

The formulations of the preparations of the vaccines which contain the aluminum-containing adjuvants contain the antigens, adjuvants, buffer solutions/excipients, preservatives, stabilizing agents, etc. and there are interactions between many kinds of antigens, adjuvants and buffer solutions/excipients. Vaccine preparations should be realized by the process of many steps such as preparation of the antigens, preparation of the buffer solutions, preparation of the adjuvants, antigen-adjuvant adsorption, final preparation, etc. The formulation development and adsorption process study of the preparations should be conducted based on the understanding of the related mechanisms, characteristics of the antigen and product requirements.

(1) Formulation Development of the Preparations:

Because of the different characteristics of different antigens, various kinds of mechanism of action of aluminum-containing adjuvants and antigens and significant differences of the microenvironment on the surface of the aluminum-containing adjuvants under different buffer system conditions, the most suitable formulation of the preparation to adsorb the specific antigen to the specific adjuvant should be based on the concrete analysis of concrete problems. The effects and content of each component in the formulation of the preparation should be specified, the selection basis for the adjuvants, buffer solutions, salt concentration, pH and other excipients should be provided, the influences on the adjuvant-antigen interactions, binding efficiency, immunogenicity, vaccine potency and stability should be studied and the binding mechanism of the antigen and adjuvant should be analyzed.

The determination of the antigen content, adjuvant content and antigen/adjuvant proportion usually include two stages such as preclinical and clinical studies. The preclinical studies mainly study the influences of different preparation formulations on the aspects such as adsorption ratio of the finished products, animal immunogenicity, controllability of the manufacturing process, etc. and then the preparation formulation should be further screened and determined through clinical trials.

It should be emphasized that the relationship between the adsorption ratio and immunogenicity is varied with products. The immunogenicity of the adjuvant-adsorbed antigens is affected by various factors: adsorption ratio, adsorption strength, desorption rate after exposure in vivo [15]. Therefore, the range of the adsorption ratio should be determined based on the data of the comprehensive process validation, preclinical/clinical studies and stability.

(2) Adsorption and Preparation Process Studies:

Adjuvant-antigen binding may be either an independent working procedure before preparation of the final bulk (e.g., form the monovalent adsorption bulk first) or concurrently conducted with the preparation process of the final bulk.

The production mode of adjuvant-antigen adsorption mainly uses the antigen and the prepared aluminum-containing adjuvants (prepared or purchased) to carry out the adsorption. The adsorption process parameters to be studied and specified include: antigen concentration, aluminum concentration, adsorbed buffer system, salt ion concentration, pH, add addition order, sample addition speed, adsorption time, temperature, stirring time, stirring speed and so on.

In-situ adsorption can also be adopted for the adjuvant-antigen adsorption. Because the concurrent generation and control of the adjuvants and adjuvant-antigen complex are involved in the in-situ adsorption, it is difficult to carry out process characterization and

production process control by steps. Sufficient selection basis should be provided if it is proposed to adopt the in-situ adsorption process. Besides the adsorption process parameters described above, attention should also be paid to more process operation parameters which affect the antigen-adjuvant binding, for example, type of the washing buffer solution, proportion of the precipitate and washing solution, washing times, etc in the process development and process control of in-situ adsorption. In addition, it is recommended to pay attention to the impacts of the local heat release of the chemical reaction of the in-situ adsorption on the quality attributes and immunogenicity of the antigen-adjuvant conjugates and immunogenicity.

It is recommended to carry out the study of the antigen-adjuvant adsorption kinetics in the formulation study of the preparations, adsorption process studies and adsorption process validation. Determine the binding speed and process conditions of adsorption (e.g., pH, ion strength, buffer system, adjuvant and antigen proportion, adsorption time and adsorption temperature, etc.) through the kinetic studies. Further validate the process performances through the study of adsorption kinetics in the process of process validation. It is encouraged to adopt new techniques to carry out related studies.

On the basis of process validation, the critical process steps of

adsorption and preparation should be identified and suitable process control strategy should be established so as to ensure the process consistency[10].

The products such as monovalent adsorption bulk after adsorption of antigen and adjuvant can be used as the intermediate products and stored under appropriate conditions and then preparation and filling of the preparations should be subsequently conducted. Sufficient study should be conducted for the filling process of the vaccines which contain aluminum-containing adjuvants, for example, resuspending conditions of the intermediate products before filling, holding conditions for the product consistency during the filling period (including the interruption in continuous filling and in case of accidents). Specific and controllable filling process parameters should be established and sufficient validation should be conducted so as to ensure the uniformity of the finished products of filling.

