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Specific Issues in the Design and Implementation of an Efficacy Trial for a Lyme Disease Vaccine

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Lyme disease is an emerging infection that has now become the most commonly reported vector-borne disease in the United States. In the 20 years since its initial description, scientific and technological advances have led to candidate vaccines for the prevention of Lyme disease. Recombinant outer surface protein A (OspA) vaccines have been successful in protecting mice in tick-challenge experiments. A candidate OspA vaccine has been found to be safe and immunogenic in phase I and II studies. This article describes some of the lessons that were learned and some of the unique obstacles encountered in the design and implementation of a large phase III efficacy field trial. Pivotal trials of vaccines for Lyme disease can be a major investment of time and resources for subjects, investigators, and sponsors. If properly conducted, they also present unique opportunities to expand our knowledge of the disease.

In January 1995, SmithKline Beecham Pharmaceuticals initiated a prospective phase III multicenter, double-blind, randomized, placebo-controlled study to evaluate the efficacy, safety, and immunogenicity of a lipoprotein–outer surface protein A (OspA) vaccine for prevention of Lyme disease (LD). Initiating this pivotal trial presented a formidable challenge because of a large number of issues not usually encountered in vaccine trials. In this article we discuss the lessons and unique obstacles encountered in undertaking such a venture and the steps taken to address these issues.

Background

LD is an emerging infection with growing public health consequences in parts of the United States and Europe [1]. LD was first recognized as a distinct clinical entity in 1975, following investigation near Lyme, Connecticut, of a cluster of cases originally thought to be juvenile rheumatoid arthritis [2]. *Borrelia burgdorferi* was subsequently identified as the causative organism and is now known to be transmitted by the bite of an infected *Ixodes* tick. OspA, a 31-kD protein, is a major surface protein of the spirochete [3] and has been identified as a promising vaccine candidate [4].

Since the original description of the disease in 1975, the protean clinical manifestations of LD have been well described and characterized. In the United States, the first recognized sign is usually erythema migrans, a characteristic expanding annular rash with central clearing that occurs at the site of the tick bite in ~60%–80% of cases. Late-stage disease, which can occur weeks to years following infection, may cause complex rheumatologic, neurological, and cardiac manifestations [5].

These variable manifestations can make definitive diagnosis problematic and present difficulties in determining case definitions for use in vaccine efficacy trials. The long latency period for the appearance of symptoms also has implications for a trial, since prolonged surveillance must be employed.

Vaccine Development

Recombinant DNA technology was used to express the OspA of *B. burgdorferi* (ZS7 strain) in lipidated form for use as an antigen in a vaccine for the prevention of LD. The ZS7 strain belongs to the genospecies *B. burgdorferi* sensu strictu, as do virtually all eastern North American strains of the agent of Lyme disease. Preclinical studies showed that this vaccine, adsorbed onto aluminum hydroxide, was able to protect C3H/HeJ mice when challenged by naturally infected ticks collected from the northeastern United States, an area where LD is intensely endemic [6].

In small animals, minimal protective antibody titers have been documented [7]. The protection conferred by the vaccine may occur through two complementary mechanisms. Circulating antibodies to OspA are produced that are capable of neutralizing spirochetes within the tick mid-gut, even before transmission [4, 8]; they may also kill the organisms directly in the host [4]. Tick transmission studies have also indicated a significant degree of vaccine-induced cross-protection between spirochetes from different regions of the U.S. [9].

Following the initial phase I studies, a double-blind, placebo-controlled dose-range study was conducted in 350 healthy adult residents of three New England islands on which LD is highly endemic. An OspA antibody response was detected in >97% of subjects receiving vaccine [10]. In a trial conducted in patients who had previously had LD, the safety and reactogenicity profile of the candidate vaccine was similar to previous obser-

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vations: all doses were well tolerated, although mild local reactions (mostly soreness at the injection site, in 40%–85%) were common [11].

