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CHAPTER

9

Global Regulatory Guidelines for Vaccines

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INTRODUCTION

Nonclinical safety testing provides evidence that a particular vaccine is safe to be administered to humans in clinical trials and is conducted prior to and in some cases concurrent with these trials. The animal studies afford clinicians data on potential toxicities and organs or organ systems that may be affected as a result of the vaccine itself, impurities or contaminants, components of the formulation or interactions thereof, or toxicities resulting from the expected immune response to the vaccine. The toxicity data are also used to define the dose that may safely be administered to humans and a no-observed-effect level, or a dose at which no toxicologically relevant findings were identified. The caveats to these studies for vaccines as well as other types of molecules are that there may be rare subpopulation toxicities that may only be addressed in humans and in general animal models may not always reflect what might be observed in humans.

In order to ensure that nonclinical studies supporting human clinical trials for vaccines are conducted in a robust and consistent manner across the industry it was recognized that widely accepted, comprehensive guidelines were required. Some nonclinical development guidelines, such as the European Medicines Agency (EMA) guideline approved in 1998 [1], as well as the United States Code of Federal Regulation (US CFR) and individual country-specific compendia that at least mention nonclinical studies specific for vaccines have been available for many years. However, the information was either of insufficient detail or more geared toward requirements for lot release or IND (Investigational New Drug) submission. Therefore, in 2002, members of academia, industry, and US and European regulatory agencies convened to discuss the drafting of the first World Health Organization (WHO) guideline for nonclinical evaluation of vaccines. The WHO guideline would be a comprehensive document providing more detail on items such as animal models, study design (dose, route
of administration, dosing schedule), and endpoints. In addition, it would include specific methods for the evaluation of additional components of vaccine formulations (adjuvants, excipients), vaccines administered via non-routine routes of administration and specific types of vaccines including combination vaccines. This guideline is now available and widely used throughout the industry as the gold standard for designing nonclinical vaccine programs.

In addition to the WHO guideline, many guidance documents are also available that outline the regulatory expectations for traditional as well as novel types of vaccines (Table 9.1). The original EMA guideline is still in effect and applies to all vaccines, as do the WHO [2], Chinese [3], and Japanese [4] guidance documents. In some cases these guidelines also detail country-specific requirements. Guidances are also available to provide more specific detail for particular vaccines that will be administered to pregnant women or women of childbearing age.

| Vaccine type                        | Guideline                                                                                     | References |
|------------------------------------|----------------------------------------------------------------------------------------------|------------|
| All vaccines                       | EMA: Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (1997) | [1]        |
|                                   | Worldwide: WHO Guidelines on Nonclinical Evaluation of Vaccines (2005)                      | [2]        |
|                                   | China: State Food and Drug Administration, China Technical guidelines for preclinical research on preventive vaccines. Notice No. 140 (April 2010) | [3]        |
|                                   | Japan: Japanese Guideline for Non-clinical Studies of Vaccines for Preventing Infectious Diseases, (PFSB/ELD Notification No. 0527-1, May 27 2010) | [4]        |
|                                   | India: Drug and Cosmetics Act, 1940 and Drug and Cosmetics Rule, 1945 (2005)                 | [12]       |
| Vaccines for pregnant women and WCBP | FDA: Guidance for Industry. Considerations for Developmental Toxicity Studies for Preventative and Therapeutic Vaccines for Infectious Disease Indications (2006) | [5]        |
| Adjuvants                          | EMA: Guideline on Adjuvants in Vaccines for Human Use (2005)                                | [6]        |
| DNA vaccines                       | FDA: Guidance for Industry. Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (2007) | [7]        |
|                                   | WHO: Guidelines for Assuring the Quality and Nonclinical Safety Evaluation of DNA Vaccines (2005) | [8]        |
| Recombinant DNA vaccines           | FDA: DRAFT Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (1985) | [9]        |
| Viral vectored vaccines            | Guideline on Quality, Nonclinical and Clinical Aspects of Live Recombinant Viral Vectored Vaccines (2010) | [10]       |
| Combination vaccines               | EMA: Note for Guidance on Pharmaceutical and Biological Aspects of Combined Vaccines (1998) | [11]       |

WCBP, women of childbearing potential.
potential [5], those with adjuvants [6], DNA [7–9] and viral-vectored vaccines [10] and those combined with other vaccines [11]. In addition to the implementation of more routine non-clinical evaluations of vaccines, programs focused on the safety of vaccines have been implemented such as the Vaccine Adverse Event Reporting System (VAERS), a post-marketing surveillance system developed in 1990 by the Centers for Disease Control (CDC) and the US Food and Drug Administration (FDA) that tracks adverse events that occur after the administration of US-licensed vaccines.

