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Sequential stages of clinical trials and overview of issues to be considered

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Introduction

We live in an exciting era in which the fruits of molecular biology and biotechnology are yielding a profusion of new and improved candidate vaccines, delivery systems, and adjuvants that have the potential to control many of the infectious disease scourges of the developing world. Indeed, it has been estimated recently that there are over 350 vaccine candidates currently under development against nearly 100 infectious diseases [1].

Discovery of a new vaccine candidate and demonstration of its safety, immunogenicity, and protectivity in animal models are, however, only the first steps toward licensure and introduction of the vaccine into public health practice. The longest phase of development for most vaccines before licensure, and arguably the most uncertain, laborious, and expensive phase, comprises clinical testing of the vaccine in humans [2]. At a minimum, this clinical testing must demonstrate the vaccine to be acceptably safe and suitably protective in the population that will ultimately be targeted for the vaccine in public health practice [3].

Over the years, regulatory agencies have adopted a standard paradigm for the manner in which new vaccines are tested in humans. A key feature of this paradigm is the phased fashion in which the testing occurs. This chapter describes the phases of vaccine evaluations in humans, including several considerations for the phased testing of vaccines in developing countries.

Rationale for phased testing of vaccines in humans

Vaccines have had a remarkable track record of safety. Nevertheless, even when manufactured flawlessly, some vaccines have caused serious side-effects [4]. The mechanisms for these side-effects are diverse and are sometimes related to such problems as vaccine-induced immunopathology, as occurred with early-generation measles and respiratory syncytial virus vaccines [5, 6]. At times the mechanism may be obscure, as was the case for

intussusception induced by quadrivalent rhesus reassortant vaccine against rotavirus diarrhea [7, 8]. Whatever the mechanism, the point to be noted is that such severe side-effects are not always predictable. To minimize potential injuries to subjects caused by vaccine side-effects during re-licensure trials, vaccines are tested in a phased manner. In this phasing, early evaluations are conducted in small numbers of subjects, so that if reactions are observed, they will affect a minimum number of volunteers. And early evaluations are typically conducted in the least vulnerable subjects, often healthy adults, so that if the reactions occur, their severity will be minimized. Successive evaluations of a particular vaccine are then conducted in progressively larger numbers of volunteers, and, as confidence in the safety-profile of the vaccine increases, in subjects in the ultimate target group, including persons who are more vulnerable (e.g., infants). With these successive studies, the complete ensemble of information about the vaccine's safety, immunogenicity, protectivity, and sometimes additional characteristics (e.g., transmissibility) is accrued. Moreover, the large number of subjects ultimately studied ensures estimation of these features in a statistically precise fashion, and, for side-effects, in a way that enables detection of relatively rare events.

Clinical trials for licensing a new vaccine candidate are generally planned in three phases. In the Code of Federal Regulations (USA), the phases of clinical trials are described by using Arabic numerals (Phase 1, Phase 2, Phase 3), while in World Health Organization publications, Roman numerals are generally used (Phase I, Phase II, Phase III). In this paper, we use Roman numerals to describe the phases of clinical trials.

Phase I trials

Phase I trials of experimental vaccines are the first human studies to be conducted after preclinical studies have demonstrated suitable safety, potency, immunogenicity, and, when possible, protectivity. The primary purpose of a Phase I trial is to rule out the possibility of frequent vaccine side-effects. Additional goals include preliminary assessment of vaccine immunogenicity, determination of an appropriate dose and regimen, and, for live vaccines, measurement of vaccine shedding. Phase I trials are typically small, often on the order of 10–50 subjects, usually enroll healthy adults, and may be done with preliminary formulations of the vaccine. Depending on whether there are concerns about potential severe side-effects and the need for biological containment of excreted vaccines, such studies may be done on an inpatient or an outpatient basis. For example, a genetically attenuated, live oral vaccine candidate might well be tested initially on an inpatient basis with containment if there is concern about transmission of fecally excreted vaccine organisms, or about the genetic stability of the candidate during the course of fecal shedding [9]. Often, Phase I studies are designed in an uncontrolled fashion.

Phase II trials

For vaccine candidates that are found to yield promising findings in Phase I trials, Phase II trials may be undertaken. The primary goals of Phase II trials are to evaluate vaccine safety and immunogenicity in larger numbers of subjects, and ultimately in the target population for whom the vaccine is intended. For live vaccines, Phase II trials may also be designed to evaluate vaccine shedding and transmissibility. Phase II trials may also further evaluate different vaccine doses and regimens.