These early studies not only provided important clinical and laboratory data but also were valuable learning experiences with regard to issues such as determination of case definition and documentation of infection. They also provided encouraging signs for the concept of an efficacious vaccine and reinforced the notion that a very large field trial would be necessary to prove efficacy.

Epidemiological Data

As in most vaccine trials, identifying the population at risk is a critical component. As far as LD is concerned, defining this population is particularly challenging because of several factors, including considerable variation in attack rates, even within areas of endemicity; seasonal transmission; year-to-year variability in incidence; and the need for outdoor exposure by subjects.

LD has rapidly become the most commonly reported vector-borne disease in the United States [12]. Surveillance for LD was initiated by the Centers for Disease Control and Prevention (CDC) in 1982. Since that time there has been at least a 19-fold increase in reported cases. The reason for this dramatic increase is multifactorial and includes increased awareness by health care providers, increased contact between humans and deer ticks, and improved reporting of cases since LD became a reportable condition in the United States in 1991.

Although the true incidence of LD in the United States is unknown, the overall reported incidence rate was 5.2 per 100,000 for 1994. Eight states reported greater rates per 100,000: Connecticut, 62.2; Rhode Island, 47.2; New York, 29.2; New Jersey, 19.6; Delaware, 15.5; Pennsylvania, 11.9; Wisconsin, 8.4; and Maryland, 8.3. The cases in these states accounted for 11,476 (88%) of the cases reported nationally [13]. These figures are strikingly higher than those for 1993. Evidence of seasonal variation has been well documented. In 1993, a total of 8,285 cases of LD were reported to the CDC by 44 states, 15% less than the number of LD cases (9,677) reported in the continental United States during 1992 [14].

There is considerable variation even within areas of endemicity. In 1992, Connecticut had a statewide incidence of 53 cases/100,000, with some communities reporting rates exceeding 250 cases/100,000 [15]. In a prospective cohort study conducted in one Connecticut county, clinical and asymptomatic *B. burgdorferi* infection in schoolage children was evaluated during three tick seasons (1990–1992). The incidences of clinical LD and asymptomatic *B. burgdoferi* infection were 10.1 and 3.8 cases/1,000 person-years, respectively [16].

The high incidence in some areas facilitated selection of sites. However, in order to obtain a representative cross-section of affected geographic areas, study sites in communities with lower incidence rates were chosen as well. Thirty-one sites in 10 states were selected for the pivotal trial. The distribution by geographic area was as follows: New England states (Connecticut, Rhode Island, Massachusetts, and Maine) accounted for 19 sites and 60% of the study population; Mid-Atlantic states (New York, Pennsylvania, New Jersey, Delaware, and Maryland) accounted for 11 sites and 39% of the study population; and 1 site in the Midwest (Wisconsin) accounted for 1% of the study population.

In addition to affecting site selection, the variation in reported rate and the estimation of the true incidence of the disease made it difficult to determine the appropriate sample size. With reported seasonal attack rates that vary in most publications from 0.1% to 4.0%, the sample size required to detect vaccine efficacy would vary significantly. It was decided to base the sample size and power calculations on a conservative estimate of an LD seasonal attack rate of 0.5%.

Eight thousand subjects (4,000 per group) would provide ample power for the primary endpoint analysis. While this number of subjects should provide reasonably tight confidence intervals, it will not be sufficient to determine vaccine efficacy against rare manifestations of LD with comfortable precision. The cost and feasibility of conducting a trial involving a huge number of subjects must be balanced against the potential statistical shortcomings.

Timing of Vaccinations

The complex enzootic cycle of *B. burgdorferi* has been well defined and accounts for the seasonal occurrence of LD [17]. This has implications for studying the disease, since the logistics of timing the vaccinations for all the enrollees must be addressed and decisions regarding the number of tick seasons to be evaluated must be made. With >10,000 subjects enrolled, it was decided that two tick seasons would provide a comfortable amount of safety data and sufficient exposure to assess the vaccine efficacy following primary immunization with 2 doses 1 month apart, as well as following a booster dose administered 1 year later. Prolonged surveillance was employed because of the long latency period for the appearance of some of the symptoms (e.g., rheumatological and neurological manifestations) and because it will assist in addressing the issue of whether vaccination alters or attenuates the disease process.