Vaccine manufacturers should also be aware that specific countries may have guidelines outlining their expectations for the nonclinical evaluation of vaccines that may differ significantly from the WHO, EMA, Japan and China guidelines. One example of this is India [12] where vaccines are considered as "drugs" in their nonclinical guidelines (Table 9.1). As a result, the toxicology studies required for vaccines are more extensive and challenging to design compared to well-accepted vaccine-specific programs. For example, the Indian guidance requires studies to be conducted in two species using two routes of administration (e.g. intramuscular and subcutaneous), dose ranging, and the identification of a maximum tolerated dose (MTD). In contrast to small-molecule drugs, vaccines are not metabolized via P450 enzymes, so multiple species (as typically required for toxicology testing of small molecules to ensure the animal model is representative of the metabolism observed in humans) should not be needed. In addition, nonclinical immunogenicity studies are typically conducted in more than one species and this would allow for the identification of species-specific differences that would need to be evaluated from a toxicological perspective. Given that the route of administration of most vaccines is parenteral (e.g. intramuscular or subcutaneous) with the goal of inducing a depot effect where the vaccine antigens are retained at the site of injection so that they may have sufficient opportunity to interact with the immune system, a single, relevant route of administration (i.e. the anticipated clinical route) should be adequate to induce an immune response and injection site reactions should be evaluated in studies utilizing the relevant route of administration. As the mechanism of action of vaccines is well understood (i.e. induction of specific immune response to vaccine antigens), and the expected immune response in a relevant species is generally associated with transient toxicity and little if any off-target toxicity is observed, dose ranging for the purposes of identifying an MTD may be of little value. Furthermore, dose-ranging studies expected to be conducted according to the Indian guidance would require testing vaccine formulations specifically prepared for toxicology studies, instead of a formulation representative of that to be tested in the clinic. Attempting to perform dose ranging or identify an MTD may require either administering dose volumes exceeding those acceptable from an animal welfare perspective or formulating the vaccine so that it is more concentrated to allow the administration of higher doses while maintaining an acceptable dose volume. In the case of unacceptable dose volume administration, effects at the injection site will very likely be observed based on mechanical trauma alone and potentially mask any reactogenicity resulting from the vaccine itself. If the vaccine is formulated at a higher concentration, the material would no longer be representative of the clinical formulation and would make injection site reactions difficult to interpret.

In general, toxicity data obtained from studies in which the vaccine is administered at dose levels or concentrations far exceeding the clinical dose may be misleading and not indicative of the safety of the vaccine. Instead of using multiples of predicted human dose of a vaccine candidate in toxicology studies (as is routinely done for small molecules), testing a human
equivalent dose (using the clinical formulation) is considered by most regulatory authorities adequate for establishing safety margins of vaccines based on differences in body weight between humans and animal models. For example, a full human dose of 0.5 mL administered to a 0.3 kg rat provides an approximate 16-fold safety margin for a 5 kg human in the case of an infant vaccine.

Discrepancies between guidelines in different countries pose a great challenge to global vaccine manufacturers. Conceivably, future interactions with the Review Committee on Genetic Manipulation (RCGM) and Drugs Controller General of India (DCGI) would facilitate discussions to address the points raised about the differences in expectations for toxicology studies of vaccines, and the possibility of aligning the recommendations with the WHO guideline.

An additional consideration which manufacturers should be aware of is country-specific requirements when attempting to initiate clinical studies or file submissions to register vaccines already marketed (in other countries) when the guidelines for nonclinical evaluation of vaccines were not in place and toxicology studies with vaccine candidates were not required prior to entry into clinical trials. Some countries (e.g. Japan, Russia) may require that thorough reviews of any available animal data even if they are not traditional nonclinical toxicity data, be included in clinical trial applications (CTAs). If this information is deemed insufficient, requests may be made to conduct routine nonclinical toxicity studies prior to the initiation of clinical studies and/or worldwide marketing application (WMA) submission to fulfill regulatory expectations outlined in the current guidelines. In light of the existing clinical data and proven safety records in humans for vaccines on the market, it is difficult to justify a retroactive conduct of nonclinical toxicity studies with such vaccines. It is important that vaccine sponsors clearly communicate reasons for the lack of toxicity data for vaccines authorized for marketing, primarily in Western countries, in the years prior to the requirements for nonclinical toxicity testing of vaccines, and emphasize the existing clinical data as the most relevant evidence of vaccine safety. Moreover, carrying out toxicity studies with vaccines that have been safely administered to millions of humans would be counter to current worldwide expectations on reductions in animal use.

Vaccine development begins with nonclinical studies in which immunogenicity is evaluated as an indication of potential efficacy in humans. According to the current regulatory requirements, once the vaccine candidate is determined to be suitable for clinical testing, nonclinical toxicity studies are conducted to support clinical studies. Prior to the conduct of nonclinical toxicity studies, vaccines are subjected to a battery of tests to ensure that the material meets minimum specifications for characteristics such as mass, identity, purity, potency, sterility, and safety. The main portion of the nonclinical toxicity studies precedes the introduction into humans during clinical trials and further studies may be conducted to support later trials as earlier trials are ongoing. These studies should be designed on a case-by-case basis but there are minimum requirements that may be applied to most vaccines. When conducting toxicity studies for a particular vaccine, vaccine type and indication must be taken into consideration when selecting the species and strain of the animals to be used, formulation to be administered, route and method by which the vaccine will be administered and number of doses and dose levels. These details are incorporated into the overall design of the studies and are applied to each type of study conducted. After all nonclinical and clinical testing has been conducted, the vaccine is determined to be safe and efficacious in humans,
and marketing authorizations are obtained, product quality characterization and safety testing are conducted for each lot as part of release procedures prior to human use.

