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The contemporary vaccine development process involves sequential assessment of safety, immunogenicity, and efficacy in phase 1, 2, and 3 clinical trials. The process is not always linear or straightforward, and adaptive flexible clinical trial designs can increase the likelihood of a successful outcome. Because the efficacy of vaccines for a number of the diseases discussed in this volume cannot be tested directly in humans, alternative approaches have been developed to address this challenge. The major elements of typical clinical trial protocol are discussed, and a checklist of essential documents supporting the trial is provided. As novel vaccine approaches and technologies emerge, the regulatory and ethical considerations will need to be revisited and adapted to respond to the ever-changing landscape.

INTRODUCTION

Vaccines are considered among the most valuable and cost-effective tools for the control of infectious diseases; indeed, universal immunization of infants and children against a variety of pathogens was considered one of the top ten achievements of the 20th century (CDC, 1999). Major scientific, technical, ethical, and regulatory principles and/or processes underpinning the vaccine development and evaluation process have been defined; however, challenges posed by the threats of bioterrorism and emerging infectious diseases, as well as technological advances in the composition, formulation, and delivery of vaccines, expose gaps in policy and practice that require ongoing consideration and refinement of approaches to the evaluation of new vaccines. The purpose of this chapter is to provide a brief overview of the approach to the evaluation of candidate vaccines in humans, with a particular attention to issues related to vaccines for biodefense and emerging and neglected diseases. The major focus will be on clinical trials and protocol development in the US, although many of the principles, regulations, and practices have been harmonized across the globe. A brief overview of related regulatory issues and preclinical vaccine development is provided; however, detailed discussions of these topics can be found elsewhere in this volume.

HISTORICAL CONSIDERATIONS

In the US, vaccines are regulated as biologicals, although vaccines are legally defined as drugs under the Food, Drug and Cosmetic Act (FDCA). The ethical principles, and the regulatory agencies and requirements that guide product development and clinical research in the 21st century evolved progressively over the 20th century. In many cases, the development of codified guidelines and the establishment of a regulatory authority were spurred by a tragic accident or a clear ethical breach (for reviews of Historical Considerations, see Baylor and Midthun, 2004; Borchers et al., 2007).

Regulatory Issues

In 1901, a number of children became ill and died after treatment with diphtheria antitoxin contaminated with tetanus toxin. This episode resulted in the first legislation designed to regulate the purity and potency of biologicals—the Biologics Control Act (BCA) of 1902. In 1938, over 100 people died after ingesting an elixir of sulfanilamide containing diethylene glycol (antifreeze), leading to the enactment of the FDCA (Ballentine, 1981). Provisions of the FDCA required manufacturers to submit safety data to the Food and Drug Administration (FDA) prior to registration. The 1962 Kefauver–Harris Amendments to the FDCA required that efficacy data also be submitted. The 1944 Public Health Service Act incorporated the BCA into section 351 of the US Code of Federal Regulations (CFR), which gave the federal government the authority to license biologicals and manufacturing facilities. In 1955, incomplete inactivation of a poliovirus vaccine resulted in the development of polio in a number of children (the “Cutter Incident”); this led to the establishment of the Division of Biologics Standards (DBS) within the NIH (Offit, 2005). The authority vested in the DBS was transferred to the FDA in 1972. The organization that is currently responsible for regulating biologics is the Center for Biologics Evaluation and Research (CBER) at the FDA. Title 21 of the CFR contains regulations pertaining to biologicals, including product standards, manufacturing, labeling, licensing, advertising, investigational use, and protection of human subjects (informed consent, nonclinical laboratory studies, and Institutional Review Boards, or IRBs). Part 312 of Title 21 CFR contains regulations related to the Investigational New Drug Application (IND). The CFR is updated each year to reflect changes in policies and procedures. Additional guidance documents related to vaccines are published by the FDA, as appropriate.

During the 1990s, an international group comprised of scientists, regulators, ethicists, and pharmaceutical representatives convened to discuss standards for designing, implementing, documenting and reporting clinical trials. The International Conference on

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Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) established guidelines for the conduct of clinical research (Good Clinical Practice, or GCP), many of which have been adopted by the US FDA (FDA, 1996). The ICH GCP provides a unified standard to ensure the quality of clinical trial data and the protection of human subjects for the European Union, Japan, and the US for clinical trials that will support licensure of new vaccines and drugs. For research related to the evaluation of vaccines for emerging and neglected diseases, implementation of GCP in developing countries may pose unique challenges. Acosta et al. (2007) conducted a multinational clinical trial of Vi polysaccharide typhoid fever vaccine in Asia among 200,000 individuals, during which implementation of GCP required adaptations in order to comply with the goals of the guidelines.

**Ethical Issues**

The revelation that Nazi physicians tortured and experimented on prisoners during World War II culminated in the publication of the Nuremberg Code in 1947 (Macrae, 2007). The Code is recognized as the first set of ethical guidelines for human research to be recognized by the international community. In 1965, the Declaration of Helsinki articulated additional responsibilities of investigators to research subjects, and paved the way for the development of IRBs.

Continued violations of informed consent were summarized in a *New England Journal of Medicine* article written by Dr. Henry Beecher (1966). This article and the subsequent expose of the ethical violations perpetrated during the Tuskegee Syphilis Study led to the 1974 National Research Act, which established IRBs and the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. After considering the principles and practices of human research in the US, the commission published the Belmont Report in 1979. This report defined the three major ethical principles (and their related clinical trial applications) pertaining to human research: respect for persons (informed consent); beneficence (risk-benefit assessment); and justice (equitable subject selection). Recommendations of the National Bioethics Commission from 1981 related to research involving human subjects were adopted into federal law in 1991. The so-called Common Rule (45 CFR 46 Subpart A) articulated the federal policy for protection of human subjects who are participating in clinical trials. Additional protections for pregnant women and fetuses, prisoners, and children are outlined in Subparts B, C, and D of 45 CFR 46. The Council for International Organizations and Medical Sciences, formed in 1949, recently published updated international ethical guidelines for research involving human subjects (CIOMS, 2002).