The storage stage, storage conditions and storage time of the intermediate products should be specified[2]. Sufficient stability study should be conducted for the intermediate products.

2. Study of the Quality Characteristics of the Adjuvant-Antigen Conjugates

As a whole, the compatibility and interference between the adjuvant and antigen and between the adjuvants (if the adjuvant system is used) should be evaluated[4]. For the study of the quality

characteristics of the adjuvant-antigen conjugates, attention usually should be paid to:

Adjuvant-antigen binding level and the consistency, for example, the adjuvant-antigen adsorption ratio, adsorption strength and the hold status after adsorption and during the whole shelf life.

Integrity of the antigen in the conjugates before and after adsorption, for example, structure change of the antigen before and after adsorption and after desorption, antigen aggregation after adsorption, influences of adsorption on stability (degradation) of the protein antigen and polysaccharide antigen and study of the influences of adsorption on other physicochemical properties of the adjuvant-antigen conjugates, for example, the physicochemical parameters such as particle size[2]. It is encouraged to adopt advanced technical methods to study the influences of adsorption on the antigen structure[14].

The biological activity of the adjuvant-antigen conjugates (detected by the biological method) is the most critical comprehensive index, in particular, it can be used as the supplement of the study of the physicochemical properties in case the correlation of the components of the adjuvant-containing vaccines/physicochemical with the immunogenicity/efficacy, for example, carry out the in vivo/in vitro potency study and carry out the study of the impacts of the total antigen/free antigen content and adsorption strength on

potency and so on.

If the vaccines are premixed with the adjuvants and jointly packaged, the potency of the finished products should be determined. If separated packages have been adopted for the antigen components and adjuvant components, the potency and antigen content of the antigen intermediate products before adsorption should also be determined besides the determination of the potency of the finished products after adsorption.

3. Stability Study of the Adjuvant-Antigen Conjugates

The stability study of the adjuvant-antigen conjugates and finished products can refer to the *Guideline for Stability Studies of Biological Products*. Reasonable study protocol should be designed according to the product characteristics in combination with the situations of storage, packaging and transportation.

The antigen desorption during the shelf life may affect the immunogenicity and safety of the products. Therefore, besides the routine release indexes, special attention should be paid to the aspects such as degree of dissociation of the antigen from the adjuvant, antigen degradation and integrity (e.g., change of the molecular weight of the antigen, change of the free polysaccharide) [2,16] and potency for the stability study of the adjuvant-antigen conjugates.

(III) Container Closure System

Assessment and compatibility study of the selected packaging containers and closure system should be conducted by referring to the *Technical Guideline for Compatibility Studies of Chemical Drug Injections and Pharmaceutical Glass Packaging Containers (Trial)* [17] and *Technical Guideline for Compatibility Studies of the Chemical Drugs and Elastomeric Seals (Trial)* [18].

The influences of the compatibility between the adjuvant and other components and efficacy and stability of each component should be studied in the development process of the combined vaccines so as to determine the package form of the component, for example, single package or combined package.

(IV) Release Specification

1. Test Items

According to the specific situations of the composition and manufacturing process of the vaccines, the quality control of the adjuvant-antigen conjugates may include the control of the intermediate products before adsorption, quality control of the adsorbed monovalent bulk (if any), final bulk and finished products. The tests of the above stages are the necessary basis to ensure the quality of the final finished products of vaccines, but the test items of the specific test stage can be comprehensively considered according to the requirements for the test items of each stage, feasibility of the test methods and requirements for batch release and

some items which cannot be tested for the finished products (e.g., purity, residual impurities, etc.) can be detected at the early stage. The quality control of the adsorbed products usually include the following several stages:

(1) Monovalent Adsorbed Bulk

The quality control indexes of the monovalent adsorbed bulk usually include but not limited to antigen content, protein content, adsorption ratio, adjuvant content, pH, bacterial endotoxin, sterility, etc.

(2) Final Bulk

The control test of the final bulk usually should at least include the sterility test and the test items such as the adjuvant content and adsorption ratio can be tested in the finished products.

(3) Finished Products

The quality control indexes of the finished products usually include the in vivo potency, in vitro potency, completeness test of adsorption and other necessary items required by the pharmacopoeia.