### TOXICITY ASSESSMENT

#### Study Design

The purpose of nonclinical toxicity studies is to identify potential target organ effects that may predict human toxicity. The guidelines state that toxicity studies should be designed to match the clinical regimen as closely as possible with regard to dose levels, route and frequency of administration and any delivery device planned for clinical use. The toxicity assessment may be stand-alone or included in other studies that incorporate toxicity endpoints and includes an evaluation of local tolerance. Toxicity is evaluated following single and repeat dosing. Separate studies may be conducted for this purpose or evaluations may be performed following the first dose on a repeat-dose study. With some exceptions (e.g. immunogenicity assays may be qualified but not validated), health authorities require that studies supporting clinical studies be conducted under Good Laboratory Practice (GLP) conditions [13]. In general, the study is designed such that reversibility may be monitored (i.e. the inclusion of recovery groups).

Guidelines indicate that the material to be evaluated in nonclinical studies should be representative of the clinical formulation and, if possible, the actual clinical material manufactured under current Good Manufacturing Practices (cGMPs) [14,15] used. However, it is acceptable to use non-GMP material that has been tested to meet minimum release specifications including potency [16], sterility [17], purity [18], identity [19], endotoxin, and bioburden as well as stability in addition to other parameters used for characterization. In vitro versions of these tests are preferred but in some cases animal testing is required (e.g. General Safety/Abnormal Toxicity [20,21] and rabbit pyrogenicity testing [18,22,23]). Testing for stability typically includes the conduct of the same in vitro and/or in vivo tests used for release at predefined intervals such as 12, 24, and 36 months and can also be used to determine the shelf-life of a particular product. If during clinical development the formulation changes, the expectation of health authorities is that the nature of the change be evaluated to determine what, if any additional nonclinical testing would be required.

#### Animal Model Selection

Most regulatory authorities recognize that one relevant animal species is generally sufficient. Relevance is minimally defined as being capable of developing an immune response to the vaccine and ideally being sensitive to the biological effects of the pathogenic organism or toxin the vaccine is designed to protect against. If the animal model selected for toxicity studies has not been evaluated in nonclinical immunogenicity studies, it may be prudent to confirm species relevance using a pilot immunogenicity study in the selected toxicity model using the anticipated clinical dose prior to conducting GLP toxicity studies. Outbred animals of proven health status are used. Situations in which more than one species is required include those where the mechanism of protection induced is not well understood or there are
species- or strain-specific differences in the pharmacodynamic effects of the vaccine. Health authorities recommend that ten animals per gender be used routinely for rodents and fewer for non-rodents (non-human primates, rabbits). In recovery groups, fewer animals than used for the interim evaluation may be justified. Per the WHO guideline, rodents should be 6–8 weeks of age at study start and rabbits should be 3–4 months of age.

Dose, Route, Controls

Typically, only a single dose level is evaluated in nonclinical studies and matches the highest dose to be used in clinical studies per guidance from health authorities. If this is not feasible based on animal welfare concerns or the dose level does not provide a safety margin, a dose may be selected that exceeds the human dose on a mg/kg basis and elicits an immune response. Although an evaluation of a dose–response and the identification of an MTD are not required, if toxicity is observed it is recommended by regulatory authorities that lower doses be evaluated. According to the WHO guideline, the number of doses administered to the test animals should be equal to or greater than the number of doses proposed in humans (typically one more dose than anticipated in the clinic is administered to the animals, i.e. “n+1” doses). Given that the clinical dosing interval may be quite extended (i.e. months) the frequency of dosing in animal studies may be shortened relative to that proposed for clinical trials and based on the kinetics of the immune response rather than matching the clinical dosing schedule. To this end, doses are typically administered at defined intervals of 2–3 weeks. Health authorities recommend that repeat-dose studies be conducted even in cases where the vaccine will be administered as a single dose clinically. However, single dose only studies may be justified in some cases, for example, where vaccine-induced antibodies are expected to neutralize a live virus vector, thereby limiting the expression of the protein of interest or when the immune response may affect species-specific proteins in the vaccine formulation.

Guidelines indicate that the route of administration should match that planned for use in clinical studies and additional routes of administration may be useful if unexpected toxicity is observed using the selected route. A negative control group is included and if appropriate an active control group (i.e. formulation without antigen) may be included if no historical data on a particular adjuvant or excipient is available. A recovery group is included to investigate reversibility of any adverse effects and to screen for possible delayed adverse effects.