A frequently used strategy for Phase II trials of vaccine candidates intended for infants is to initiate the studies in an older age group, often adults, and to conduct successive studies in progressively younger age groups, with the transition to each younger age group contingent on satisfactory results from the study of the previous age group. In contrast to Phase I trials, which are often done with preliminary formulations, Phase II trials typically evaluate the final formulation of the vaccine, since data from studies of preliminary formulations are not usually eligible for consideration by regulatory authorities in their deliberations about vaccine licensure. Phase II trials are typically larger than Phase I trials, sometimes enrolling 200 or more subjects. By the time that a vaccine candidate has reached Phase II, concerns that would mandate testing it on an inpatient basis have typically been resolved, and outpatient studies are the norm. In contrast to Phase I trials, Phase II trials are conventionally designed as randomized, controlled trials, and control groups typically receive a placebo or an active agent to permit blinding.

For live vaccine candidates, there may be a concern about unintended transmission of the vaccine strain from vaccinees to non-vaccinees with whom they are in contact. Special Phase II trials are sometimes conducted to assess the transmissibility of such vaccine candidates. For example, a Phase II study of the live oral cholera vaccine, CVD03-HgR, in Jakarta, Indonesia, randomly allocated pairs of sibling children within households to either vaccine or placebo, and judged transmissibility of the vaccine candidate by the rate of fecal excretion of the vaccine strain and seroconversion to the vaccine in placebo recipients [10].

Phase II trials may also be done to address environmental concerns about genetically engineered, live vaccine candidates. For CVD03-HgR, for example, open sewers outside the Jakarta homes of children who had received the vaccine were sampled to evaluate whether there was any detectable persistence of the vaccine strain during the 36–40 hours after vaccination [10].

A recent trend in vaccine evaluation is to use Phase II trials to obtain initial data on the level of vaccine protection against targeted, naturally occurring infections. What distinguishes these Phase II trials from conventional Phase III trials (*vide infra*) is that they are smaller in size (although usually larger than Phase II trials geared only to the assessment of safety

and immunogenicity) and less able to evaluate vaccine protection with suitable statistical precision than well-designed Phase III trials. Also in contrast with Phase III trials, such Phase II trials may be used to evaluate vaccine prototypes that will be later modified or augmented into final vaccines for licensure (e.g., a vaccine against a single serotype, when a multi-serotype vaccine will be required for the final vaccine), and may attempt to obtain estimates of vaccine protection based on prevention of a surrogate endpoint (e.g., the use of HIV viral load as a surrogate for the rapidity of HIV disease progression).

For certain vaccines, studies are done in volunteers to evaluate the clinical protection against an intentional challenge with the target pathogen. Such studies are sometimes termed Phase IIb trials. In these studies volunteers are typically allocated at random to receive the vaccine or a comparison agent, usually a placebo, and are then challenged at a defined interval after vaccination with an inoculum of the pathogen predicted to cause the target disease in nearly 100% of the control group. The comparative attack rate of the target disease in vaccinees *versus* controls provides an estimate of the conventional measure of vaccine protective efficacy (PE) = (1 minus the relative risk of the disease in vaccinees *versus* controls) × 100%. Estimates of vaccine protection in Phase IIb studies, which are typically small, often conducted in ca. 20–30 subjects, may be helpful in triaging vaccines that are deserving of study in larger and more expensive Phase III trials [11, 12].

In addition to providing estimates of vaccine protection, Phase IIb trials can serve to provide data on vaccine safety, vaccine immunogenicity, vaccine shedding and transmissibility (for live vaccines), and preliminary assessments of immunological responses that correlate with protection. Because such studies entail intentional challenge with pathogens, subjects for the studies should always be healthy adults. The intentional challenge also limits Phase IIb trials to infections for which there is no risk of severe acute complications, significant sequelae, or chronic infection if appropriate therapy is administered promptly upon recognition that the challenge has resulted in infection. Examples of pathogens for which Phase IIb studies have been successfully carried out include cholera, diarrheagenic *Escherichia coli*, *Shigella*, Rocky Mountain spotted fever, malaria, and influenza [13]. It is imperative that such studies be carried out by staff who are highly skilled in the diagnosis and treatment of the infections under study, and it is usually desirable to conduct the challenge phase of these studies under inpatient conditions.

