19

International Safety Regulations for Vaccine Development

Robert V. House
DynPort Vaccine Company LLC, Frederick, USA

19.1 Introduction

The introduction of vaccines in 1796 arguably resulted in one of the greatest increases in survival and quality of life for humanity of any medical intervention in history. The routine use of immunization has spared the lives of countless individuals who would otherwise have died of communicable disease. Unfortunately, this has resulted in an increasing state of complacency, or worse. Vaccines are blamed for maladies without strong scientific evidence, and usually with no evidence at all (Halsey, 2001; Offit, 2011). An anti-vaccine lobby is gaining strength on these specious accusations, with the tragic result that parents are being encouraged to limit or even reject vaccination of their children. Played to its logical conclusion, this trend would almost certainly result in an increase in preventable disease.

19.2 What "Toxicities" have been Attributed to Vaccination?

A variety of maladies have been attributed to the use of vaccination. Before we discuss how toxicity is assessed and how this assessment is regulated, it is useful to examine a few of these attributions.

Nonclinical Safety Assessment: A Guide to International Pharmaceutical Regulations, First Edition.
Edited by William J. Brock, Kenneth L. Hastings and Kathy M. McGown.

19.2.1 Immune System "Overload"

Some have opined that administration of multiple vaccines to children is somehow overloading their immune system, leading to an inability to resist naturally-acquired infections. But can the immune system in fact be quantitatively overloaded? Based on the work of Offit *et al.* (2002), we must consider a few facts. The potential immune repertoire of a neonate is approximately 10^9 to 10^{11} antigens, and the B-cell capability of neonates is approximately 10^7 B-cells per mL of circulating blood. The total "load capacity" of a neonate is therefore approximately 10^4 vaccines simultaneously, assuming 100 antigens/vaccine and 10 epitopes per antigen (10^3 epitopes). However, based on a standard vaccination regimen, children are now exposed to approximately 125 vaccine antigens (although not simultaneously). By contrast, in 1960, there were over 3200 antigens per five vaccines total. In the intervening 50 years there has been no evidence of massive immune dysfunction related to vaccine exposure.

19.2.2 Increase in Allergy/Atopy

Another dubious idea is that vaccination disrupts the immune response in such as way to increase the incidence of allergic disease. Much of the basis for this is the so-called "hygiene hypothesis", which states that preventing childhood infections (which are generally Th1-type immune responses) results in an immune response more strongly biased toward a Th2 (allergic) response. However, this is an immunological fallacy for a variety of reasons (Offit and Hackett, 2003). First, other strong Th2-biased responses (such as immune changes seen in pregnancy) do not predispose toward allergies. Secondly, helminth infections decrease, rather than increase, allergies. Finally, the increased prevalence of Th1 and Th2 conditions are not geographically exclusive. In fact, several major, well-controlled clinical trials have failed to detect a correlation between allergy and vaccination.

This is not to say that allergic reactions to vaccines don't occur; while such reactions are rare (few cases per every 10 000 vaccinations), they can occur to various vaccine components such as animal proteins (ovalbumin, chicken proteins, gelatine, calf lymph); yeast proteins (recombinant products); preservatives (thimerosal); adjuvants (aluminium); and antibiotics. Reactions can also occur to packaging (latex in stoppers). It is important to understand that allergy *to* a vaccine component is not synonymous with allergy caused *by* vaccination.

19.2.3 Autism

Perhaps the most contentious issue regarding vaccination is a supposed association with autism. This association was originally based on a now-notorious paper (Wakefield *et al.*, 1998) which attributed the development of autism to immunization with the Measles-Mumps-Rubella (MMR) vaccine. From this inauspicious beginning, a considerable number of studies on this alleged association have been published, with the great majority clearly discounting any causal association. The issue reached its zenith in 2010 when *The Lancet* published a retraction of the original paper (Anonymous, 2010). Sadly, although the weight of scientific evidence has now refuted any association between vaccination and autism, this is likely not to be the end of the public controversy given the special

interests involved, not the least of which are distraught parents seeking a cause for their children's disorder(s).