To potentially be protected against *B. burgdorferi* infection, subjects must have developed the minimal protective antibody titer as predicted by the preclinical studies. The phase II studies revealed that 4 weeks after the second dose of 30 μg of the lipoprotein-OspA vaccine, this level of antibody was easily achieved. Therefore, subjects received two doses of vaccine 4 weeks prior to the earliest potential exposure period. This tight timetable presents a major constraint on LD vaccine efficacy trials.

In the northeastern United States, *Ixodes scapularis,* the deer tick, feeds on humans from April/May through September/October. Consequently, the vaccinations had to be initiated in

late January to early February, followed by the second dosage in late February to early March, in order to have potentially protective titers established by April/May. This translated into vaccinating >10,000 subjects in a 4–6-week window twice in a period of 3 months. Educating, coordinating, and monitoring such an undertaking required a tremendous effort on the part of study and site personnel and represented a potential drain on resources. The 98.8% rate of compliance for subjects receiving both doses is a tribute to the efforts at each site.

Another issue regarding timing of vaccination, which was not initially appreciated, was the potential exposure of vaccine to extreme temperatures during shipping in January and February. This was addressed by insulating the shipping containers to avoid freezing and attaching a thermal recording device to each shipment package.

Enrollees

Vaccine trials usually require that subjects be in good health and be compliant with the protocol procedures. Trials involving LD also require that subjects have potential exposure to ticks. Outdoor exposure such as biking or hiking was emphasized in advertisements for potential subjects in order to obtain enrollees with maximal potential tick exposure. Regional and local advertising through all available media venues in the community was implemented very actively by individual investigators and by the sponsor. Investigators were also offered an incentive for surpassing enrollment milestones, which further stimulated active recruitment.

Remaining in an area of endemicity for at least 1 month during the LD transmission season was incorporated as an inclusion criterion for subjects to be considered for the per-protocol efficacy analysis. Every effort was made to continue to obtain follow-up information on subjects who left areas of endemicity, in order to ensure that they had not developed signs or symptoms of LD as a result of the long latency period following potential exposure.

Undoubtedly, one of the factors that helped us achieve our enrollment goal of >10,000 subjects in a 2-month period was the anxiety that LD has instilled into the affected communities. In many areas, confusion regarding the diagnosis, treatment, and potential sequelae has heightened the fear of contracting LD. Although subjects were fully instructed that this was a placebo-controlled trial and that efficacy had yet to be proven, this did not dampen the enthusiasm and the willingness of the subjects and, in some cases, of the entire community to participate.

Case Definition

Another critical component in any trial is defining the primary endpoint and the case definitions. For most vaccine trials, a well-defined clinical endpoint is identifiable, usually in the form of culture, serological assay, or recognized clinical signs and symptoms. LD presents special problems since the clinical manifestations are protean, and laboratory testing is problematic as well. Cultures, PCR, and determinations of T cell–mediated responses provide evidence of *B. burgdorferi* infection but are not routinely available options except in some research laboratories. ELISAs are commercially available but lack sufficient sensitivity and specificity for use in efficacy trials. Western blot (WB) assays are specific but need standardization.

Since no single laboratory or clinical parameter can be utilized to identify all suspected cases, criteria have been developed by the CDC. The CDC criteria, however, were developed as a surveillance tool, which frequently necessitates a compromise between sensitivity and specificity to reach the optimal surveillance objective. For example, cases of LD presenting as a flulike illness or involving asymptomatic seroconversion are not included in the CDC definition.

All these subsets must be captured in an efficacy trial. The CDC criteria were therefore deemed to be inadequate for the purpose of conducting a pivotal efficacy trial. The final protocol case definition included several clinical and serological subsets, with the primary analysis being dependent upon objective evidence of infection.