2. Test Method

The aluminum-containing adjuvant adsorption will have specific influences on detection of the antigen components and whether the test methods and related test results will be affected after preparation should be studied[19]. Some special focuses of attention are listed as follows:

Some process related impurities cannot be accurately detected for the finished products after aluminum adsorption, for example, residual DNA, host cell protein, etc. They should be detected at the suitable sensitive steps.

Some test items (e.g., determination of in vitro potency, etc.) of the finished products after aluminum adsorption usually should receive the treatment for desorption and corresponding methodology study and methodology validation should be conducted. The desorption method should be established based on the understanding of the adsorption mechanism of the antigen adjuvant. Suitable and feasible desorption methods should be selected. Methodology validation should include the studies of the influences of the desorption process on the antigen conformation, antigen recovery rate after desorption.

For the vaccines which contain the aluminum-containing adjuvants, the in vitro potency test of the finished products mainly reflects the influences of the antigen on the potency of the finished products and therefore the determination of content of the adjuvant, determination of the adsorption ratio and determination of in vivo potency usually should be conducted for the finished products. Carry out comparability analysis of the in vivo and in vitro potency data of adequate batches after marketing with clinical batches so as to evaluate the feasibility to replace the in vivo potency by the in vitro potency.

(V) Combined Vaccines

The Guideline should be jointly considered with the *Technical Guideline for the Preclinical and Clinical Studies of the Combined Vaccines*.

Combined vaccines may contain many kinds of adjuvant-antigen conjugates, Many aspects such as compatibility of the antigen, adjuvant, buffer system and excipients as well as the influences on the test method should be comprehensively considered for the research and development of the preparations. The compatibility between the adjuvant and other components as well as the adsorption capacity for each kind of component should be studied. The adsorption efficiency and dynamics of concurrent adsorption of many components should be considered [16]. If some antigen components will not be adsorbed before preparation, the influences of the adjuvant on such non-adsorbed antigen (e.g., whether it will be adsorbed after preparation) and influences of such non-adsorbed antigen on the adjuvant-antigen complex) (e.g., whether the adsorbed component has desorption after preparation) should be assessed[2,16]. The buffer systems which are suitable for all the antigen-adjuvant adsorption products should be chosen in the development process of the preparations of the combined vaccines. It is suggested to adopt the method of matrix analysis to study the potential influences of different preparation formulations on the

adsorption, competition and interactions of all the antigens.

The mixing strategies of different antigen components in the production process of the combined vaccines mainly include joint adsorption, sequential adsorption and parallel separated adsorption, Suitable mixing strategy should be chosen according to the controllable degree of the process and degree of competition of the antigen so as to ensure the relatively good immunogenicity of the antigens of the combined vaccines and the repeatability of the production.

(VI) Vaccines Containing Many Kinds of Adjuvant Components

The vaccine preparations may contain many kinds of adjuvants, for example, different adjuvants and adjuvant system (e.g., the marketed adjuvant system, etc.) [6] are adopted for different antigen components and one kind of the aluminum-containing adjuvant which actually exists in the forms of several kinds of aluminum-containing adjuvant in the preparation formulation. The pharmaceutical study part of the aluminum-containing adjuvants in such adjuvant system can refer to the related content of the Guidelines. For other adjuvant components except the aluminum-containing adjuvants, related pharmaceutical studies can refer to the requirements of the related foreign technical guidelines for new adjuvants and the format and content of the application dossiers can refer to the requirements for studies of the

pharmaceutical excipients.

Besides the above separated pharmaceutical studies for each kind of adjuvants, the studies of the mutual binding and interference between the adjuvants[4], mutual interference of each kind of adjuvant and each antigen and mutual interference situations between the adjuvant-antigen conjugates should also be studied for the vaccines containing the adjuvant system. The study indexes such as pH change should be used to prove that chemical equilibrium has been reached for the adjuvants in the preparations or the subsequent equilibrium will not affect the product quality and a continuous steady state of the above interactions should be ensured during the whole shelf life.

IV. Nonclinical Studies

As a whole, related animal models should be adopted to study the factors which may affect the efficacy and safety of the vaccines such as suitable proportion of adjuvant/antigen, immunization procedure and immunization route for the nonclinical studies.

Ideal animal models should show the protective effects on the critical attacks of the pathogens (infectious diseases). If related animal models are not available, a kind of animal which can induce the immune response similar to the expected human immune response can be chosen [2].