It is abundantly clear that the ethical considerations and regulatory guidelines for the conduct of clinical research are inextricably intertwined: ethical considerations drive the need to regulate the conduct of clinical trials to ensure the protection of human subjects and the quality and integrity of the data generated. Seven ethical requirements that should be fulfilled in the conduct of clinical research have been proposed by Emanuel et al. (2000): the research should provide information that will advance science and knowledge (value); scientific validity; fair subject selection; favorable risk-benefit ratio; independent review; informed consent; and respect for enrolled subjects. The increasing complexity and redundancy of the oversight of contemporary clinical trials reflects the commitment to accomplish these goals. A simplified prototypical organizational structure for a vaccine research clinical trial is shown in Fig. 12.1. Further details regarding the roles and responsibilities of the participants in the clinical trials process are provided below.

**STAGES OF VACCINE DEVELOPMENT**

**Overview**

The major stages of vaccine development are outlined in Fig. 12.2. The initial stage is referred to as the pre-IND (investigational new drug) stage. Activities conducted in this stage culminate in the production of a vaccine that can be evaluated in humans—the IND stage. Vaccines that are shown to be safe, pure, and effective in humans may then be licensed for use (licensing and postmarketing stage).

**Pre-IND Stage**

Once a public health need for control of a disease by means of vaccination has been identified, detailed studies designed to understand the pathogenesis of infection and to identify immune responses associated with protection are conducted. Studies may involve assessment of infection and immunity in humans if the disease occurs naturally at a frequency high enough to permit their evaluation (interpandemic influenza, malaria, tuberculosis, and others); however, detailed evaluations of other rare and lethal diseases—particularly those included in the Centers for Disease Control and Prevention (CDC) Category A biothreat list—must
be studied in animal models that might predict the pathology and immune responses that occur in humans (smallpox, tularemia, inhalational anthrax, hemorrhagic fever viruses, etc.). Candidate vaccine formulations containing or expressing epitopes that can elicit immune responses associated with protection are then designed and evaluated in vitro and in animals, where their safety and immunogenicity profiles are established. A critical part of the pre-IND stage is the development and validation of a manufacturing process.

**IND Stage**

Candidate vaccines typically undergo a sequential series of evaluations in humans. Three major phases are identified, as summarized in Table 12.1. In practice, the scheme is not so straightforward: phase 1 and phase 2 clinical trials may overlap or be combined,
and multiple phase 1 and/or phase 2 evaluations may be necessary for adjustment of dosage, change in regimen, and evaluation in other age and/or risk groups, even after pivotal phase 3 studies have demonstrated efficacy. Close and ongoing communication between the sponsor, regulatory agencies, investigators, and IRBs is critical throughout the IND stage, including pre-IND meetings, end-of-phase 2 meeting, pre-Biologic Licensing Application (BLA), and BLA meetings to facilitate the timely progression through the clinical trials process.

Phase 1 Clinical Trials

The first evaluation of a candidate vaccine in humans is referred to as a phase 1 clinical trial. Typically less than 100 healthy young adult subjects are enrolled in phase 1 trials. In fact, the number may be considerably smaller for novel products, in which case investigators may elect to inoculate only a handful of subjects to exclude the possibility of unexpected reactogenicity or toxicity (Keitel et al., 1993a). The major goal of the phase 1 trial is to assess safety and tolerability; however, preliminary immunogenicity assessments and dose-ranging information can provide valuable information regarding dosage selection for subsequent evaluations. Although the design of phase 1 clinical trials of drugs often is open-label, many contemporary phase 1 clinical trials of candidate vaccines are randomized, double-blind, placebo-controlled trials. The phase 1 trial may be performed in stages, where a small cohort of subjects is vaccinated and observed for a period of 1–4 or more weeks before the remainder of the study cohort is vaccinated in stage 2. This approach reduces the exposure of subjects to the occurrence of unexpected toxicity. For vaccines that are produced in the US whose ultimate use will be in other countries (such as malaria vaccines), the initial evaluation occurs in the US (phase 1a), followed by phase 1b testing in a healthy population in the target country. Inclusion of placebos in this and other phases of development reduces bias in the assessment of adverse events (AEs) and serious AEs (SAEs), and provides internal controls for laboratory assessments of immune responses following immunization.

Because the number of subjects enrolled in a phase 1 trial is small, these studies lack statistical power to detect AEs that occur at a low rate. Nevertheless, small, carefully monitored phase 1 trials can identify unexpected and/or unacceptable toxicities that require reformulation or reevaluation of a potential candidate (Keitel et al., 1999; Edelman et al., 2002). In some circumstances, specific monitoring of subjects in phase 1 or phase 2 trials for evidence of infection after immunization is relevant, particularly if there are concerns that the vaccine might elicit immunopathologic responses when the vaccinated subject is naturally infected. Immunopathology resulting in severe atypical infection and/or death occurred among persons immunized with an inactivated measles vaccine and in infants given an inactivated vaccine to prevent respiratory syncytial virus (RSV) vaccine (Polack, 2007). Similar concerns exist regarding the potential for dengue virus and SARS coronavirus vaccines to elicit immunopathologic responses (Edelman et al., 2003; Deming et al., 2006).

Although not specifically relevant for this chapter, an exploratory IND study may precede a typical
Phase 1 evaluation for the assessment of drugs and therapeutic biologicals (FDA, 2006). The exploratory IND study option is characterized as a small early phase 1 trial that has no therapeutic or diagnostic intent; rather, the goal is to assess feasibility for further development of a drug or biological. Goals of this type of study may include determination of the mechanism of action of a drug in humans; evaluation of the pharmacokinetic profile of the agent; selection of one from a group of promising candidates; and exploring biodistribution characteristics using imaging techniques. Because these studies pose lower potential risk to subjects, exploratory IND studies require less or different preclinical support than typical phase 1 studies.