Endpoints

Routine parameters indicated in nonclinical guidelines (clinical signs, body weights, food consumption, serum biochemistry, hematology, and gross and histomorphological examinations) should be included in toxicity studies in order to evaluate the toxicity of the candidate vaccine, with an emphasis on immune parameters such as local inflammatory reactions, effects on draining lymph nodes and the induction of immune system biomarkers such as cytokines and chemokines. Data are collected both during the treatment period (1–3 days following the first and last dose) and at the end of the recovery phase. Health authorities require that the immune response to the vaccine be measured to confirm the relevance of the animal model. A pathology evaluation is included with an emphasis on injection sites, immune organs and draining and distant lymph nodes. The extent of the pathology evaluation is
determined by the amount of nonclinical and clinical data available on the candidate vaccine and/or adjuvant/excipients present in the formulation.

**Local Tolerance**

Given that most vaccines are administered via either intramuscular or subcutaneous routes, a common adverse finding is local reactogenicity. In order to predict the likelihood of these reactions in clinical studies, regulatory guidelines indicate that an evaluation of local tolerance including histopathology at the site of injection should be included either as part of the toxicity study or as a stand-alone study. While some local reactogenicity is anticipated, if an unacceptable level is observed, additional studies designed to evaluate mechanism of toxicity may be needed.

**ADDITONAL TOXICITY ASSESSMENTS**

**Special Immunological Investigations**

Although an immune response to the candidate vaccine is generally part of the mechanism of action, there may be circumstances where an immune response may result in toxicity (e.g. autoimmunity due to similarity between vaccine antigens and host proteins, precipitation of immune complexes, exacerbation of disease, or hypersensitivity). In such cases, additional studies designed to evaluate the mechanism of toxicity may be required by health authorities. Given the complexity of such studies and the questionable predictive nature of the endpoints for actual pathology, vaccines anticipated to cause such effects should be studied carefully.

**Developmental Toxicity Studies**

Many vaccines are indicated for use during childhood and would therefore not require nonclinical developmental toxicity studies. However, unless a sound scientific argument can be made indicating such studies are unnecessary, regulators require that preventative and therapeutic vaccines for infectious diseases indicated for women of childbearing potential and pregnant women be evaluated in nonclinical studies. The purpose of these studies is to assess adverse effects on the pregnancy status and/or the developing embryo/fetus as a result of maternal immune modulation inherent in the biological activity of the vaccine antigen and/or constituents of the vaccine product (e.g., adjuvants and excipients). Developmental toxicity studies may also be triggered by findings in repeat-dose toxicity studies indicative of effects on reproductive organs or distribution/persistence of plasmid or viral vector-based vaccines to gonadal tissues. Health authorities indicate that the need for fertility studies should be evaluated on a case-by-case basis. According to regulatory guidelines, in cases where the vaccine is either very similar to or a combination of vaccines on which developmental toxicity studies have already been performed, additional developmental toxicity studies may only be needed if significant changes have been made to components such as new adjuvants or excipients or concerns exist regarding increased toxicity upon mixture of the individual components in the case of a combination vaccine.
The timing of developmental toxicity studies differs for various populations. Regulatory authorities recommend that data from nonclinical developmental toxicity studies for vaccines indicated for use in pregnant women be available prior to enrolling pregnant women in clinical trials. Women of childbearing potential may be enrolled in clinical trials prior to the conduct of nonclinical developmental toxicity studies provided precautions are taken to avoid pregnancy during the trial. For this population, a single developmental toxicity study is conducted, typically concurrent with Phase III clinical trials, but this timing may vary depending on the country in which the clinical studies are conducted.

While the guideline for Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility, ICH S5(R2), 2005 [24] is useful as a reference tool, given the complexities of the evaluation of vaccines, there are components of the testing paradigm that are distinct for vaccines. Per the WHO guideline, the nonclinical testing strategies described in ICH S5(R2) [24] may need to be altered such that the particular vaccine being studied is adequately evaluated. Previous clinical experience, including data from immunogenicity studies in pregnant women is taken into account when designing nonclinical studies as such data may be helpful in risk assessment but may not preclude the need for nonclinical studies. Data from previous nonclinical studies may be used to aid in species and dose selection for developmental toxicity studies as well as interpretation of study results.

Regulatory agencies recommend an early dialogue to gain concurrence with the planned developmental toxicity evaluation but a general outline of the studies and endpoints is available [2,24]. Typical models include the rat, rabbit, or mouse and one species is generally sufficient. Non-human primates should be used only in cases where no other alternative is available.