Another case definition issue that arose during the phase II study was the criteria for WB positivity. The CDC/Association of State and Territorial Public Health Laboratory Directors Working Group criteria for defining WB positivity [18] were incorporated into the phase III protocol along with a definition of seroconversion. Indeed, one significant advantage of a prospective trial is that both prestudy and poststudy sera can be available for testing.

The protocol case definitions ultimately required extensive diagnostic testing and documentation. As soon as subjects developed symptoms suggestive of possible LD, they were asked to make a site visit for an acute evaluation. This evaluation was based on the medical history, physical examination, WB assays and testing of sera for titers of antibody to OspA, and additional testing depending upon the symptomatology. In the event of rash, photographs and skin biopsy specimens (for culture and PCR for *B. burgdorferi*) were obtained in standardized fashion.

Arthrocenteses were performed if clinically indicated. Photographs were also obtained to document facial palsy. Radiculopathy, syncope, and meningitis or encephalopathy were evaluated and documented with the appropriate laboratory test: nerve conduction studies, electrocardiography, and lumbar puncture, respectively.

Subjects were asked to return to the site 2–4 weeks later for a follow-up visit to assess their condition and obtain a convalescent serum sample for WB testing.

In addition, each vaccinee had sera drawn at baseline and after both transmission seasons for WB testing to detect seroconversion. The WB specimens at month 12 from all vaccinees were collected from February to April and needed to be tested

before the busiest time of the second transmission season, when acute and convalescent serum results for new suspected cases would be awaited anxiously by subjects. Collecting such extensive data to document a case required a willingness on the part of all the investigators to devote the necessary resources to such an effort. The number of well-documented confirmed cases (~1% of the participants after the first transmission season) is a tribute to their efforts.

In the phase II studies it became evident that a significant percentage of skin lesions clinically thought to represent obvious erythema migrans could not be confirmed with culture, PCR, or determination of seroconversion. Therefore, in the phase III study, all patients with possible erythema migrans rashes—no matter how "classic"—had to undergo full diagnostic evaluation. Adequate photographic documentation of the lesions was also important, and careful consideration was given to selection of the photographic guidelines and equipment. Different categories were assigned to those erythema migrans lesions confirmed by laboratory testing vs. those about which there was only a strong clinical suspicion.

Surveillance

Once case definitions were determined and an algorithm constructed to obtain the supportive data, the question of surveillance for the detection of suspected LD cases among >10,000 vaccinees was addressed. Enrollees were given wallet-size cards as well as refrigerator magnets as a reminder of their participation in the study. We believed that a postcard system was most likely to ensure continued participation on the part of the enrollees as well as to capture all the information needed to identify potential cases that might not otherwise be available.

Postcards were sent to each vaccinee five times during the first transmission season of the trial. Each postcard listed a series of questions regarding the appearance of potential LD symptoms and other significant health problems. If any symptom was checked off, the site contacted the subject for additional information and arranged a visit, if necessary. The subjects always had the option of contacting the investigator regarding their symptoms.

More than 54,000 postcards were mailed to enrollees, and an impressive 90% were returned to the sites. An additional 7% of enrollees provided information when contacted by telephone. Only 3% of the postcard data were not retrieved, which again serves as witness to the outstanding compliance of the study participants.

At the end of the first transmission season, after all the suspected cases had been identified, the sites were surveyed to determine the number of suspected cases identified specifically through the postcard system. Fewer than 1% of the suspected cases were identified via this mechanism, a finding confirming the efficiency of the surveillance system. Undoubtedly, the postcards served as a constant reminder to the enrollees of their participation in the trial. Since the postcard system was so successful, it was repeated with modifications during the second transmission season.