Pay attention to the effects of the aspects such as immunogenicity,

potency and protective efficacy of the vaccines for the nonclinical efficacy. Nonclinical safety evaluation is very important to determine the suitable adjuvant/antigen proportion and safe dose, immunization procedure and route and determine the unknown or potential adverse reactions to be detected at the beginning of the clinical trials. Because the finished products of the antigens, adjuvants and vaccines may cause safety problems, the safety evaluation of the adjuvant-containing vaccines should include the toxicity study of the adjuvants and the finished products of the vaccines. The nonclinical safety studies should be conducted according to *Good Laboratory Practice* (GLP) for drugs.

The nonclinical efficacy, safety and pharmacokinetics studies of the separated adjuvants are usually not required for the traditional aluminum adjuvants.

Nonclinical studies of combination of the aluminum-containing adjuvants with the target antigens should comply with the related guidelines for pharmacological and safety studies of the vaccines. Scientifically evaluate the safety and efficacy of the aluminum-containing adjuvants according to the characteristics of the aluminum-containing adjuvants and antigens based on the principle of “concrete analysis of concrete problems” in order to reduce the risk for use of the clinical population.

1. For the vaccines without change of the aluminum-containing

adjuvants and antigens contained in the marketed vaccines: carry out the comparability study of immunogenicity, potency and protective efficacy with the marketed vaccines first. If the type and content of the aluminum-containing adjuvants and antigen are same as those of the marketed vaccines, the safety studies of local tolerability and hypersensitivity can be conducted and compared with the marketed vaccines.

2. For the vaccines with change of the type and/or content of the aluminum-containing adjuvants and antigen content compared to the marketed vaccines: the comparability study of the immunogenicity, potency and protective efficacy, local tolerability and allergy with the marketed vaccines should be conducted and the results should be compared with the marketed vaccines. Repeated doses toxicity test should be considered if the safety risk may be increased.

3. For the vaccines with combination of the aluminum-containing adjuvants with new antigens: Systematic nonclinical safety and efficacy studies should be conducted, including the necessity of the added adjuvants, proportion of the adjuvants and vaccines, immunogenicity (including the exploration of the immunization schedule and immunization route, etc.), efficacy studies of potency and protective efficacy as well as immunotoxicity, single dose and repeated doses toxicity in one kind of the sensitive animal species, local tolerability and allergy safety studies. Evaluate the necessity to

carry out the reproductive toxicity test according to the mechanism of action of the antigen and intended population. Related safety pharmacology studies should be conducted if the antigens have the safety risk of the cardiovascular, respiratory and nervous systems.

V. Clinical Studies

The clinical trials of the vaccines with aluminum-containing adjuvants should comply with the related requirements of Chinese Good Clinical Practice (GCP) and the general technical guidelines for vaccines[20-22].

Though there is human use experience for many years for combination of the aluminum-containing adjuvants and vaccine antigens, there is no option and/or preparation mode which can be directly used for all the vaccines. Reasonable adjuvant - antigen screening and optimization of the vaccine formulation is the key to improve the immune response of the vaccine and enhance the protective efficacy. Because each kind of the combination of adjuvant - antigen is unique, sufficient nonclinical studies should be conducted for research and development of the aluminum-containing adjuvants so as to learn the mechanism of action of various adjuvants and the impacts on the immune response. Corresponding studies usually should be conducted for the type of the aluminum-containing adjuvants, optimal dose formulation of the aluminum-containing adjuvants-antigen and safety and efficacy of

vaccines according to the product category and determine the formulation of the drug product proposed to be used for the clinical trials at the nonclinical study stage. If reliable animal models or immune indexes for prediction of the immune effect are lack of, completion of related exploratory study in the early clinical trials should be considered[23]. For research and development of the mixed/polyvalent vaccines, sufficient evidences for presence or absence of aluminum-containing adjuvants should be provided for each kind of antigen component.

For the vaccines with aluminum-containing adjuvants, various aluminum-containing adjuvants and vaccine agents should be used as whole to carry out the safety and efficacy evaluation in the clinical study. For research and development of the vaccines with aluminum adjuvants, it should be considered that addition of the aluminum-containing adjuvants can enhance the immune response and will not obviously increase the local and systemic adverse reactions of inoculation at the same time, namely, the potential benefits from the booster immunization response brought by use of the aluminum-containing adjuvants must exceed the risks brought by it and the safety risk should be clinically acceptable.