As with repeat-dose toxicity studies, regulatory guidelines indicate that the animal model chosen should be relevant (i.e. have the ability to mount an immune response) and generally the same species is used for repeat-dose and developmental toxicity studies. In addition to the ability of the test animal to mount an immune response, it is also important to evaluate maternal antibody transfer by measuring vaccine-induced antibody in cord or fetal blood to verify exposure of the embryo or fetus to maternal antibody. Given the importance of the immune response in establishing the relevance of the preclinical model, it is expected that information about the onset and duration of the antibody response be obtained. Guidelines indicate that these samples should be collected prior to exposure to the vaccine being studied, on the day of cesarian sectioning and at the end of the weaning period. While there are many ways to evaluate the immune response, antibody measurements are routinely used as a marker for vaccine-induced effects. Repeat-dose toxicity studies in non-pregnant animals may be used for this purpose unless evidence suggests that the immune response differs in pregnant animals. As with repeat-dose toxicity studies, guidelines dictate that the route of administration mimic the clinical route, the maximal human dose should be administered and the material used should be identical to or representative of the final clinical formulation. If administration of the full human dose is not feasible, a dose that exceeds the full human dose on a mg/kg basis and elicits an immune response should be used. Dosing frequency will depend on the onset and duration of the immune response of the particular vaccine being studied but in general episodic dosing is recommended. Regulatory authorities indicate that in cases where the lack of a
relevant animal model hinders the assessment of an immune response, developmental toxicity studies may still be of value with respect to potential embryo/fetal toxic effects of the vaccine components/formulation.

For preventative and therapeutic vaccines the safety concern is generally related to development and growth of the embryo and fetus. Therefore, the primary focus is on the detection of adverse effects on the pregnant/lactating female and development of the embryo/fetus and the offspring following exposure of the pregnant female to the vaccine from implantation through the end of pregnancy. Animals are routinely exposed to the vaccine in the pre-mating stage and during the period from implantation until closure of the hard palate and end of gestation to evaluate effects on organogenesis (Stages C, D, E per ICH S5(R2) [24]. Pre-mating and booster dose administration as needed ensure exposure of the embryo or fetus to the vaccine-induced immune response as well as components of the vaccine formulation. Postnatal follow-up from birth to weaning is recommended to evaluate normality of growth, body weight gain, suckling activity, and viability. To this end, one subgroup of females is subjected to cesarian section to allow uterine and fetal examinations and a second subgroup is allowed to litter with postnatal follow-up of the offspring. A sufficient number of animals is used to allow an evaluation of at least 40 animals per group with an allocation of 20 animals each to the cesarian and littering subgroups. Control groups such as PBS or others designed to evaluate certain aspects of the formulation such as adjuvants or excipients are included. Guidelines indicate that typical endpoints providing data on prenatal and postnatal development including maternal functions such as those described in ICH S5(R2) [24] should be included. Routine toxicity endpoints evaluated include clinical observations including data on general appearance and body weights, local tolerance, and food consumption as well as study-specific endpoints such as duration of pregnancy, abortions, premature deliveries, and parturition. In the cesarian section group, maternal observations include a necropsy for macroscopic evaluation as well as recording the number and distribution of corpora lutea, implantation sites, viable and nonviable fetuses, and early and late resorptions as well as a gross evaluation of the placenta. Fetal examinations in the cesarian group include body weights of live fetuses and an evaluation of viable fetuses for gross external, visceral, and skeletal alterations. To the extent possible, late resorptions and nonviable fetuses are also examined for gross external alterations. The gender of all fetuses is determined via internal evaluation. Maternal observations in the natural delivery group include a determination of the duration of gestation and parameters such as the fertility, gestation, and live birth indices. Following the pre-weaning period, dams are sacrificed so that a gross necropsy of the thoracic, abdominal, and pelvic viscera may be performed. The distribution of implantation sites and any observed abnormalities is recorded. Guidelines indicate that a cause of death and pregnancy status be determined for all animals that die or are sacrificed early. Aborted fetuses and/or delivered pups are examined as feasible. Offspring allowed to mature through mating (F1 generation) are evaluated for normal growth, body weight gain, and nursing activity. Functional observational evaluations are also conducted, viability and lactation indices recorded and genders determined. Gross necropsies are performed at the end of the study and any pups that are sacrificed or die early are evaluated for abnormalities or cause of death as appropriate.

While the lack of effects in developmental toxicity studies may not be predictive of human response due to factors such as species-specific differences in the immune
response, different developmental time lines and differences in placentation, studies should be designed to optimize predictive value. If a cause for concern exists such that the vaccine being studied may be immunotoxic or if the studies conducted indicate that other adverse effects may be observed, one could consider other parameters that may be incorporated into the study design such as an evaluation of lymphocyte subsets or other specific biomarkers.

Genetic Toxicity/Carcinogenicity

Regulatory guidelines generally do not require genotoxicity and carcinogenicity studies for vaccines based on limited exposure to vaccine antigens following administration, intermittent administrations with significant intervals between dosing, and a limited number of repeated administrations over the lifetime of an individual receiving the vaccine. Exceptions to this may be cases where novel components such as adjuvants or excipients are included in the vaccine formulations. If it is determined that genotoxicity studies are required, tests for mutations and chromosomal damage are conducted prior to the introduction of the vaccine into humans and the full battery of tests may be conducted in parallel with clinical trials per the Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, ICH S2(R1), 2011 [25].