Phase 2 Clinical Trials

Vaccine candidates that have favorable safety and immunogenicity profiles in phase 1 trials may progress to expanded phase 2 trials. Several hundred healthy subjects may be enrolled into phase 2 trials. The major goals of phase 2 trials are to assess safety and to develop optimal regimens for immunization. Typically several dosage levels are evaluated; different immunization regimens (number of doses and interval between doses) also may be explored. Most phase 2 trials are randomized, double-blind, placebo-controlled trials. Although larger numbers of subjects are evaluated, phase 2 trials still lack statistical power to detect events that occur in a low proportion of subjects. Initial phase 2 trials are usually conducted among persons who are at low risk for acquiring the target disease; therefore, additional phase 1 and phase 2 trials may be necessary to assess safety and immunogenicity among populations who are at high risk for the disease, in which pivotal efficacy trials will be conducted. For several emerging and neglected diseases, the preliminary efficacy of a vaccine can be assessed in a human challenge model, including malaria, influenza, and cholera (Couch et al., 1971; Epstein et al., 2007; Tacket et al., 1999). These carefully controlled experimental inoculations of subjects with the target pathogen can provide proof of concept that immunization with a vaccine candidate confers protection prior to large phase 3 clinical trials. In the pediatric population, rechallenge of vaccinated subjects with homologous or related live attenuated influenza virus vaccines (LAIV) provided supportive evidence that intranasal immunization with LAIV would confer protection against subsequent infection with wild-type influenza viruses (Belshe et al., 2000). Phase 2 trials may also incorporate plans to assess the concomitant administration of other vaccines, biologicals, or medications. For example, administration of a new vaccine to infants in the context of the increasingly complicated childhood immunization schedule poses complex problems; evidence that simultaneous administration of licensed and experimental vaccines does not interfere with protective responses to components in either product is necessary (Rennels et al., 2000).

Phase 3 Clinical Trials

Once a vaccine candidate has been shown to be safe and to possess acceptable reactogenicity and immunogenicity profiles, assessment of the efficacy can be undertaken in phase 3 clinical trials. The type and size of the population to be studied will depend on epidemiology of the target disease (population at risk for disease and the disease attack rate) and the level of protection conferred via immunization. A phase 3 clinical trial for prevention of disease that occurs at a low incidence may require hundreds of thousands of subjects, such as the US field trial of inactivated poliovirus vaccine in the 1950s (Francis, 1955); whereas phase 3 trials of vaccines for prevention of high-incidence infections (such as RSV in infants) theoretically would require no more than hundreds of subjects, particularly if the vaccine was expected to be highly efficacious.

The assessment of efficacy of vaccines against agents of bioterrorism, as well as vaccines for candidate pandemic influenza, may be problematic. For example, smallpox, inhalational anthrax, hemorrhagic fever viruses, tularemia, plague, and influenza A/H5N1 do not occur naturally at a frequency high enough to permit controlled evaluation of clinical vaccine efficacy prior to licensure of vaccines. The FDA final rule entitled “New Drug and Biological Drug Products: Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible” (otherwise referred to as the “Animal Rule”) was published to address this circumstance (FDA, 2002). The rule permits use of animal efficacy data when collection of human efficacy data is not feasible. Safety and immunogenicity data in humans and animal efficacy data can be used to support licensure when several conditions are met, as follows: (1) the pathophysiology of the infection is reasonably well understood; (2) the pathogenesis and immune responses in one or two animal species are expected to predict these responses in humans; (3) the immunogenicity endpoint(s) correlated with protection in the animal(s) are related to human immune responses; and (4) immunogenicity

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endpoints in animals and humans are sufficiently well understood to permit selection of a regimen that would be expected to predict protection in humans. For other diseases where correlates of protection are reasonably well defined (for example, serum HAI antibody level against influenza viruses), surrogate markers can be used to support licensure (FDA, 2007a). Finally, in order to facilitate the approval of vaccines for such severe, life-threatening illnesses, additional mechanisms for expedited review and accelerated approval have been developed by the FDA.

Phase 4 Clinical Trials

Regulatory authorities are increasingly requiring additional studies to be conducted after market approval. Phase 4 studies may also be initiated by the sponsor for a variety of reasons. While a vaccine appears safe after it has been studied in thousands of individuals, rare adverse events may only be observed after hundreds of thousands or even millions of people have been vaccinated, as occurred with the first-generation live attenuated rotavirus vaccine (Murphy et al., 2001). The long-term safety or continued efficacy of a vaccine may be unknown at the time of licensure, and the benefit of immunization of special populations that may have been underrepresented or not studied in the IND stage may be of interest. Unfortunately, phase 4 clinical trials typically are not randomized, and they may be uncontrolled. Nevertheless, several safety surveillance systems have been established to facilitate the early detection of potential rare, serious reactions to vaccines, including the Vaccine Adverse Event Reporting System (VAERS), the Vaccine Safety Datalink (VSD), and the American Academy of Pediatrics Practice Research Office Settings (PROS) (Ellenberg et al., 2005). Design options for phase 4 studies include case-control studies and cohort studies.

TABLE 12.2 Checklist of essential clinical trial protocol elements

| Title page          | Statement of compliance         |
|---------------------|---------------------------------|
| Signature page      | Protocol summary                |
| Background and rationale | Purpose and objectives        |
| Study design and endpoints | Study population: Description, inclusion/exclusion criteria; recruitment and retention |
| Study agent/interventions | Study agent/interventions |
| Study procedures/evaluations | Study schedule                |
| Study schedule      | Assessment of safety: Safety parameters, reporting requirements, halting rules |
| Clinical monitoring structure: Site/safety monitoring plan, and safety reviews | Statistical consideration: Sample size; data analyses, etc. |
| Quality control and quality assurance | Ethics/protection of human subjects: IRB, informed consent; confidentiality, etc. |
| Data management     | Appendices: Personnel roster; table of procedures; lab processing flow sheet, etc. |

Source: Adapted from NIAID protocol template guidance (NIAID, 2006). Courtesy: NIAID.
scientific rationale for selection of the vaccine candidate should be discussed. A concise description of the study agent should be provided, including summaries of preclinical studies and relevant clinical studies. Finally, potential risks and benefits of immunization with the investigational agent should be delineated.