19.3 How Vaccines are (Slightly) Different from Other Biopharmaceuticals

Most of the subsequent discussion in this chapter will deal with regulations covering preventive vaccines; that is, vaccines that are developed specifically to prevent infection. Where appropriate, any differences that are specific to therapeutic vaccines will be noted.

Unlike almost all other medicines, preventive vaccines are routinely administered to large numbers of otherwise healthy individuals. Most vaccine recipients are also very young, so consideration must be given to the status of the recipient's developing immune system. Thus, the risk–benefit ratio for vaccines must be weighted even more heavily in the direction of safety. Does this imply that vaccines should be completely safe? Not at all; as with any other medical intervention, vaccines have inherent risks that should be anticipated, tested for and controlled. As with other medical products, it is necessary to establish standardized regulations that provide a clear process for assessing safety, with sufficient flexibility to accommodate the complex biology and immunology that is the basis of immunization.

In the past, safety testing for vaccines was not quite as thorough compared to other medical products since vaccines were generally perceived as safe and were administered at most only a few times over the course of an individual's lifetime. This concept has changed with proliferating technologies that have resulted in a diversity of vaccine candidate formats. Modern vaccines include recombinant proteins and peptides, conjugated molecules, genetic constructs (such as DNA vaccines), and recombinant cells, as well as traditional live and attenuated whole organisms (which are becoming increasingly rare). In addition, recent advances in immunology have demonstrated the importance of innate immunity and have led to an explosion of new adjuvant technologies, which must be evaluated in combination with specific antigens, further increasing the complexity of studies required to assess overall safety.

19.4 Regulatory Framework for Assessing Safety of Vaccines

Vaccine development in the US follows an essentially three-tiered regulatory structure. At the highest level are legal statutes written by Congress and promulgated by the Executive branch. Examples of such statues include the Public Health Service Act (42 USC 262-63) Section 351, the Federal Food, Drug and Cosmetic Act (21 USC 301-392) and the Food and Drug Administration Amendments Act. Beneath this general framework are more specific FDA rules published in the Code of Federal Regulations (CFR). Examples that are pertinent vaccines include Protection of Human Subjects (21 CFR 50), Institutional Review Boards (21 CFR 56); and Good Laboratory Practices (21 CFR 58). These regulations should be familiar to anyone engaged in developing medical products. The most specific understanding of vaccine development comes at the most proximate level, published as FDA guidance documents. The specifics of guidance documents related to safety will be discussed below.

Vaccines are subject to specific regulations enforced by the FDA, and licensure is based on successful submission of a Biologics License Application (BLA). Section 351 of the Public Health Service Act, 42 USC 262 states that "the Secretary shall approve a biologics license application on the basis of a demonstration that the biological product that is the subject of the application is *safe, pure and potent* and the facility in which the biological product is manufactured, processed, packed or held meets standards designed to assure that the product continues to be safe, pure and potent (emphasis added).

For vaccines, the manufacturing and testing necessary to ensure the characteristics of the product remain unchanged and are paramount. In terms of safety assessment for vaccines, toxicological testing in animals forms only a part of the overall continuum of testing, and one must be cognizant of myriad regulations that cover many aspects of the development lifecycle. Testing thus should be developed early in the lifecycle and be robust enough to ensure that product characteristics of safety, purity and potency meet all appropriate standards, not only at the time of licensure, but also continue to meet these standards post-licensure.