The pivotal clinical trials of the vaccines which contain aluminum-containing adjuvants should follow the randomized, blind and control principles and should be designed according to the

characteristics of the antigen and adjuvant components. For most of the vaccines for which efficacy can be evaluated with the immunogenicity surrogate endpoints, the immunogenicity indexes related to protection should be mainly evaluated in the clinical trial. If immunogenicity cannot be used as the surrogate endpoint, the clinical protective efficacy of the vaccines with aluminum-containing adjuvants should be evaluated. It is encouraged to carry out the correlation evaluation between immunogenicity and protective efficacy in the target populations. If the span of the age group is relatively big, stratification should be conducted or many independent clinical trials should be conducted in advance in the study design.

In the Guideline, the clinical trial design can be considered according to the following categories[2]: 1) The vaccines with combination of the aluminum-containing adjuvants and marketed antigens, including the vaccines without change of the type and content of the antigens and adjuvants of the marketed vaccines as well as the vaccines with increase, decrease and replacement of the antigen content and type and/or content of the adjuvant and 2)the vaccines researched and developed with combination of the aluminum-containing adjuvants and new antigens for research and development of the vaccines with aluminum-containing adjuvants. Specifically pay attention to the following aspects:

1. For research and development of the aluminum-containing adjuvants and the marketed antigens, dose exploratory study usually should not be separately conducted for the formulation of the aluminum adjuvant and antigen during the clinical study period of the vaccines with aluminum-containing adjuvants if the type and content of the antigen and adjuvants of the marketed vaccines are unchanged, and the formulation and dose range of the aluminum-containing adjuvant - antigen can refer to the marketed similar products.

If the content of the aluminum-containing adjuvants should be increased, decreased and adjusted, and the type of the aluminum-containing adjuvants should be changed for replacement carry out the clinical trials to prove the rationality of the post-change using quantity of the adjuvants and the combination formulation with the antigen through clinical trials according to the actual change situations under the premise with the support of the preclinical study data.

The study of the impacts of the aluminum-containing adjuvants on the immune response of the antigen should consider the impacts of the aluminum adjuvants (such as aluminum hydroxide and aluminum phosphate) and the buffer system on the efficacy and safety of the vaccines with aluminum-containing adjuvants.

2. For research and development of the vaccines with combination

of the aluminum-containing adjuvants with new antigens or it is proposed to add the aluminum-containing adjuvants in the vaccines of the marketed antigen/many kinds of antigens, consider to include necessary comparability clinical study for example, whether additives should be added, different adjuvant-antigen formulation contents and different types of adjuvants in the design of the clinical research and development plan so as to explore and prove the necessity for addition of the aluminum-containing adjuvants and rationality for the adjuvant-antigen formulation because the preclinical studies of the vaccines with aluminum-containing adjuvants are difficult to sufficiently predict the immune benefits which are beneficial for human.

For research and development of the vaccines with combination of the marketed antigen with proposed addition of aluminum-containing adjuvants, the marketed antigen vaccine should be chosen as the control, and superior results should be obtained for efficacy, and safety risk has not been increased. The clinical advantages should be reflected after weighing the advantages and disadvantages. If the purpose to use aluminum-containing adjuvants is to meet the urgent need of public health, reduce the needed quantity of antigen under the specific conditions, i.e., use the limited antigen dose to inoculate so that immune protection can be achieved in the population as much as

possible and acceptance of the clinical results of the non-inferiority design can be considered.

Standardized method should be used to collect and provide important safety study data in the clinical trails of the vaccines with aluminum-containing adjuvants [24, 25]. For adverse reactions, attention usually should be paid to the local reactions related to inoculation, for example, pain, redness and swelling, induration, granulomatous inflammation, aseptic abscess as well as the systemic reactions such as fever, allergic reaction and so on. Attention should also be paid to other unknown or potential adverse reactions of the vaccines with aluminum-containing adjuvants, for example, the potential immune-mediated inflammatory diseases (pIMDs). Long-term safety monitoring should be continuously conducted after marketing of vaccines.

When the impacts of the aluminum-containing adjuvants on immune response and long-term safety are studied, the effects of the adjuvant components on antigen should be comprehensively assessed and illustrated.

The clinical studies of the vaccines with aluminum-containing adjuvants with combination with the aluminum-containing adjuvant system or other novel adjuvants (including the combination of the marketed antigens or various antigens with adjuvants) should also refer to the related requirements of Chinese and foreign guidelines

such as *Technical Guideline for Clinical Trials of Vaccines*, *Technical Guideline for the Preclinical and Clinical Studies of the Combined Vaccines* and so on[20, 21].