Objectives and Purpose

The objectives and purpose of the trial should be clearly and explicitly stated. For phase 1 trials, the primary objectives will be to assess the safety, tolerability, and reactogenicity of a vaccine, whereas assessment of immunogenicity is a secondary objective. For combined phase 1/2 and phase 2 clinical trials, safety and immunogenicity may be coprimary endpoints. Exploratory endpoints may also be included, such as the effect of age, race, or gender on immune responses (Keitel et al., 2006). Combined phase 1/2 trials may be proposed when the vaccine candidate represents a variant of a previously licensed construct, such as a subvirion influenza vaccine for prevention of avian influenza (Treanor et al., 2006). Efficacy and safety typically are the primary endpoints for phase 3 trials.

Study Design

The study design then should be described. For clinical trials of candidate vaccines, the study design typically is a randomized, double-blind, controlled clinical trial—one example of a parallel group design. Phase 1 safety and tolerability studies often utilize a titration design, where ascending dosages of the experimental agent are sequentially administered to new cohorts of subjects (Gorse et al., 2006). Cluster-randomized clinical trial design is occasionally employed to assess vaccine efficacy. In this circumstance, larger groups of individuals (such as nursing homes or schools) are randomized to an intervention, rather than individual subjects, and the clinical endpoints are ascertained for vaccinated subjects (Rodrigues et al., 2005), or for a subset of the cluster, such as the contacts of healthcare workers in a closed setting (Hayward et al., 2006). Phase 1 clinical trials historically were open-label; however, in recent years most phase 1 clinical trials of vaccines have been randomized and blinded. The value of a placebo control in clinical trials has been described; however, a licensed control vaccine may be used rather than a placebo, particularly for phase 3 clinical trials in children. In a recent phase 3 clinical trial of pneumococcal conjugate vaccine in infants, a meningococcal type C vaccine served as the control vaccine (Black et al., 2000). In this case, the meningococcal control vaccine provided potential benefits to the study participants. Flexible adaptive design methods frequently are employed in the development of vaccines; these incorporate plans for modifications of the clinical trial design that are made before or during the conduct of the research (Chow and Chang, 2007). Adaptations to ongoing trials may include prospective adaptations, such as interim analysis, stopping rules for early termination due to futility/safety concerns/efficacy, or sample size re-estimation; ad hoc adaptations such as changes in inclusion and exclusion criteria, dosage or regimen alteration, and trial duration; or retrospective adaptations at the end of the study but before unblinding, including changing the study endpoint or altering the statistical hypothesis (superiority to noninferiority). The goal of this approach is to permit modification based on accumulated evidence to alter trial design to increase the probability of success without undermining the validity of the trial (Gallo et al., 2006). Such adaptations may require modifications of the study hypotheses, protocol amendments, and sample size recalculations.

Clinical trials are also classified as single-center or multicenter studies. Phase 1 studies often are single-center studies; however, multicenter trial design may be used for any phase of clinical vaccine development. Multicenter study design provides several advantages: enrollment of subjects is expedited, and the results of the trial are likely to be more generalizable. Study endpoints need to be clearly identified. For phase 1 trials, safety, tolerability, and reactogenicity primary endpoints may include the frequencies and severities of injection site reactions (pain, tenderness, redness, and swelling) and systemic reactions (fever, chills, headache, myalgia, arthralgia, etc.), as well a laboratory evidence of adverse reactions (hematologic, biochemical, and other). For phase 2 clinical trials, specific immune responses at defined time points after immunization typically characterize the primary endpoints; safety and reactogenicity may be primary or secondary endpoints. For phase 3 clinical trials, protection against laboratory-confirmed infection and/or disease is the primary endpoint, and the major safety assessment may be the frequency of SAEs associated with administration of the investigational agent.

Study Population

A detailed description of the proposed study population and the number of subjects to be studied must be provided; specifically, characteristics (age range,
health status, ability to provide informed consent, etc.) of potentially eligible persons (Inclusion Criteria) and factors that would render an individual ineligible (Exclusion Criteria) should be explicitly enumerated. For some phase 1 trials, screening for eligibility may include medical history, physical examination, and laboratory screening for evidence of good health (normal hematologic and biochemical parameters, and no evidence of active hepatitis B, hepatitis C, or HIV infection). Information regarding serosusceptibility to the candidate pathogen may be necessary. For example, a phase 1 clinical trial of a dengue virus vaccine may require evidence of no prior infections caused by these viruses (Edelman et al., 2003), and a phase 1 or 2 clinical trial assessing the immunogenicity of LAIV may focus on persons with low or absent levels of preexisting immunity to the candidate vaccine (Keitel et al., 1993b). For phase 3 clinical trials, it is necessary to identify a population in which the infection or disease occurs at a high enough frequency to assess the ability of a vaccine to protect. For example, pivotal phase 3 trials of an inactivated hepatitis A vaccine were conducted in specific US communities where the rate of hepatitis A infections in children was high (Werzberger et al., 1992).

Human subjects considerations may include a description of certain behaviors and/or concomitant medications that would exclude a subject. For most clinical trials of vaccines, women who are capable of bearing children must consent to certain birth control measures. The US Department of Health and Human Services (DHHS) has published a guidance regarding research in pregnant women: for research conducted in this population, there must be direct benefit to the woman or her fetus or there must be only minimal risk to the fetus, and information cannot be obtained any other way. For many phase 1 clinical trials, use of prescription medications is not permitted. Clinical trials of vaccines that potentially could be transmitted to others in the community raise special concerns. Recent reevaluations of smallpox vaccines posed concerns with regard to transmission of the vaccine virus from subjects to their contacts (Frey et al., 2002). In this case, persons who had household or other significant contacts with young infants, people with eczema, pregnant women, and immunocompromised individuals were excluded from participation.