Phase III trials

For vaccine candidates found to be suitably safe and immunogenic in Phase II trials, Phase III trials may be done to provide rigorous evidence about

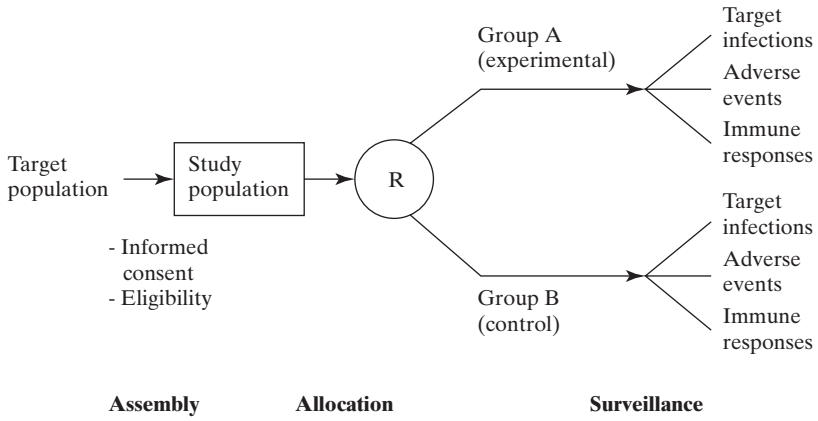

Figure 1. A simplified schematic of a Phase III vaccine trial designed as a two-group, randomized, controlled trial. In such a trial, the study population is assembled from a target population and is then randomized to receive an experimental vaccine or a control agent. The experimental and control groups are followed longitudinally and concurrently to detect the comparative occurrence of target infections, adverse events, and immune responses in the two groups to assess vaccine protection, vaccine safety, and vaccine immunogenicity, respectively.

vaccine protection against naturally occurring infections, and to provide additional data on vaccine safety in larger numbers of vaccinees. Phase III studies are designed as randomized, controlled trials with clear hypotheses, and are conducted in the target group for whom vaccine licensure is desired and in a population that normally experiences the target infection. These studies thus constitute pivotal evaluations that provide the basis for decisions about whether to license a vaccine for use in public health practice [14].

Figure 1 diagrammatically depicts the design of a Phase III trial comparing two groups. In such a trial participants are recruited from a target population and are enrolled for the study after acquisition of informed consent and ascertainment of eligibility. Prior to the trial, a formal randomization scheme is developed to allocate subjects to an experimental vaccine group or a control group, and this randomization scheme is used to allocate the compared agents to consenting subjects who are eligible for participation. To ensure blinding of investigators and subjects to the identities of the compared agents, controls may receive an inert placebo or an active vaccine. If the latter is chosen, it is typically an agent that is identical in appearance to and given with the same regimen as the experimental vaccine, but does not elicit immune responses that are known to protect against the pathogen targeted by the experimental vaccine. After randomization, subjects in the compared groups are followed concurrently with uniform surveillance procedures to detect target infections and adverse events, and the

comparative rates of these events in the two groups form the basis for the assessment of vaccine PE and safety. Similarly, participants, or a subsample of participants, are assessed immunologically at baseline and at a defined interval after dosing.

Because Phase III trials must provide statistically meaningful estimates of vaccine PE (calculated in the same way as described above for Phase IIb trials) and because the target disease outcomes to be prevented by vaccination in the trials are typically rare in occurrence, Phase III trials are often quite large, sometimes enrolling tens of thousands of subjects. In addition, because it may be necessary to measure vaccine PE over several years following vaccination in order to provide information necessary to convince regulatory and public health authorities, Phase III trials may entail maintenance and follow-up of study populations for long durations. The large size, lengthy duration, prospective conduct, and extensive quality control and quality assurance procedures needed for Phase III trials make them extremely expensive, often costing millions of dollars.