What do these terms mean in practice? As defined in the Code of Federal Regulations (21 CFR 600.3(p)), safety is "the relative freedom from harmful effect to persons affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time". Purity is defined in CFR 21 CFR 600.3(r) as "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances". Finally, potency is defined (21 CFR 600.3(s)) as "the specific ability or capacity of the product, as indicated by appropriate laboratory test or by adequately controlled clinical data obtained through administration of the product in the manner intended, to effect a given result". [Note that this "given result" does not necessarily imply the efficacy of the vaccine.] It is appropriate therefore to discuss these preliminary issues of quality before covering more traditional concepts of safety (toxicity) testing.

19.4.1 Quality Testing

A commonly-used expression in vaccine development is "the process is the product". Perhaps more than any other type of medicinal product, including even other biopharmaceutical products, the production process (and consequently the various quality checks to ensure consistent production) of vaccines is intimately associated with the regulatory process and, indeed, the ability to license the product. As stated above, it is crucial that manufacturers are able to demonstrate that a vaccine is safe, pure and potent. The term "safety" in this case subtends a number of different things, all of them covered by various regulations and guidelines. The key concept is "product characterization"; for vaccines, it is crucial that the product's biochemical/biophysical characteristics are well understood and documented, and that the manufacturing process is likewise well characterized to facilitate consistency in product over time. Special considerations should be given to adventitious agent testing, examination for unwanted (extraneous) materials, and evaluation of stability under various storage conditions.

Product characterization is assessed by designing and implementing lot release testing. While the exact list of required lot release assays is dependent on the vaccine itself, some examples include the following:

- *Sterility tests* to detect microbial contamination, whether bacterial or fungal;
- *General safety tests*, to detect extraneous toxic contaminants (a "blunt force" assay generally performed in guinea pigs and mice);
- *Identity tests* to confirm identity of the product. The type of test used is usually dependent on the vaccine characteristics but can include amino acid analysis (AAA), Western blots, SDS-PAGE, or immunologic assays;
- *Potency* measurements (*in vivo* or *in vitro*) to evaluate chemical composition of the vaccine product, its antigen content, and its immunogenicity. These three characteristics are, not surprisingly, closely related.
- *Purity* determinations including percent moisture, presence of endotoxin, and biochemical tests such as SDS-PAGE. Tests should also be included to determine the efficacy of removing process contaminants.

Whereas many vaccines are now based on recombinant proteins or peptides, many still are based on inactivated or attenuated live organisms. Such vaccines represent a difficult challenge; as described earlier, the acceptable risk–benefit ratio for vaccines, while historically high, is now much more conservative. As a consequence, it is crucial that strong demonstration of attenuation and lack of reversion be demonstrated. Various approaches are available to accomplish attenuation with a high degree of confidence (Kimman, 1992; Frey, 2007), and animal models are being developed to assess safety (Levenbook, 2011).

FDA Guidelines that cover various aspects of vaccine manufacture and testing include: Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (FDA, 1985); Guidance for Industry, Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product (FDA, 1999); Draft Guidance for Industry, Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (FDA, 2006a) and Guidance for Industry Process Validation: General Principles and Practices (FDA, 2011). In Europe, guidance is found in: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. CPMP/ICH/138/95 (CPMP, 1995); Note for Guidance on Pharmaceutical and Biological Aspects of Combined Vaccines CPMP/BWP/477/97 (CPMP, 1998) and Guideline on Quality, Non-clinical and Clinical Aspects of Live Recombinant Viral Vectored Vaccines EMA/CHMP/VWP/141697/2009 (EMA, 2010). Additional guidance is found in Guideline of Pharmaceutical Aspects of the Product Information for Human Vaccines. EMA/CMP/BWP/2758/02 (EMA, 2003).

19.4.2 Toxicology Testing

Following the initial safety assessment provided by the battery of tests required to demonstrate purity and characterization, nonclinical safety studies must be performed prior to initiation of human clinical trials. As with any other medicinal product, vaccines must be tested for safety in well-controlled studies. In the US, Federal regulations are quite

specific: 21 CFR 312.23(a)(8) states that " . . . adequate information about the pharmacological and toxicological studies . . . *in vivo* or *in vitro studies* should be conducted on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required vary with the duration and nature of the proposed clinical investigations".