Safety Pharmacology

Most vaccine guidelines indicate that safety pharmacology studies are only needed if data from nonclinical or clinical studies suggest that the vaccine may affect physiological parameters such as central nervous system (CNS), respiratory, cardiovascular (CV) or renal function. One notable exception is the Japanese guidance [4] which states that safety pharmacology studies are required for all vaccines (novel as well as previously marketed). However, this guidance does indicate that a stand-alone safety pharmacology study may be reduced or eliminated if specific endpoints have been included in repeat-dose toxicity studies. Furthermore, based on the author’s personal experience, the requirements for nonclinical safety pharmacology studies may also be limited or omitted if safety pharmacology endpoints (e.g. ECG evaluations) have been included as part of the clinical evaluation “overseas” prior to the conduct of clinical studies in Japan. In the event that safety pharmacology studies are required, given that repeat-dose toxicity studies are typically conducted in rats or rabbits, an assessment of CV parameters as part of the general toxicity study is not well established and rarely feasible. Therefore, in practice a separate safety pharmacology study would have to be conducted for this purpose. It is highly desirable to perform safety pharmacology studies in the same species as that used in the general toxicology studies in order to put toxicity responses into perspective with other study data. Where this is not possible (e.g. toxicology species is the rabbit), studies in non-human primates or other relevant species would need to be conducted. While the design of safety pharmacology studies for vaccines should be considered on a case-by-case basis, general descriptions of the design of these studies may be found in Safety Pharmacology Studies for Human Pharmaceuticals, ICH S7A, 2000 [26].
Pharmacokinetics

While health authorities recognize that traditional pharmacokinetic evaluations are not required for vaccines, if, for example, the formulation contains novel adjuvants or alternative routes of administration are used, local deposition and distribution studies are conducted to evaluate retention time at the site of administration and to determine where the vaccine and its components are distributed, respectively.

SPECIAL CONSIDERATIONS

Adjuvants

The purpose of an adjuvant [6] is to boost the immune response to the antigens contained in the vaccine. Given the regulatory and public scrutiny surrounding the use of adjuvants it is important to provide a rationale for the need for an adjuvant supported by evidence in a relevant animal model (e.g. character of the immune response, protection against a lethal challenge of the pathogenic organism, or information from the literature). Since not all adjuvants are designed to elicit the same type or duration of immune response, separate immunogenicity and toxicological evaluations are conducted. Per regulatory guidelines, pharmacokinetic studies are not required. If no toxicity data exist, full toxicological evaluations on the adjuvant alone should be conducted similar to new chemical entities [27,28]. If more than one adjuvant is to be used, an evaluation of each alone and in combination is conducted. Though the species selected for these studies should ideally be the same as that used for proof-of-concept, species selection is dependent upon the antigen and adjuvant combination and any potential species specificity associated with the adjuvant itself. In the adjuvant-alone studies, evaluations for local tolerance, the induction of hypersensitivity and anaphylaxis as well as pyrogenicity [18,22,23] are recommended. The adjuvant is administered at intervals reflecting the proposed clinical use and systemic toxicity is evaluated including a full necropsy and tissue collection for histopathology. The evaluation should include a dose response but the highest dose may be selected based on expected clinical use rather than establishing an MTD. Other endpoints are included as outlined in guidelines for the evaluation of new chemical entities [27,28]. If the adjuvant is intended for use in pregnant women or women of childbearing potential, reproductive toxicity studies are conducted. The dosing regimen reflects the intended clinical plan and the vaccine is administered both prior to and during pregnancy. While adjuvants of a biological nature are treated as biotechnology products and therefore do not require genotoxicity testing per ICH S6 [29] synthetic adjuvants are subject to the standard battery of genetic toxicity testing per ICH S2 [25].

Health authorities require that the clinical formulation containing both the adjuvant and the antigen be evaluated to ensure that the combination is compatible, demonstrates consistent adsorption and does not exert a synergistic effect on toxicity. Characterization of the immune response is also included such as a dose response of both adjuvant and antigen as well as comparative studies designed to evaluate the immune response of the vaccine in the absence of the adjuvant.
Additives (Excipients and Preservatives)

Per regulatory guidelines, additives are evaluated in a manner similar to adjuvants in that in the absence of toxicity data full toxicity studies are conducted as with new chemical entities [27,28] and the final clinical formulation is evaluated to ensure compatibility, etc.

FORMULATION/DELIVERY DEVICES

Guidelines dictate that the vaccine formulation and delivery device intended for clinical studies be used in nonclinical toxicity studies. If this is not possible, for example because of the lack of an appropriate animal model, pilot studies are conducted to develop such a model prior to the conduct of nonclinical toxicity studies.