V. Changes of the Aluminum-containing Adjuvants of the Marketed Vaccines

Changes of the supplier, manufacturing site and preparation process of the raw materials of the aluminum-containing adjuvants or supplier of the purchased aluminum-containing adjuvants of the marketed vaccines may have adverse influences on safety and efficacy of the vaccines. The comprehensive recognition of product and process knowledge is an important basis to control the change risks. The manufacturing process of the aluminum-containing adjuvants should be continuously monitored after marketing approval. Monitor the critical process parameters and product quality characteristics and ensure the stability of the product quality and manufacturing process and continuously enhance the understanding of the manufacturing process of the aluminum-containing adjuvants, and impacts on quality of the aluminum-containing adjuvant and finished products of vaccines. It is recommended to periodically analyze the quality stability of the aluminum-containing adjuvants in combination with the trend analysis of the aluminum-containing adjuvants and vaccines and ensure the correlation between the quality characteristics of the

aluminum-containing adjuvants and efficacy of the final products in combination with the actual production experience in routine production of vaccines.

The changes of the aluminum-containing adjuvants should be sufficiently assessed by referring to related technical guidelines and comparability study should be conducted. Besides that the assessment should be conducted for quality of the aluminum-containing adjuvants before and after change, it is more important that the comparability study will bring should be conducted for the influences of changes of the adjuvants on the adsorption products and vaccine products so as to prove that changes of the aluminum-containing adjuvants have not caused adverse influences on safety, efficacy and quality controllability of the adsorption products and finished products of the vaccines.

If the quality comparability study results cannot prove that the quality is comparable and changes may affect the efficacy and/or safety of the product, it is necessary to carry out the animal safety efficacy study to compare with the pre-change products. The evaluation methods and/or indexes of safety and efficacy can be selected according to the characteristics of different vaccines, and the extended immunotoxicity test can be considered for the efficacy aspect, and local tolerability and allergy test usually should be considered in the aspect of safety, and carry out the repeated doses

toxicity study if necessary[26].

If the above preclinical study results cannot prove that the changes of the aluminum-containing adjuvants have not produced adverse influences on efficacy and/or safety of the vaccine, it is necessary to carry out necessary bridging clinical study so as to further validate the safety and efficacy of the vaccines with aluminum-containing adjuvants.

Glossary

Adjuvants: Refer to the substances which can assist the antigen response and mediate the immune responses and types.

Adjuvant System: Refers to the specific combination formed by many kinds of immunoregulation molecules and presentation system which can obtain better and wider protective efficacy of the vaccine for the specific pathogens and target populations through the effective adjustment of the innate immune or/and acquired immune reactions.

Aluminum-containing Adjuvants: Refer to the aluminum salts or mixture of the aluminum salts which play the role of adjuvant, for example, aluminum hydroxide, aluminum phosphate, amorphous aluminum hydroxyphosphate sulfate and mixture system of the and the in above aluminum-containing adjuvants.

Aluminum-containing Adjuvant System: Refers to the compound

adjuvant system formed based on the aluminum-containing adjuvants and other adjuvant components or presentation system (e.g., CpG, MPL, lipidosome, QS21, etc.), for example, AS04 adjuvant system.

Vaccines which Contain Aluminum-containing Adjuvants: Refer to the vaccines prepared by adsorbing, binding or mixing of the aluminum-containing adjuvants and antigens. Besides the aluminum-containing adjuvants, the vaccines may also contain the adjuvant system. However, unless otherwise specified, the "vaccines with aluminum-containing adjuvants" in the Guideline only refer to the vaccines formed by aluminum-containing adjuvants and antigens.

Co-adsorption: Many kinds of antigens and adjuvants can be jointly mixed for adsorption. The production step of this method is simple, however, the competitive adsorption between different antigens may result in relatively lower adsorption rates of partial antigens and may affect the interbatch consistency.

Sequential Adsorption: Sequentially add the single antigen into the aluminum-containing adjuvants and buffer solutions. The addition order of the antigen may affect the adsorption ratio of each antigen.

Parallel Separated Adsorption: Single antigens will separately adsorb with the adjuvant and will then be mixed or mixed with the antigens which do not need adsorption in advance. This method can control

the degree of adsorption of the single antigen relatively well and reduce the interbatch difference of the adsorption ratio. The separately adsorbed adjuvant-antigen conjugates usually have relatively good stability and should be used according to the needs. However, more in-process controls and release testing are needed.

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