While regulations require that safety testing be performed, regulators have routinely recognized that the complex characteristics of vaccines would preclude the implementation of regimented requirements; thus, vaccines tend to be covered by guidance documents. This flexibility acknowledges that the study design for safety assessment should be case-by-case and driven by not only the unique character of the vaccine candidate, but the body of existing literature to devise a coherent and comprehensive plan.

With this in mind, there are a number of things that should be considered when designing preclinical safety assessments for vaccines. One should bear in mind that preclinical safety studies should help determine a safe and immunogenic dose for human clinical trials and to enable entry into those trials. Carefully-designed preclinical studies will inform what types of safety parameters should be included in clinical studies, as well as identify any potential target organ toxicities. In addition, study designs should incorporate as many clinical parameter considerations as are practical, such as the intended target population (and any existing clinical data or literature that would inform potential toxicities), how the route of administration might affect anticipated toxicity, and any novel or unique features of the vaccine candidate. As an example, a proposed vaccine against botulinum neurotoxin would likely include evaluation for potential neurotoxicity of the vaccine itself.

The balance of this chapter will focus on FDA, EMA, and WHO regulations. These regulations are generally congruent, and in most cases regulatory agencies will default to WHO guidelines where applicable. While regions other than the US and Europe have their own internal regulations governing the development of vaccines, compliance with the three entities listed above is generally necessary to facilitate international trade in vaccines.

19.4.3 General Toxicology Study Design Considerations

Regulatory guidelines for vaccines are relatively harmonized between the US and Europe. In Europe, the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CHMP, 1997) provides a fairly comprehensive description of preclinical safety testing for vaccines; however, this guidance has increasingly been superseded by the WHO guidelines on nonclinical evaluation of vaccines, WHO Technical Report Series No. 927, Annex 1 (2005), which is very similar although slightly more detailed. For US regulations, the WHO guidance is the default regulation. The following sections are based on this guidance.

The obvious first issue in study design is the selection of which animal model to use. The issue of what constitutes a relevant animal species is one that deserves careful consideration prior to any safety assessment. A primary consideration should be that the species chosen should be "responsive" to the vaccine; that is, the animal should develop an immune response following vaccination. Ideally, the animal should be sensitive to the pathogenic organism or toxin against which the vaccine is being developed. In general,

the FDA has required only one relevant animal species, although exceptions might be necessary on a case-by-case basis. The majority of times the relevant model tends to be a small animal (rodents or rabbits); nonhuman primates are generally not used unless the vaccine candidate characteristics dictate this model.

The route of administration, dose and dosing frequency chosen for toxicity studies should duplicate the intended route of clinical administration as closely as possible. This can be difficult when using small animal models, particularly if novel administration devices are employed. Generally, a full human-equivalent dose is preferred by regulators. However, many vaccines are administered in a dose of 500 μL; while this volume can be given intramuscularly in humans, it is impractical for rodents. Dividing this volume into multiple dose sites is one strategy, although admittedly less than ideal. Route of administration can also become a problem with mucosal administration, particularly with nasal vaccines where the human nasal environment is not easily replicated in animals. Finally, dose frequency (for vaccines which require multiple vaccinations, such as recombinant vaccines) might not be practical in animals with a short life span such as rodents. In these cases, an abbreviated or compressed schedule is usually acceptable. Although dose is an important concept in toxicology, it is probably less important for safety assessment of vaccines as long as the amount administered to animals is demonstrably immunogenic.

Vaccine toxicology studies can be conducted using either single-dose or repeated-dose paradigms, depending on existing knowledge about the vaccine candidate. For repeated-dose studies the number of doses should generally be N + 1 (N = number of doses to be given to humans) in an effort to gain a margin of safety, and it is preferable to utilize a range of dose concentrations. Each study should include a concurrent control group (the formulation matrix lacking only the relevant antigen is generally used), and if an adjuvant is employed an adjuvant-only formulation can be used.