Measurement of immune responses in Phase III trials is done for two purposes. First, it is necessary to document that the vaccine tested in a Phase III trial elicited the level of immune response expected on the basis of earlier studies. Comparison of immune responses in the vaccine and control groups permits estimation of the proportion of immune responders among the experimental vaccine group that can be attributed to receipt of the vaccine. If vaccine PE proves unexpectedly low in the trial, this assessment will be crucial in helping to explain these results. Assessments to assess whether immune responses were as robust as expected usually require evaluation only of a small subsample of subjects participating in the trial. Second, it is often desirable that a trial define an immunological correlate of vaccine protection – a level of short-term immune response to vaccination that demarcates vaccinees who are protected against the target infection. Immunological correlates of protection are best defined by comparing immune responses to the vaccine in vaccinees who develop the target infection (breakthroughs) *versus* vaccinees who do not.

Definition of immunological correlates of vaccine protection is very important because such correlates permit assessments of the protection of the tested vaccine and ones suitably similar to it in small, short-term studies with immunological endpoints, without resort to full-scale, Phase III efficacy trials with clinical infection endpoints. Phase III trials usually provide the only opportunity before licensure for determining an immunological correlate of protection. Unfortunately, determination of immunological correlates within Phase III trials can be logistically demanding and costly. These problems result from four considerations: 1) it is necessary to contrast short-term immune responses in vaccinees who develop the target infection *versus* those who do not in order to determine an immunological correlate of protection; 2) at the time of vaccination and collection of specimens for immunological assessments it is unpredictable which vaccinees

will acquire the target infection ("breakthrough events"); 3) most Phase III trials have very few breakthrough events; and 4) blinding of Phase III trials prevents knowledge of who received the vaccine and who received the control agent. The implication of these considerations is that it may be necessary to obtain suitable specimens from virtually every participant in the trial to ensure that immunological assessments will be available for a large enough number of vaccine breakthroughs to enable statistically meaningful assessments. Moreover, even when arrangements are made to collect the necessary specimens from the required number of subjects, measurement of immune responses in these specimens may not necessarily yield an immunological correlate of the vaccine protection observed in the trial, sometimes for obscure reasons [15].

Other considerations for phased testing of vaccines for developing countries

When in the phased sequence a vaccine should be tested in developing countries

Experience has demonstrated that the performance of a vaccine in affluent populations in industrialized countries cannot be assumed to generalize to persons living in developing-country settings. Examples of vaccines that performed less well in developing-country than in industrialized-country populations have included oral polio vaccine, certain live oral rotavirus and cholera vaccines, and parenteral, polysaccharide-diphtheria protein conjugate *Haemophilus influenzae* type b (Hib) vaccine [16–19]. It is therefore important that new vaccines be tested in developing countries prior to their licensure in these settings.

This raises the question, for vaccine candidates developed in the industrialized world, of when in the phased sequence the candidate should be tested in the developing world. Traditionally, it has been argued, partly over concerns about using poor populations in the developing world as "human guinea pigs", that trials in the developing world should commence only when a vaccine has been fully evaluated and licensed in the industrialized world. Recent changes in thinking have challenged this view. It is now recognized that deferring trials in developing countries to this extent carries the unwanted consequence of significantly delaying the availability of vaccines to populations in developing countries. For example, polysaccharide-protein vaccines against Hib, which have been licensed for over a decade in the United States and other industrialized countries and have nearly eliminated invasive Hib disease in children in many of these settings, are used only to a limited extent today in the developing world [20]. This inequity in use of new-generation vaccines has elicited calls for parallel testing, rather than sequential testing, of new vaccines in industrialized- and developing-

world populations. Parallel-testing of new vaccines in the industrialized and developing worlds is now being pursued for both experimental HIV and rotavirus vaccines [21, 22].

Another important trend affecting the testing of vaccines in developing-country populations is the emergence of highly qualified vaccine producers in the developing world, some capable of developing innovative new vaccines. Clearly, it is appropriate that the initial groups for testing of an experimental vaccine developed by such producers be selected from the population of the country in which the vaccine is developed.