Suspected LD Cases

All subjects who developed symptoms of LD were asked to return to the investigator's site for further evaluation. This evaluation included the obtaining of acute and convalescent serum samples for OspA antibody testing and WB assays. Depending upon the symptoms, other specimens (synovial fluid, CSF, skin biopsy) were obtained for culture, PCR, or testing for T cell–mediated immunity.

All of the testing was performed by one central laboratory (that of Dr. Allen Steere), and the challenge was to provide the investigator with the results within 48 hours. The coordinating physician (Dr. Steere) and the site investigators remained blinded throughout the study since the WBs were interpreted by the laboratory technicians only. Dr. Steere and the investigators received the test results in blinded fashion, without mention of the presence or absence of a 31-kD (OspA) band.

At the initiation of the study, the concern was that suspected cases would not come to the investigator's attention—hence the need for constant surveillance. Early in the first transmission season, it became obvious that there was a very high rate of recall among the participants and that the challenge would be to process all the specimens in the required time frame. Ultimately, >10% of the total study population was evaluated for suspected LD; >2,500 specimens were submitted to the laboratory for this subpopulation alone.

In addition, the same laboratory was responsible for performing systematic WB testing on each vaccinee as previously described. Collecting data on these subjects, processing their specimens, notifying sites of the results, and entering the information into the database became a monumental task. All of this was a direct result of our desire to apply a very high level of suspicion in order to capture all the suspected cases and to accurately identify and categorize documented cases of LD.

Data Management

Conducting a trial of this magnitude required that both the sponsor and the sites, especially those with enrollments of 900 and 1,200 enrollees, be prepared to deal with the tremendous volume of data. Since it would have been impractical to have paper case-report forms filled out and manually entered into a database, an electronic remote data-entry system was used. Although additional up-front time was required to design screens and arrange training for site and sponsor personnel, the on-site computer system appears to be a worthwhile investment.

Another major benefit is access to the data in "real time," allowing up-to-date safety surveillance. The number of adverse events collected for >10,000 subjects followed for 2 years also

justified the use of the remote entry system. In fact, the number of adverse events was so large that it could be considered "too much background noise." In this regard we are indebted to the Data Safety Monitoring Board (DSMB) and Dr. Steere, whose advice on evaluating the adverse events and especially the serious adverse events has been invaluable. Dr. Steere has also coordinated and monitored all laboratory activities, including assay validation, sample testing, and the reporting of results.

Data Safety Monitoring Board

The DSMB was created prior to trial initiation for the purpose of having an independent committee oversee the conduct of the study. While such oversight committees are not unusual, the composition of this DSMB was uniquely tailored to meet the needs of conducting an LD vaccine trial.

Dr. Allen Steere has been involved with LD since the initial reports of a cluster of cases involving childhood arthritis in 1975 [2]. As the coordinating investigator for this study, he has reviewed the serious adverse events, suspected cases of LD, and other issues of particular clinical and theoretical concern and has reported his findings to the DSMB.

The members of the DSMB are experts in LD, vaccinology, biostatistics, and infectious diseases, and one member is from the CDC. We have benefited from their wealth of knowledge and experience in dealing with vaccine and epidemiological trials as well as from their previous interactions with regulatory agencies.

The DSMB is updated regularly to review the status of the trial. Its members have been invaluable in dealing with multiple issues, including adverse events (as previously noted), requests for unblinding individual subjects, quality assessments of specimen handling, and confirmation that suspected cases of LD qualify as confirmed cases according to the case definition. The DSMB has also reviewed the preliminary report and recommended that the placebo recipients should be offered vaccine at the end of the study.

Conclusion

A potential vaccine for LD has presented a unique opportunity for vaccinologists and LD experts. LD presents unique problems in study design because of its variable manifestations, its long latency period for the appearance of symptoms following infection, and the lack of available specific diagnostic tests. Attempting a large pivotal trial involving such a condition required a major investment for everyone involved, particularly the subjects, investigators, and sponsor. It also represents a major opportunity to learn more about the epidemiology as well as the clinical and laboratory manifestations of LD.

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