ALTERNATE ROUTES OF ADMINISTRATION

Animal Models

In selecting an appropriate animal model for vaccines delivered through alternate routes of administration (nonparenteral routes such as oral, intradermal, etc.) health authorities recommend that the anatomy of the animal models, in particular relative to the site of administration, be taken into consideration such that one may ensure the animals are properly exposed to the vaccine. If an appropriate animal model is not available it may be necessary to employ several studies in different animal models to adequately evaluate toxicity.

Dose

The dose administered may differ between the parenteral and alternate route identified. Therefore, regulatory guidelines indicate that dose ranging studies may be required to determine the appropriate dose and dose volume to be administered via the alternate route chosen.

Endpoints

In addition to endpoints typically included in routine toxicity studies (see previous section on Toxicity Assessment), it is recommended that additional evaluations be considered to determine whether administration would affect specific target organs associated with a particular route of administration such as the lungs in inhaled vaccines.

Immunogenicity

Vaccines administered via alternate routes may affect the immune system differently and result in immune responses that involve protective antibodies and/or cellular immunity. Therefore, guidelines indicate that in addition to serological assays, other tests such as T- and B-cell responses and cytokine production should be considered. Assays for the assessment of local and systemic responses at distant sites may also need to be developed.
SPECIAL CONSIDERATIONS FOR PARTICULAR TYPES OF VACCINES

In addition to the safety concerns to be addressed routinely for vaccines in general, there are special considerations that are taken into account for specific types of vaccines such as live attenuated, DNA, viral vectored, and combination vaccines.

Live Attenuated Vaccines

Live attenuated vaccines contain a version of the living virus that has been weakened so that it does not cause serious disease in people with healthy immune systems. Examples of live attenuated vaccines include the measles, mumps, and rubella vaccine (MMR) and varicella (chickenpox) vaccines. Guidelines indicate that the nonclinical evaluation of live attenuated vaccines includes an assessment of the degree and stability of attenuation and assays be implemented that distinguish attenuated from fully virulent and ideally partially virulent strains to assess reversion. The production process is also designed in such a way as to assess the stability of attenuation. The likelihood of exchange of genetic information with nonvaccine strains is also determined. If the wild-type virus is neurotropic or the vaccine has been passaged through neural tissue, health authorities require that an evaluation of neurovirulence during nonclinical development be included in a model capable of distinguishing between wild-type and fully or partially attenuated strains. Small animal models may be acceptable for this purpose provided they are susceptible to wild-type virus. Programs including live attenuated vaccines based on genetically modified organisms also include an environmental risk assessment including the possibility of shedding of vaccine organisms following administration.

DNA Vaccines

Plasmid DNA vaccines are defined as purified plasmid preparations containing one or more DNA sequences capable of inducing and/or promoting an immune response against a pathogen [7]. Guidelines indicate that nonclinical toxicity studies for DNA vaccines following GLP guidelines [13] should be conducted prior to the initiation of Phase I clinical studies. While nonclinical toxicity studies for these vaccines are carefully considered based on product-specific characteristics, planned clinical studies, and previous experience with similar constructs, in general studies are conducted per the recommendations in the WHO guidelines for nonclinical evaluation of vaccines [2]. Per the WHO guideline for DNA vaccines [8], there are several potential safety issues specific to the administration of plasmid DNA into humans: (i) the injected DNA taken up by cells of the host may integrate into the host’s chromosomes and cause an insertional mutagenic event, (ii) the long-term expression of a foreign antigen may result in an undesired immunopathological reaction, (iii) the use of genes encoding cytokines or co-stimulatory molecules may pose additional risks, (iv) antibodies against the injected DNA itself may be formed and these may contribute towards undesired autoimmune reactions; (v) the expressed antigen may itself have biological activity; and (vi) expression of other gene sequences such as those for antibiotic resistance, in mammalian or bacterial cells may pose a risk. Therefore, in addition to studies designed to evaluate systemic toxicity and...
local reactogenicity, regulatory authorities require that studies designed to evaluate plasmid biodistribution, persistence, and integration be conducted using sensitive nucleic acid detection techniques such as quantitative polymerase chain reaction (qPCR)-based assays. Some of the risks associated with DNA integration include tumorigenesis and chromosomal instability and these evaluations serve to determine the tissue distribution following administration and the potential for the vector to integrate into the host genome. A panel of tissues including the blood, heart, brain, liver, kidney, bone marrow, ovaries/testes, lung, draining lymph nodes, spleen, and muscle and subcutis at the site of administration is evaluated. Given that the same plasmids encoding different antigens behave similarly in vivo, health authorities may waive the requirements for biodistribution studies for vaccines for which studies have already been conducted and have an acceptable biodistribution/integration profile. These evaluations are conducted at both early and late time points. The possibility of distribution to or expression in germline cells must be evaluated unless otherwise justified. Persistence of plasmids in gonadal tissue over time would trigger additional evaluations in tissues such as ova and sperm cells as well as studies to determine the effect on fertility and general reproductive function. Embryo–fetal and perinatal toxicity studies may also be required if women of childbearing potential are to be exposed to the product. DNA vaccines utilizing novel vectors, formulations, methods of delivery, routes of administration, or any other modifications expected to significantly impact cellular uptake and/or biodistribution may require these studies and regulatory agencies recommend that input be solicited on specific needs prior to the conduct of any studies. Integration studies are only conducted if it is determined that the plasmid persists at levels greater than 30,000 copies per µg of host DNA by study termination.