Progression of phased studies

Conventional descriptions of phased trials often portray a seamless progression of trials from Phase I to Phase II, and from Phase II to Phase III. In reality, the vast majority of vaccine candidates that are tested in Phase I studies do not progress all the way through Phase III testing, because of disappointing findings or because of a lack of sufficient resources to support the increasingly expensive studies in successive phases [2]. While unwelcome findings at any stage may lead to termination of the clinical development of a vaccine, this need not always be the case. For example, the live-attenuated, oral cholera vaccine, CVD103-HgR, was shown to be safe and highly immunogenic when given as a single dose of 5×10^8 organisms in an extensive series of studies in North American volunteers. However, when the vaccine was subsequently tested in Thai adult volunteers, it proved erratically immunogenic [23]. To address this problem, the vaccine developer raised the dose by one log, to 5×10^9 organisms, and found the vaccine to be suitably immunogenic but still safe in a variety of settings in developing countries [9]. The CVD103-HgR experience illustrates that successful vaccine development may require detours in the phased sequence of clinical trials in order to accommodate necessary adjustments in vaccine dose, schedule, or even formulation in response to findings that emerge during the course of clinical studies. However, because vaccines that appear promising in developed countries not infrequently fail to perform as well in developing countries, and because such detours can greatly increase the expense of clinical development, completion of clinical testing of vaccines for developing countries may be exceptionally challenging.

Alternative endpoints for vaccine licensure

The conventional sequence of phases usually culminates in one or more Phase III trials that are designed to provide pivotal evidence on the clinical protection conferred by the vaccine against the targeted, naturally occur-

ring, infectious disease. Sometimes, however, proof of clinical efficacy in a Phase III trial may not be necessary for licensure of a vaccine. For example, CVD103-HgR vaccine against cholera was licensed in Europe for use in travelers on the basis of Phase I-II studies, together with Phase IIb trials showing a high level of protection against experimental cholera in North American volunteers [24]. However, it is unlikely that Phase IIb evidence from industrialized countries will be taken as a basis for licensure of vaccines in developing countries, in part because of the discrepancies between vaccine performance in developed *versus* less-developed settings and in part because of the uncertainties about whether protection in Phase IIb studies predicts the performance of a vaccine in populations experiencing endemic disease.

In addition, if there is an accepted correlation between a certain immune response (e.g., a serological antibody titer) to vaccination and clinical protection, this immune response may sometimes be used as *prima facie* evidence for judging whether the vaccine is likely to be sufficiently protective to warrant licensure. For example, PRP-CRM_{197} diptheria toxin and PRP-*Neisseria meningitidis* outer membrane protein (OMP) conjugate vaccines against Hib were licensed for infants on the basis of Phase III evidence of clinical protection against invasive Hib disease. Subsequently, PRP-tetanus toxoid conjugate vaccine was licensed for infants in the United States largely on the basis of proven safety and the attainment of protective serum antibody titers to PRP, without corresponding evidence of clinical protection from a Phase III trial [25, 26]. Serological endpoints are also commonly used as a basis for licensure of combination vaccines for which the component vaccines have already been licensed and seroresponse criteria for these components have been established [27].

Some vaccines of public health importance are directed to diseases that cannot be reliably predicted in populations at risk and that cannot be ethically studied in Phase IIb trials. Examples include vaccines against some bioterrorism agents such as anthrax and plague, and vaccines against certain epidemic diseases such as SARS and Ebola. In these situations, decisions about licensure will have to depend on pre-clinical data, demonstration of safety in humans, and assessments of immune responses in humans (even if such immune responses are not known with certainty to be correlated with clinical protection of humans against the target disease).

Good clinical practice

In recent years, considerable attention has been devoted to the ethics, scientific quality, conduct, documentation, and reporting of clinical trials. Several organizations have produced guidelines for these activities, which are subsumed under the rubric of "Good Clinical Practice (GCP)". Guidelines for GCP have been published by the World Health

Organization, the United States Food and Drug Administration, and the International Conference on Harmonization (ICH), among others [13]. Table 1 presents the essential elements of GCP in guidelines issued by the International Conference on Harmonization [28].

The promulgation of GCP has had several implications for vaccine trials in developing countries. Increasingly, the world of vaccines is becoming globalized. Vaccines targeted for a global market may be tested in developing countries to provide initial licensure. For example, a live oral human rotavirus vaccine developed by a major multinational vaccine producer has recently received its initial licensure in Mexico. Moreover, vaccines produced by manufacturers in developing countries are now being used throughout the world. Indeed, the majority of all doses of measles vaccines now administered to children in the world are produced in India.