Reversibility of any adverse effects should be evaluated in repeated-dose studies. This is generally accomplished using a staged post-mortem design in which toxicologic evaluations are performed shortly (within days) after cessation of exposure, as well as following a recovery period (two weeks is often used). To economize in use of animals and other resources, this recovery group might include only a control and high-dose group.

19.5 Parameters Monitored

End-point assessment for vaccine studies is very similar to any other biopharmaceutical product, with some minor exceptions. First, immunogenicity of the vaccine should always be assessed concurrent with toxicologic end-points; this serves to demonstrate that the vaccine is having its intended effect in conjunction with assessment of any adverse effects. Second, the local toxicity of vaccines should be assessed; this local reaction is generally termed “reactogenicity” and can routinely be measured using a standard or modified Draize scoring system. Local reactogenicity must be determined following administration of a full human dose (same volume to be used clinically) so the animal model must be able to accommodate an injection of this volume.

Routine toxicological end-points are integral to vaccine safety assessment. These include clinical observations, serum chemistries, haematologic analysis, body and organ weights, and histopathology. Histopathology is generally done on the full standard battery

of tissues, with particular emphasis on "pivotal" organs such as the liver, brain, kidneys and so forth. In addition, special emphasis should be placed on histopathology of immune system organs and tissues (spleen, thymus, bone marrow and lymph nodes, both distant and those draining the site of injection).

19.5.1 Safety Testing for Adjuvants

Another way in which vaccines differ from most other medicines is in the use of adjuvants, which act to improve or "boost" immunogenicity, possibly through engagement of the innate immune response (Leroux-Roels, 2010). In general, adjuvants have limited, if any, function exclusive of their role in improving vaccines and, as such, are not licensed separately; rather, adjuvants are licensed in conjunction with the vaccine to which they are added (Sesardic, 2006). In general, regulatory agencies will expect to see data substantiating the need for an adjuvant, particularly in clinical trials; if there is no compelling reason to include an adjuvant, it should not be included. Before a consideration is made to include an adjuvant in a new vaccine candidate, the adjuvant should be tested separately as a new chemical entity, and the toxicology studies employed should be appropriate to the class of compound that the adjuvant represents. Recommended testing approaches for adjuvants are given in Guideline on Adjuvants in Vaccines for Human Use, EMA/CHMP/VEG/134716/2004 (EMA, 2005). While not strictly mandated by regulations, immunotoxicology assessment is a good idea given that the function of an adjuvant is often to "prime" the immune response (Brennan and Dougan, 2005). An explanatory note for "Guideline on Adjuvants in Vaccines for Human Use" was issued by EMA (EMA, 2006).

19.5.2 Reproductive Toxicology

In the United States, reproductive toxicity testing is described in Draft Guidance for Industry: Considerations for Reproductive Toxicity Studies for Preventive Vaccines for Infectious Disease Indications (FDA, 2006b).

Reproductive toxicology has long been a requirement for drug development; however, it is only in the past decade or so that regulations have been promulgated for this type of testing for vaccines. Reproductive toxicology testing is necessary when a vaccine could be administered to females of child-bearing potential; at present (2013) no testing requirements have been established for males regarding vaccine development. Standard reproductive toxicology testing paradigms established for drugs may not be appropriate for vaccines. Rather, studies should use an appropriate animal model, usually a species being used for other safety and efficacy studies. In general, small animal models are employed for these studies, although recently approaches have been proposed using nonhuman primates (Martin and Weinbauer, 2010). Study design is expected to evaluate both embryo–foetal and postnatal/neonatal toxicity. An understanding of the kinetics of antibody formation (and vector clearance in the case of live/attenuated vaccines) is crucial, with peak kinetics of antibody expression occurring during the period of organogenesis. For this reason, selection of animal model becomes of particular importance since the vaccine candidate should be immunogenic in the model of choice. Reproductive toxicity testing for vaccines is generally conducted using Phase 3 human dose and schedule.