The methods for test article allocation should also be described. For most phase 1 and phase 2 clinical trials of vaccines, the subjects are randomized to receive one of several dosage levels of vaccine or placebo. Ideally, randomization should not occur until the subjects have been qualified for participation. Typically the randomization occurs in blocks of a prespecified number that represents a multiple of the number of test articles. For example, if there were four dosage levels of vaccine and a placebo, then the block size might be 5, 10, or 15. If the block size chosen were 5, then the subjects would be randomized 1:1:1:1:1. Block randomization can reduce the risk of unequal group sizes. In some circumstances the probability of receiving one product differs from the probability of receiving another. For example, in an efficacy study to be conducted in children, an investigator may wish to reduce the number of subjects randomized to receive the placebo, and the randomization scheme selected may be 2:1 (vaccine:placebo). The vaccine group assignments for subjects should be concealed from the subjects and from investigators to reduce bias in the assessments performed after vaccination (so-called double blinding). Additional measures can be taken to reduce the potential for imbalances in baseline characteristics of enrolled subjects, such as stratification of subjects according to age, prior receipt of a related vaccine, etc., prior to randomization.

Study Agent/Interventions

The clinical trial protocol should contain basic information regarding the characterization of the vaccine formulations—including dosage(s), packaging, labeling, storage; preparation, administration, dosing, and accountability methods for each study product, including placebo and/or control preparations. More complete descriptions of study vaccines, including manufacturing information, preclinical and clinical safety, immunogenicity, and efficacy should be provided separately in the Investigators’ Brochure (IB). Information regarding the use of concomitant medications, including prohibited medications, should be detailed. For example, during phase 1 clinical trials concomitant use of prescription medications may be prohibited. During phase 2 clinical trials, concomitant use of certain medications may be allowed, such as antihypertensive medications or antidepressants. In general, concomitant use of immunosuppressive, immunomodulatory, or cytotoxic drugs would be prohibited in any clinical trials of live attenuated vaccines.

Study Procedures and Evaluations

A description of the proposed clinical evaluations then follows. In phase 1 or 2 clinical trials, detailed and frequent physical assessments of the injection site and systemic responses may be indicated, as well as review of subject records of clinical responses following
immunization. The intensity of study assessments will vary according to the nature of the study product: more frequent and detailed assessments would be indicated for novel products whose safety profile is undefined. Periodic collection of blood, nasal, fecal, or other samples to assess for the occurrence of toxicity, or to determine the frequency, magnitude, and/or duration of shedding of a live vaccine candidate may also be indicated (Piedra et al., 1993; Taylor et al., 1997). These laboratory assessments should be tailored to the particular needs of the protocol, and should be based on the pathogenesis of the disease, the vaccine under evaluation, and information collected in the pre-IND stage. Brief descriptions of the type(s) of specimens to be collected, methods for specimen collection, preparation, handling, storing, and shipping (if applicable) should be outlined; detailed procedures should be provided in a separate Manual of Procedures (MOP) for each study. For phase 3 trials, clinical follow-up is specifically targeted at ascertaining whether the vaccine prevents infection or disease and capturing the occurrence of SAEs; however, limited prospective safety assessments may be included (perhaps only in a subset of subjects) to expand the safety database (Oxman et al., 2005).

Study Schedule

Once the specific clinical and laboratory procedures for assessing safety, immunogenicity, and/or efficacy have been described, a detailed study schedule indicating the timing of various assessments/procedures/interventions should be outlined, including those that will be conducted at screening, enrollment, and each follow-up visit. Clinical assessments and laboratory procedures that should be completed in the event the subject’s participation in the trial is terminated early should be described, as should those for women who become pregnant during the study. Finally, a description of clinical and laboratory assessments to be performed in the event the subject returns for an unscheduled visit (for example, for a severe vaccine reaction) may be appropriate. A summary table or figure outlining study procedures is particularly useful.

Assessment of Safety

As described above, the clinical trial protocol should detail the specific safety parameters that will be monitored during the course of the trial, along with the methods and timing for their assessment and reporting. Standardized definitions for AEs and SAEs have been formulated (FDA, 1996): an AE is defined as any untoward medical event that occurs in a subject who has received a study agent. SAEs include death, life-threatening events, hospitalization or prolongation of hospitalization, congenital anomalies, and events that result in permanent disability. Each AE and SAE that is recorded should be assessed for its severity and relationship to vaccination. The grading of AEs is usually based on the interference with activity, where 1 = mild, does not interfere with usual activities; 2 = moderate, interferes somewhat with usual activities; and 3 = severe, incapacitating. The severity grading system of laboratory abnormalities should be established before the initiation of the trial. The FDA has suggested guidelines for toxicity grading scale for healthy adults and adolescents enrolled in vaccine clinical trials (FDA, 2007b).

Contemporary standards for assessing the relationship of the AE to immunization have narrowed the choices to “associated” and “not associated.” All AEs that are temporally related to administration of the investigational agent and have no alternative etiologies to explain the event are considered associated. SAEs should be reported promptly to the sponsor; in turn, the Sponsor must notify the FDA in a timely fashion.

Prospective guidelines for discontinuation of individuals from further vaccinations, as well as halting rules for the clinical trial should be outlined. Typical circumstances for discontinuation of an individual include severe and/or hypersensitivity reactions following immunization, development of an Exclusion Criterion during the trial, and failure or inability of the subject to comply with study procedures. Note that every attempt should be made to continue to follow these subjects for safety assessments. Rules for halting a phase 1 clinical trial may include the occurrence of one or two hypersensitivity reactions or SAEs associated with the investigational agent or the occurrence of moderate or severe reactogenicity among a predefined proportion of subjects.

Finally, the safety oversight plan should be described. For single-center trials or other small clinical trials, a Safety Monitoring Committee (SMC) consisting an Independent Safety Monitor (ISM) from each site and an independent member with expertise relevant to the protocol has the responsibility to review trial results periodically and on an ad hoc basis, and to make recommendations to the Sponsor regarding the conduct of the clinical trial. For larger, multicenter trials, a Data and Safety Monitoring Board (DSMB) consisting of site ISMs and individuals with clinical and statistical expertise relevant to the protocol is constituted to review study progress and advise

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the Sponsor. The SMC or DSMB may recommend terminating a trial because of unexpected or severe toxicity; continuation of a trial after review of AEs that triggered halting rules; alteration of sample size based on interim analyses; or any other protocol modifications that are deemed necessary to complete the trial successfully.