With the trend toward globalization of vaccines, increasing emphasis has been placed on conducting clinical trials in developing countries in a manner that conforms to international GCP standards. While the benefits of GCP trials are unquestionable, rigid adherence to GCP standards can be a double-edged sword. The expense of clinical trials has risen rapidly in recent years [29]. A portion of this increased expense arises from the extensive documentation and auditing requirements demanded by regulatory agencies as a component of GCP, together with the expense of proprietary products developed to ensure compliance with regulatory requirements, such as proprietary computer software for data management and electronic reporting of trials. While this increased expense can be borne by producers anticipating major markets for their vaccines in affluent markets, it constitutes a disincentive to the clinical testing of vaccines against "orphan diseases" affecting developing countries, for which lucrative markets are not foreseen.

The ethical guidelines for clinical trials in developing countries have been the subject of recent controversy. The ethical principles of respect for persons, beneficence, and justice, which underlie modern clinical trials, are well accepted [30]. Nevertheless, several issues continue to be debated, of which three are touched on here [31–33]. One issue concerns the threshold for the risk/benefit ratio for a vaccine to be tested in developing countries. For example, a live oral, quadrivalent rhesus rotavirus-reassortant vaccine was licensed and then withdrawn from the United States market by its manufacturer because of an association between vaccination and the rare occurrence of intussusception [7, 8]. Understandably, this action had a chilling effect on the testing of this vaccine in developing countries. A meeting of experts organized by the World Health Organization acknowledged the rationale for the company's withdrawal of this vaccine in the United States, where rotavirus diarrhea is a cause of morbidity but not of significant mortality. Nevertheless, there was agreement among participants in the meeting that the burden of mortality of rotavirus in developing countries provided an ethical justification for continued evaluation of this vaccine in these settings [34].

Table 1. Principles of Good Clinical Practice (GCP). From [28]

1. Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with GCP and the applicable regulatory requirement(s).
2. Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
3. The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Clinical trials should be scientifically sound and described in a clear, detailed protocol.
6. A trial should be conducted in compliance with the protocol that has received prior Institutional Review Board (IRB)-Independent Ethics Committee (IEC) approval-favorable opinion.
7. The medical care given to and medical decisions made on behalf of subjects should always be the responsibility of a qualified physician or when appropriate, a qualified dentist.
8. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
10. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.
11. The confidentiality of records that could identify subjects should be protected, respecting privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
12. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.
13. Systems with procedures that assure the quality of every aspect of the trial should be implemented.

A second controversy over the ethics of trials in developing countries has focused on the use of placebos. As stated in the Declaration of Helsinki, "The benefits, risk, burdens, and effectiveness of a new method should be tested against the best current prophylactic, diagnostic, and therapeutic methods" [35]. There is agreement that a placebo would be inappropriate as a control agent for a trial if a locally licensed standard alternative vaccine exists; there is also general agreement that if such an alternative does not exist, it would be preferable to use for the control group an active vaccine against a different target infection, provided the active control does not cross-protect against the target pathogen and that use of the active vaccine maintains blinding in the trial [36]. However, it remains controversial whether a placebo can be ethically administered to the control groups in trials for which there is no suitable alternative active control vaccine and in which a licensed alternative vaccine against the target infection exists, but the alternative is not licensed in the country for the trial [37].

Yet a third controversy concerns what is owed to the participants at the conclusion of a vaccine trial. The Declaration of Helskinki states that: "At the conclusion of a study, every patient entered into the study should be

assured of access to the best proven prophylactic, diagnostic, and therapeutic methods identified by the study" [35]. Few would disagree that if the vaccine tested in a trial is found to be beneficial, it should be provided to all participants in the trial. However, controversy surrounds the boundaries for the group of persons entitled to receive the investigational vaccine at the conclusion of the study – only participants in the trial, all persons living in the same region, or all persons living in the same country? And for how long are these beneficiaries entitled to receive the vaccine?

Increasing expectations for demonstration of vaccine safety before licensure

Because vaccines are administered in public health practice to healthy individuals, most commonly children, there is a higher expectation of safety for vaccines than for many therapeutic agents. In the past, pre-licensure Phase III trials were often designed merely to evaluate the safety profile of a vaccine observed in previous studies. Accordingly, the focus of such trials was on evaluating events that were expected on the basis of earlier studies, that occurred during an interval immediately following vaccination, and that were seen with appreciable frequency. Indeed, a common tactic in older Phase III vaccine trials was to evaluate adverse events only in a small subsample of the total trial population, obviating the possibility of detecting rare but potentially significant side-effects. Modern regulatory agencies now usually require that surveillance for adverse events be undertaken for the total study population rather than for a subsample, at least for events that can be detected passively (e.g., among persons presenting to health care centers for treatment), and that this surveillance be maintained for the duration of the trial.