19.5.3 Immunotoxicity

While vaccines are clearly intended to produce benefits in humans and animals, paradoxically they could strictly be characterized as immunotoxicants, given that immunotoxicology can be defined as an agent that modulates a resting or nominal immune state in either a positive or negative manner (House and Hastings, 2004). In practical terms, vaccines are evaluated for immunomodulatory activity very early in development, limiting the possibility of adverse immune findings prior to human trials. This is not to say that the possibility of immunosuppression or hyper-stimulation of the immune system should not be considered; in fact, while not mandated by regulations, potential immunotoxicity should be considered. The current thinking on immunotoxicity assessment is covered by ICH S8, Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals (ICH, 2004). This guidance is applicable for both US and European regulatory agencies. ICH M3 (R2), Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH, 2009) also includes wording relevant to immunotoxicity testing, although not specifically in the context of vaccines.

19.5.4 Genotoxicity

In nearly all cases, genotoxicity assessment is not required for vaccines. One notable exception is for vaccines that contain DNA or other genetic elements, such as plasmid DNA. With these vaccines there could be a risk that the genetic elements may become incorporated into the host genome, may become persistent in the host, may cause adverse immunological or immunotoxicological reactions, and may even pose an environmental hazard (Schalk *et al.*, 2006). Multiple guidance documents cover this issue, including Draft Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (FDA, 2007), Guidelines for Assuring the Quality and Nonclinical Safety Evaluation of DNA Vaccines (WHO, 1998) and Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products, CPMP/BWP/3088/99 (CPMP, 2001). In addition, some useful guidance can be found in ICH S6, Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (ICH, 1998) and its addendum (ICH, 2011). Williams *et al.* (2009) have published a useful summary of approaches designed to address genotoxicity issues in vaccines.

19.6 Clinical Safety Assessment of Vaccines

Safety is always of primary importance since vaccines are most often given to healthy individuals. As a consequence, while the overall strategy for clinical trials with vaccines is similar to other medicinal products, there are a few differences. In particular, safety assessment is the principal end-point for both Phase 1 and Phase 2 trials, and remains a key end-point for Phase 3 and for post-licensure assessment. Likewise, immunogenicity is a key assessment factor for Phases 1–3, with efficacy testing usually taking place during Phase 3 trials. A recent review by Marshall and Baylor (2011) provides an excellent overview of the FDA's clinical development expectations for vaccines.

Vaccine safety continues to extend into the post-licensure period, and a variety of programmes are in place or under development to track the long-term consequences of

immunization. Examples include the Vaccine Adverse Event Reporting System (VAERS) (Iskander *et al.*, 2006) and the Vaccine Safety Datalink (Baggs *et al.*, 2011).

19.7 Summary

Vaccines are one of mankind's greatest medical advances, preventing many of the infectious diseases that have ravaged the human population throughout history. However, since they are routinely given to large numbers of otherwise healthy individuals, society has decided that evaluation of these agents must consider a near-zero tolerance for risk. Alarmingly, a vocal and increasingly influential anti-vaccine movement has begun to influence popular perception and understanding of vaccines and vaccinations around the world, often using fear and disinformation in an attempt to reduce or eliminate childhood vaccination. Clearly, there is an urgent need for a rational discussion and efforts to reverse this disturbing trend. A number of individuals have provided reasoned discussions in the literature (c.f., François *et al.*, 2005; Jacobson *et al.*, 2007; Poland *et al.*, 2009); however, these academic treatises are written for other academics. What is desperately needed is a more "popular" defense of vaccines for the general public.