Clinical Monitoring

A clear plan for monitoring the conduct of the clinical trial site(s) should be outlined in the study protocol. Specific objectives of site-monitoring visits are to review all study documentation to ensure protection of human subjects; compliance with GCP, clinical and laboratory procedures, test article administration and accountability guidelines; and accurate and complete data collection and documentation. The Sponsor may conduct monitoring visits, and may also designate an independent Contract Research Organization (CRO) to conduct monitoring visits on a regular basis throughout the trial. Early monitoring visits can be particularly valuable in order to identify systematic, unintentional deviations from the protocol.

Statistical Considerations

A detailed Statistical Analysis Plan (SAP) should be prepared that restates the study hypotheses, objective and endpoints, describes the statistical basis for the sample size selected, and outlines the methods that will be used to analyze safety and efficacy. If interim analyses are planned, then the statistical issues related to this should be discussed.

Quality Management

The clinical trial site is responsible for protocol compliance and accurate and complete data collection and recording. In a separate document, site-specific Standard Operating Procedures (SOPs) should outline the methods that will be used to ensure that these activities are accomplished, as well as methods to ensure appropriate training of the study staff. The overall quality management plan should be described in the protocol.

Ethics/Protection of Human Subjects

A description of the ethical standards that will be followed to ensure protection of human subjects should be described: in the US, compliance with 45 CFR Part 46 and ICH E6 GCP is expected. The protocol should indicate that no research (including screening) will begin until the protocol and the consent form have been IRB-approved. The consent process should be described, as should the provisions for subject confidentiality. The Health Insurance Portability and Accountability Act of 1996 (HIPAA) was enacted to improve portability and continuity of health insurance coverage; nevertheless, it contains regulations that have direct relevance for clinical research (DHHS, 2007). For example, informed consent documents are required to include extensive information on how the participant’s protected health information (PHI) will be kept private. Administrative, physical, and technical safeguards must be adopted to ensure the security of electronic PHI. Finally, special issues related to exclusion of special populations (women, minorities, and children) should be addressed.

Data Management and Record Keeping

The goals of data management are to ensure the accuracy, completeness, and timeliness of clinical trial data collection. While the primary responsibilities for data collection rest with the clinical trial site, additional oversight and data management responsibilities (quality review, analysis, and reporting) may be shared with the sponsor and a data coordinating center (DCC). The organizational structure of data management should be described in this part of the protocol. Data capture methods and internal quality checks, types of data, timing of reports, study records retention, and identification of protocol deviations and corrective actions should be addressed here, as well as in greater detail in the MOP.

Other Considerations

In addition to the major protocol elements described above, a full protocol should include a title page, a statement of compliance with GCP, and other regulatory guidelines, a signature page, a table of contents, a list of abbreviations; a protocol summary; a list of key personnel and their roles, a description of unique facilities, if applicable, a list of references, publication policy; and appropriate appendices. A list of essential documents that should be on file before the trial starts is shown in Table 12.3; additional documents should be added to the trial documentation as new information becomes available (protocol amendments, updates, IRB approvals, training certificates, CVs, screening and enrollment logs, test article accountability and shipment logs, monitoring reports, consent forms, source documents, CRFs, communications with the sponsor, etc.).
TABLE 12.3 Checklist of essential documents on file at the clinical trial site before the clinical trial begins

Signed full clinical trial protocol, and amendments, if applicable
Sample case report forms (CRFs)
IRB-approved informed consent document
Investigators’ brochure
Manual of procedures (MOP)
Information that will be given to subjects
Recruiting materials (text of advertisements, flyers, etc.)
IRB approval letter
Copy of IRB/IEC Federal Wide Assurance
Composition of IRB
FDA Form 1572 (Principal Investigator Responsibilities)
Curriculum vitae of participating investigators
Financial disclosures; other clinical trial agreements
Copy of the principal investigator’s medical license
Laboratory credentials/certifications
Laboratory reference ranges
Sample labels for investigational product
Instructions for handling investigational product and other trial materials
Shipping records for trial-related materials
Clinical trial site initiation monitoring report

Source: Adapted from ICH E6 GCP guidance (FDA, 1996).
Courtesy: U.S. FDA.

CONCLUSIONS

The success of any clinical trial hinges on the development of a carefully designed protocol. Although discussion of clinical trial implementation is beyond the scope of this chapter, it is clear that the protection of human subjects and scientific integrity of the trial design and documentation are the overarching goals of any clinical research protocol. The clinical trial protocol must document the processes that will be used to ensure that these goals are attained. Conscientious supervision of clinical trial activities is a shared responsibility of the investigators, the IRB, the sponsor, the CRO, the DCC, the safety oversight committee, and all other partners who are participating in the trial. While meticulous attention to detail and strict protocol compliance are essential, the entire study team must be flexible and prepared to respond in a timely fashion to unexpected findings. Novel approaches to the development of vaccines for bio-defense and emerging and neglected diseases will continue to evolve and will require ongoing reconsideration of the ethical and regulatory principles and practices that guide the conduct of clinical trials.

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References

Acosta, C.J., Galindo, C.M., Ochiai, R.L., Danovaro-Holliday, M.C., Laure-Page, A., Thiem, V.D., Jin, Y., Khan, M.I., Sahuto, S.M., Hanza, H.B. et al. Implementation of good clinical practice guidelines in vaccine trials in developing countries. Vaccine 2007; 25:2852–2857.

Ballentine, C. Taste of Raspberries, Taste of Death. The 1937 Elixir Sulfanilamide Incident. US Food and Drug Administration-FDA Consumer Magazine, June 1981:Issue 1–4. 1981. http://www.fda.gov/oc/history/elixir.html.

Bayl s, N. and Miduthn, K. Regulation and testing of vaccines. In: Vaccines (S. Plotkin et al., Eds.), pp. 1539–1556. Philadelphia: Elsevier Inc., 2004.

Beecher, H. Ethics and clinical research. N. Engl. J. Med. 1966; 274:1354–1360.