In addition, for DNA vaccines that encode multiple antigens, it is required that the immune response generated against a representative subset of the encoded antigens be evaluated. Additional evaluations such as cytokines in vaccines where the genes encoded are immunomodulatory and cases where sequential immunization using more than one type of vaccine will be conducted in a prime-boost regimen should be included based on input from regulatory agencies. Specific studies to evaluate autoimmunity are not recommended but a thorough evaluation of the welfare of the animals should be included in nonclinical toxicity studies.

For biologicals produced using recombinant DNA technology [9] in which the active ingredient is radically altered from the natural substance, nonclinical testing, which may include carcinogenicity, teratogenicity, and effects on fertility in some cases, is conducted on a case-by-case basis with input from regulatory agencies.

**Viral Vectored Vaccines**

Per the EMA guideline on viral vectored vaccines [10], live recombinant vectored vaccines are live viruses that express a heterologous antigen(s). The viral vectors may be replication-defective or replication-competent and some examples of viral vectors being utilized for this purpose are pox viruses, adenoviruses, alphaviruses, measles virus, yellow fever virus, and vesicular stomatitis virus. The antigens may be of viral, bacterial, or parasitic origin but generally derive from those infectious agents for which no effective vaccine exists such as human immunodeficiency virus (HIV), malaria, dengue virus, severe acute respiratory syndrome coronavirus (SARS-CoV), and ebola virus. Because the immune response elicited by live viral
vectored vaccines is specific to both the heterologous antigen and the antigens of the vector itself, evaluations of the immune response to the overall vectored vaccine is required by health authorities. In addition, human virulence of the vaccine may not be predicted from known virulence of the vector alone and studies in appropriate animal models are conducted to evaluate virulence. The nonclinical toxicity studies for live virus vectored vaccines are conducted according to the EMA [10] and WHO guidelines for nonclinical evaluation of vaccines [2], with some exceptions. Regarding species relevance, regulatory guidelines indicate that it is preferable to use the species in which an animal model of disease has been established. When evaluating the immune response to the vaccine, consideration is given to pre-existing immunity and nonprotective immunity to the vector and/or heterologous antigens. The immunogenicity evaluation also includes quantitative as well as qualitative data which characterize humoral, cell-mediated and innate immune responses, as appropriate, to both the intended protective antigen as well as the antigens of the vector. When selecting groups to be evaluated on nonclinical toxicity studies, it is required that a group consisting of the vector alone be included. If an immune response to the vector is found to interfere with the response to subsequent administrations of the vaccine, a single-dose study may be justified. Biodistribution studies are also included in the toxicity evaluation for live recombinant vaccines.

Guidelines indicate that reproductive toxicity studies should be considered in cases where the vaccine may be administered to pregnant women or if biodistribution studies show replication in reproductive organs. When reproductive toxicity studies are required, the vaccine should be administered during the most sensitive period of fetal development.

**Combined Vaccines**

While combination vaccines [11] such as inactive diphtheria, tetanus, and pertussis (DPTT) or live MMR have been available for decades with proven safety records, any new combinations of existing or novel vaccines must be evaluated nonclinically for any effect on immunogenicity, adsorption to aluminum adjuvants, and stability. Health authorities require that the quality of the immune response be evaluated as well as any potential interference and/or incompatibilities between antigens. A thorough evaluation would include a comparison of the combination product to the individual components. The requirement for studies designed specifically to evaluate safety of the combined product is determined on a case-by-case basis but generally should be conducted if there is a known or theoretical risk associated with the combination. Guidelines indicate that nonclinical testing should also be considered in the case where a novel single-component vaccine is developed from a previously licensed combination vaccine.

**SUMMARY**

Beginning in approximately 2003, nonclinical testing guidelines were implemented for vaccines. The first global document was the WHO guideline for nonclinical evaluation of vaccines and this is still considered the gold standard as it is a comprehensive document providing detail on animal models, study design, endpoints, and alternative methods. In addition to the WHO guideline, many guidance documents are also available that outline the
regulatory expectations for traditional vaccines as well as particular types of vaccines such as those that will be administered to pregnant women or women of childbearing potential, those with adjuvants, DNA and viral vectored vaccines, and those combined with other vaccines. It is highly desirable for vaccine manufacturers to apply the same strategies and methods to nonclinical testing of vaccines in the era of global development and marketing. However, while the guidelines for major countries provide fairly standard recommendations, manufacturers should also be aware that some country-specific guidance documents may require additional or different studies than expected by broadly defined requirements for the toxicology programs for vaccines.

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