As it turns out, based upon clinical data, vaccines by and large appear to be some of the safest medicinal products. Still, to address the ongoing need to ensure safety, regulations have been promulgated by the world's primary regulatory bodies (FDA and EMA/EMA) and health organizations (WHO). Through years of collaboration, many of these guidance documents and points to consider have been harmonized, providing a corpus of directives to pharmaceutical and biotechnology companies engaged in vaccine development. It is certain that as new technologies for vaccine discovery and development are employed (such as those reported by Momose *et al.* (2010)), these regulatory considerations will have to co-evolve with them. Only by doing so will vaccines continue to provide protection against infectious diseases.

References

Anonymous (2010) Retraction–Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet*, **375**(9713), 445.

Baggs, J., Gee, J., Lewis, E. *et al.* (2011) The Vaccine Safety Datalink: a model for monitoring immunization safety. *Pediatrics*, **127**(Suppl 1), S45–S53.

Brennan, F.R. and Dougan, G. (2005) Non-clinical safety evaluation of novel vaccines and adjuvants: new products, new strategies. *Vaccine*, **23**, 3210–3222.

CPMP (1995) Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. CPMP/ICH/138/95. http://bit.ly/mIaQvg. Accessed June 2, 2011.

CPMP (1997) Note for guidance of preclinical pharmacological and toxicological testing of vaccines. CPMP/SWP/465/95. http://bit.ly/mKUzXg. Accessed April 15, 2011.

CPMP (1998) Note for Guidance on Pharmaceutical and Biological Aspects of Combined Vaccines. CPMP/BWP/477/97. http://bit.ly/jTl8Rq. Accessed June 1, 2011.

CPMP (2001) Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products. CPMP/BWP/3088/99. http://bit.ly/mx0sm2. Accessed May 2, 2011.

EMA (2003) Guideline of Pharmaceutical Aspects of the Product Information for Human Vaccines. EMA/CMP/BWP/2758/02. http://bit.ly/m4moBt. Accessed June 3, 2011.

EMA (2005) Guideline on Adjuvants in Vaccines for Human Use. EMA/CHMP/VEG/134716/2004. http://bit.ly/kGqquE. Accessed April 15, 2011.

EMA (2006) Explanatory Note on Immunomodulators for the Guideline on Adjuvants in Vaccines for Human Use. EMA/CHMP/VWP/244894/2006. http://bit.ly/jlNhUs. Accessed April 15, 2011.

EMA (2010) Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. EMA/CHMP/VWP/141697/2009. http://bit.ly/9HrYJj. Accessed June 1, 2011.

FDA (1985) Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology. http://1.usa.gov/jsp3r8. Accessed April 2, 2011.

FDA (1999) Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product. http://1.usa.gov/ivhkmE. Accessed April 15, 2011.

FDA (2006a) Draft Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases. http://1.usa.gov/ii7Hqn. Accessed May 2, 2011.

FDA (2006b) Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications. http://1.usa.gov/luu4Tw. Accessed May 2, 2011.

FDA (2007) Draft Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. http://1.usa.gov/mFk7nn. Accessed May 2, 2011.

FDA (2011) Guidance for Industry: Process Validation: General Principles and Practices. http://1.usa.gov/hkVV6m. Accessed May 10, 2011.

François, G., Duclos, P., Margolis, H. *et al.* (2005) Vaccine safety controversies and the future of vaccination programs. *Pediatr. Infect. Dis. J.*, **24**(11), 953–961.

Frey, J. (2007) Biological safety concepts of genetically modified live bacterial vaccines. *Vaccine*, **25**(30), 5598–5605.

Halsey, N.A. (2001) Combination vaccines: defining and addressing current safety concerns. *Clin. Infect. Dis.*, **15**(33 Suppl 4), S312–S318.

House, R.V. and Hastings, K.L. (2004) Multidimensional immunomodulation. *J. Immunotoxicol.*, **1**, 123–129.

ICH (1998) ICH Topic S 6. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. http://bit.ly/iTczr1. Accessed May 12, 2011.