Belshe, R.B., Gruber, W.C., Mendelman, P.M., Mehta, H.B., Mahmood, K., Reisinger, K., Treanor, J., Zangwill, K., Hayden, F.G., Bernstein, D.I., Kotloff, K., King, J., Piedra, P.A., Block, S.L., Yan, L. and Wolff, M. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. J. Infect. Dis. 2000; 181:1133–1137.

Black, S., Shinefield, H., Fireman, B., Lewis, E., Ray, P., Hansen, J.R., Elvin, L., Ensror, K.M., Hackell, J., Silver, G., Malinoski, F., Madore, D., Chang, I., Kohberger, R., Watson, W., Austrian, R. and Edwards, K. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser permanente vaccine study center group. Pediatr. Infect. Dis. J. 2000; 19:187–195.

Borchers, A.T., Hagie, F., Keen, C.L. and Gershwin, M.E. The history and contemporary challenges of the US Food and Drug Administration. Clin. Ther. 2007; 29:1–16.

CDC. Ten great public health achievements – United States, 1900–1999. MMWR Morb. Mortal. Wkly. Rep. 1999; 48:241–243.

Chow, S.-C. and Chang, M. Adaptive Design Methods in Clinical Trials. Boca Raton: Chapman and Hall/CRC; Taylor and Francis Group, 2007.

Chow, S.-C. and Liu, J.-P. Design and Analysis of Clinical Trials. Concepts and Methodologies. Hoboken: Wiley, 2004.

CIOMS. International Ethical Guidelines for Biomedical Research Involving Human Subjects. Council for International Organizations of Medical Sciences. 2002: 1–59. http://www.cioms.ch/frame_guidelines_nov_2002.htm.

Couch, R.B., Douglas, R.G., Jr., Fedson, D.S. and Kassel, J.A. Correlated studies of a recombinant influenza-virus vaccine. 3. Protection against experimental influenza in man. J. Infect. Dis. 1971; 124:473–480.

Deming, D., Sheahan, T., Heise, M., Yount, B., Davis, N., Sims, A., Suthar, M., Harkema, J., Whitmore, A., Pickles, R., West, A., Donaldson, E., Curtis, K., Johnston, R. and Baric, R. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. PLoS Med. 2006; 3:e525.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
Department of Health and Human Services. Office for Civil Rights-HIPAA Medical Privacy-National Standards to Protect the Privacy of Personal Health Information. United States Department of Health & Human Services. 2007. http://www.hhs.gov/ocr/hipaa/

Edelman, R., Wasserman, S.S., Bodison, S.A., Putnak, R.J., Eckels, K.H., Tang, D., Kanesa-Thanan, N., Vaughn, D.W., Innis, B.L. and Sun, W. Phase I trial of 16 formulations of a tetravalent live-attenuated dengue vaccine. Am. J. Trop. Med. Hyg. 2003; 69:48–60.

Edelman, R., Wasserman, S.S., Kublin, J.G., Bodison, S.A., Nardin, E.H., Oliveira, G.A., Ansari, S., Diggs, C.L., Kashala, O.L., Schmeckpepper, B.J. and Hamilton, R.G. Immediate-type hypersensitivity and other clinical reactions in volunteers immunized with a synthetic multi-antigen peptide vaccine (PICS-MAPIN) against Plasmodium falciparum sporozoites. Vaccine 2002; 21:269–280.

Ellenberg, S.S., Foulkes, M.A., Midthun, K. and Goldenthal, K.L. Evaluating the safety of new vaccines: summary of a workshop. Am. J. Public Health 2005; 95:800–807.

Emanuel, E.J., Wendler, D. and Grady, C. What makes clinical research ethical? J. Am. Med. Assoc. 2000; 283:2701–2711.

Epstein, J.E., Rao, S., Freilich, D., Luke, T., Sedegah, M., de la Vega, P., Sacci, J., Richie, T.L. and Hoffman, S.L. Safety and clinical outcome of experimental challenge of human volunteers with Plasmodium falciparum-infected mosquitoes: an update. J. Infect. Dis. 2007; 196:145–154.

FDA. E6 Good Clinical Practice: Consolidated Guidance. Guidance for Industry- ICH1-58. US Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER); Center for Biologics Evaluation and Research (CBER). 1996. http://www.fda.gov/cder/guidelines.htm.

FDA. New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible. HHS17989-37998. 2002. http://www.fda.gov/cber/rules/humeffic.htm.

FDA. Guidance for Industry, Investigators, and Reviewers; Exploratory IND Studies. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER); Center for Biologics Evaluation and Research (CBER). 2006. http://www.fda.gov/cber/guidance/index.htm.

FDA. Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines. Guidance for Industry. 1-18. 2007. US Department of Health and Human Services; Food and Drug Administration; Center for Biologics Evaluation and Research. 2007a. http://www.fda.gov/cber/guidelines.htm.

FDA. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. US Department of Health and Human Services. Food Drug Administration. Center for Biologics Evaluation and Research–I-8. 2007. Guidance for Industry. 2007b. http://www.fda.gov/cber/guidelines.htm.

Francis, T., Jr. Evaluation of the 1954 poliomyelitis vaccine field trial; further studies of results determining the effectiveness of poliomyelitis vaccine (Salk) in preventing paralytic poliomyelitis. J. Am. Med. Assoc. 1955; 158:1266–1270.

Frey, S.E., Couch, R.B., Tacket, C.O., Treanor, J.J., Wolff, M., Newman, F.K., Atmar, R.L., Edelman, R., Nolan, C.M. and Belisle, R.B. Clinical responses to undiluted and diluted small-pox vaccine. N. Engl. J. Med. 2002; 346:1265–1274.

Gallo, P., Chung-Stein, C., Dragalin, V., Gaydos, B., Kram, M. and Pinheiro, J. Adaptive designs in clinical drug development—an executive summary of the PhRMA Working Group, J. Biopharm. Stat. 2006; 16:275–283.