Increasing concern about vaccine safety has also led to other changes in the design of Phase III trials. Formerly, calculation of sample sizes for such trials was usually geared to enable detection of a certain level of vaccine protection against the target illness, with an acceptable level of statistical power. Detection of rare side-effects was not a typical goal of such calculations. The recent experience with the live oral, quadrivalent rhesus rotavirus reassortant vaccine, which was withdrawn from the United States market because of an association with a small but statistically significant risk of intussusception [7, 8], has led to more stringent regulatory requirements for pre-licensure trials of newer-generation, live oral rotavirus vaccines. The United States Food and Drug Administration, for example, has required that these newer- generation vaccines be tested in numbers of infants sufficiently large to be able to detect whether intussusception is a vaccine side-effect. Current trials of these newer vaccines have therefore enrolled samples of infants many times the number enrolled in pre-licensure trials of the live oral, quadrivalent rhesus rotavirus reassortant vac-

cine, resulting in greatly increased clinical development costs. Whether the experience with rotavirus vaccines portends regulatory requirements for substantially larger Phase III trials for other new-generation vaccines remains to be seen.

The experience in the United States with the live oral, quadrivalent rhesus rotavirus reassortant vaccine illustrates yet another issue confronting pre-licensure trials. Data from pre-licensure trials of this vaccine showed a suggestive but non-significant elevation of the risk of intussusception [38]. The United States Food and Drug Administration licensed the vaccine with the proviso that the occurrence of this potential side-effect be scrupulously monitored post-licensure. This was possible because of the well-developed systems for post-licensure surveillance for vaccine side-effects established in the United States [39, 40]. Unfortunately, post-licensure surveillance systems capable of detecting and evaluating rare but serious vaccine adverse reactions are absent in most developing countries.

The weaknesses of post-licensure surveillance systems in developing countries place an even greater onus upon pre-licensure trials in these settings to provide reassurance that a vaccine will not cause rare but serious side-effects. However, the large size and great expense of trials capable of detecting rare side-effects present a major dilemma, especially for vaccines against diseases that are primarily limited to the developing world, for which resources for clinical trials are scarce. Clearly, there is an urgent need to develop improved systems of post-licensure surveillance capable of detecting rare but significant vaccine adverse reactions in developing countries.

Recently work has begun to evaluate the methodological challenges and practical feasibility of establishing large population-based, dynamic computerized databases for evaluating potential vaccine side-effects in developing-country settings. Such databases link vaccination histories to severe disease outcomes in defined cohorts, and have been used successfully in industrialized countries for rapid and credible evaluations of putative vaccine adverse reactions [40]. One such database has been successfully established on a pilot scale in Vietnam [41]. Overcoming the challenges of establishing such databases in the diverse settings of the developing world remains a significant but important research agenda.

Concluding remarks

The basic phases of testing of vaccine candidates are relatively straightforward and widely accepted. The successive phases of clinical evaluation of vaccine candidates allow for acquisition of critical information about vaccine safety, immunogenicity, excretion, transmission, and protection in an incremental fashion, while minimizing the risks to subjects who volunteer to participate in these studies.

In recent years we have seen a burgeoning of vaccine candidates against diseases of developing countries, creating breathtaking possibilities for disease prevention in these settings. At the same time, this profusion of vaccine candidates for the developing world has added a layer of complexity to the seemingly straightforward phased sequence of trials.

The factors underlying this complexity are multiple, and include considerations about when in the phased testing sequence a vaccine developed in an industrialized country should begin testing in developing countries; about whether and how to pursue the clinical development of a vaccine when disappointing clinical results are observed in developing countries; about how to meet the scientific, financial, logistical, and ethical challenges of conducting clinical trials in developing countries that conform to contemporary international standards of Good Clinical Practice; and about how to ensure that a vaccine is acceptably safe before and after licensure. The manner in which these issues are addressed in the future will have a great bearing on the success of efforts to accelerate the introduction of new-generation vaccines into programs for the poor in developing countries.

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