ICH (2004) ICH Topic S8 Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals. CHMP/167235/2004. http://bit.ly/k5Hyao. Accessed May 3, 2011.

ICH (2009) Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. S6(R1). http://1.usa.gov/8iyC3h. Accessed May 12, 2011.

ICH Topic M 3 (R2) Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. CPMP/ICH/286/95, 2009. http://bit.ly/jaOqis. Accessed May 15, 2011.

Iskander, J., Pool, V., Zhou, W. *et al.* The VAERS Team (2006) Data mining in the US using the Vaccine Adverse Event Reporting System. *Drug Safety*, **29**, 375–384.

Jacobson, R.M., Targonski, P.V., and Poland, G.A. (2007) A taxonomy of reasoning flaws in the anti-vaccine movement. *Vaccine*, **25**(16), 3146–3152.

Kimman, T.G. (1992) Risks connected with the use of conventional and genetically engineered vaccines. *Vet. Q.*, **14**(3), 110–118.

Leroux-Roels, G. (2010) Unmet needs in modern vaccinology: adjuvants to improve the immune response. *Vaccine*, **28**(Suppl 3), C25–C36.

Levenbook, I. (2011) The role of non-human primates in the neurological safety of live viral vaccines (review). *Biologicals.*, **39**(1), 1–8.

Marshall, V. and Baylor, N.W. (2011) Food and Drug Administration regulation and evaluation of vaccines. *Pediatrics*, **127**, S23–S30.

Martin, P.L. and Weinbauer, G.F. (2010) Developmental toxicity testing of biopharmaceuticals in nonhuman primates: previous experience and future directions. *Int. J. Toxicol.*, **29**, 552–568.

Momose, H., Mizukami, T., Ochiai, M. *et al.* (2010) A new method for the evaluation of vaccine safety based on comprehensive gene expression analysis. *J. Biomed. Biotechnol.*, **2010**, 361841.

Offit, P.A. (2011) Why are pharmaceutical companies gradually abandoning vaccines? *Health Affairs*, **24**, 622–630.

Offit, P.A. and Hackett, C.J. (2003) Addressing parents' concerns: do vaccines cause allergic or autoimmune diseases? *Pediatrics*, **111**, 653–659.

Offit, P.A., Quarels, J., Gerber, M.A. *et al.* (2002) Addressing parents' concerns: Do multiple vaccines overwhelm or weaken the infant's immune system? *Pediatrics*, **109**, 124–129.

Poland, G.A., Jacobson, R.M., and Ovsyannikova, I.G. (2009) Trends affecting the future of vaccine development and delivery: the role of demographics, regulatory science, the anti-vaccine movement, and vaccinomics. *Vaccine*, **27**(25–26), 3240–3244.

Schalk, J.A., Mooi, F.R., Berbers, G.A. *et al.* (2006) Preclinical and clinical safety studies on DNA vaccines. *Hum. Vaccin.*, **2**(2), 45–53.

Sesardic, D. (2006) Regulatory considerations on new adjuvants and delivery systems. *Vaccine*, **24S2**, S2/86-S2/87.

Wakefield, A.J., Murch, S.H., Linnell, A.J. *et al.* (1998) Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet*, **351**, 637–641.

WHO guidelines on nonclinical evaluation of vaccines WHO Technical Report Series No. 927, Annex 1 (2005). http://bit.ly/ksVaWB. Accessed May 5, 2011.

WHO Guidelines for Assuring the Quality and Nonclinical Safety Evaluation of DNA Vaccines (1998). Geneva, Switzerland. http://bit.ly/m3Hz1H. Accessed May 10, 2011.

Williams, J.A., Carnew, A.E., and Hodgson, C.P. (2009) Plasmid DNA vaccine vector design: impact on efficacy, safety and upstream production. *Biotechnol. Adv.*, **27**, 353–370.