Gorse, G.J., Keitel, W., Keyserling, H., Taylor, D.N., Lock, M., Alves, K., Kenner, J., Deans, L. and Gurwitz, M. Immunogenicity and tolerance of ascending doses of a recombinant protective antigen (rPA102) anthrax vaccine: a randomized, double-blinded, controlled, multicenter trial. Vaccine 2006; 24:5950–5959.

Hayward, A.C., Harling, R., Wetten, S., Johnson, A.M., Munro, S., Smedley, J., Murad, S. and Watson, J.M. Effectiveness of an influenza vaccine programme for care home staff to prevent death, morbidity, and health service use among residents: cluster randomised controlled trial. Br. Med. J. 2006; 333:1241.

Keitel, W., Couch, R., Bond, N., Adair, S., Van Nest, G. and Dekker, C. Pilot evaluation of influenza virus vaccine (IVV) combined with adjuvant. Vaccine 1999a; 11:909–913.

Keitel, W.A., Atmar, R.L., Cate, T.R., Petersen, N.J., Greenberg, S.B., Ruben, F. and Couch, R.B. Safety of high doses of influenza vaccine and effect on antibody responses in elderly persons. Arch. Intern. Med. 2006; 166:1121–1127.

Keitel, W.A., Couch, R.B., Quares, J.M., Cate, T.R., Baxter, B. and Maasab, H.F. Trivalent attenuated cold-adapted influenza virus vaccine: reduced viral shedding and serum antibody responses in susceptible adults. J. Infect. Dis. 1993b; 167:305–311.

Keitel, W.A., Kester, K.E., Atmar, R.L., White, A.C., Bond, N.H., Holland, C.A., Krzych, U., Palmer, D.R., Egan, A., Diggs, C., Ballou, W.R., Hall, B.F. and Kaslow, D. Phase I trial of two recombinant vaccines containing the 19 kd carboxy terminal fragment of Plasmodium falciparum mosquito surface protein 1 (msp-19) and T helper epitopes of tetanus toxoid. Vaccine 1999; 18:531–539.

Macrae, D.J. The Council for International Organizations and Medical Sciences (CIOMS) guidelines on ethics of clinical trials. Proc. Am. Thorac. Soc. 2007; 4:176–178.

Meinert, C. Clinical Trials Design, Conduct, and Analysis. New York: Oxford University Press, 1986.

Murphy, T.V., Gargiullo, P.M., Massoudi, M.S., Nelson, D.B., Jumaan, A.O., Okoro, C.A., Zanardi, L.R., Setia, S., Fair, E., LeBaron, C.W., Wharton, M. and Livengood, J.R. Intussusception among infants given an oral rotavirus vaccine. N. Engl. J. Med. 2001; 344:564–572.

NIAID. NIAID Protocol Template Extramural Guidance. 2006. http://www3.niaid.nih.gov/research/resources/toolkit/attachments/ProtocolTemplateEXTRAMURAL.pdf.

Offit, P.A. The Cutter incident, 50 years later. N. Engl. J. Med. 2005; 352:1411–1412.

Oxman, M.N., Levin, M.J., Johnson, G.R., Schnader, K.E., Straus, S.E., Gelb, L.D., Arbeid, R.D., Simberkoff, M.S., Gershon, A.A., Davis, L.E. et al. A vaccine to prevent herpes zoster and post-herpetic neuralgia in older adults. N. Engl. J. Med. 2005; 352:2271–2284.

Piedra, P.A., Glezen, W.P., Mbawuike, I., Gruber, W.C., Baxter, B.D., Boland, F.J., Byrd, R.W., Fan, L.L., Lewis, J.K., Rhodes, L.J. et al. Studies on reactogenicity and immunogenicity of attenuated bivalent cold recombiant influenza type A (CRA) and inactivated trivalent influenza virus (TI) vaccines in infants and young children. Vaccine 1993; 11:718–724.

Polack, F.P. A typical measles and enhanced respiratory syncytial virus disease (ERSD) made simple. Pediatr. Res. 2007; 62:111–115.

Rennels, M.B., Englund, J.A., Bernstein, D.J., Losonsky, G.A., Anderson, E.L., Pichichero, M.E., Munoz, F.M. and Wolf, M.C. Dimination of the anti-polyribosylribitol phosphate response to a combined diphtheria-tetanus-acellular pertussis/Haemophilus influenzae type b vaccine by concurrent inactivated poliovirus vaccine. Pediatr. Infect. Dis. J. 2000; 19:417–423.

Rodrigues, L.C., Pereira, S.M., Cunha, S.S., Genser, B., Ichihara, M.Y., de Brito, S.C., Hijjar, M.A., Dourado, I., Cruz, A.A., Sant’Anna, C.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 2005; 366:1290–1295.
Tacket, C.O., Cohen, M.B., Wasserman, S.S., Losonsky, G., Livio, S., Kotloff, K., Edelman, R., Kaper, J.B., Cryz, S.J., Giannella, R.A. et al. Randomized, double-blind, placebo-controlled, multicentered trial of the efficacy of a single dose of live oral cholera vaccine CVD 103-HgR in preventing cholera following challenge with *Vibrio cholerae* O1 El tor inaba three months after vaccination. Infect. Immun. 1999; 67:6341–6345.
Taylor, D.N., Tacket, C.O., Losonsky, G., Castro, O., Gutierrez, J., Meza, R., Nataro, J.P., Kaper, J.B., Wasserman, S.S., Elderman, R., Levine, M.M. and Cryz, S.J. Evaluation of a Bivalent (CVD 103-HgR/CVD 111) live oral cholera vaccine in adult volunteers from the United States and Peru. Infect. Immun. 1997; 65:3852-3856.
Treonor, J.J., Campbell, J.D., Zangwill, K.M., Rowe, T. and Wolff, M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N. Engl. J. Med. 2006; 354:1343-1351.
Wang, D. and Bakhai, A. Clinical Trials: A Practical Guide to Design Analysis and Reporting. London: Remedica, 2006.
Werzberger, A., Mensch, B., Kuter, B., Brown, L., Lewis, J., Sitrin, R., Miller, W., Shouval, D., Wiens, B., Calandra, G. et al. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. N. Engl. J. Med. 1992; 327:453-457.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY