Issues in Clinical Trials of Prophylaxis of Fungal Infections

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The validity of the results of a clinical trial is highly dependent upon the design of the trial. The definition of disease, the selection criteria for enrollment in the trial, the selection of the study and control drugs, and the end points all affect whether the information obtained from the trial ultimately is useful in making decisions in clinical practice. These factors all apply to the design of clinical trials of the prophylaxis of infectious diseases. In addition, prophylaxis trials have several important differences from the design of trials of the treatment of those same diseases. The risk-benefit analysis for trials of prophylaxis is different, in that asymptomatic patients are exposed to the drug and more patients will be exposed than will develop the disease under study. Standardization of the design of such clinical trials will allow more efficient development of new drugs and will allow clinicians to compare more accurately the safety and efficacy of prophylactic agents.

To demonstrate the safety and efficacy of an antimicrobial in the prophylaxis of an infectious disease, clinicians, as well as regulatory agencies, desire adequate and well-controlled clinical trials with clinically relevant end points. However, there are important differences between clinical practice and clinical trials and between prophylaxis and treatment trials. Here, I examine issues regarding the design of clinical trials for prophylaxis of infectious diseases. Although the focus is on the prophylaxis of fungal diseases, the general principles are similar in prophylaxis trials in bacterial, viral, and parasitic infections. I discuss the definition of prophylaxis, the general considerations in clinical trials of prophylaxis of infectious diseases, the characteristics of study drugs and control agents, and the selection of appropriate patient populations and end points.

DEFINITIONS OF STUDIES OF ANTIFUNGAL THERAPY

It is important to have clear definitions of the entity under study in a clinical trial. Clear definitions allow investigators to have consistency in the types of patients they enroll in a trial and to generalize the results to patients outside the trial, allow regulatory agencies to accurately describe the intended use of the drug in prescription drug labeling, and allow clinicians to appropriately use the label information in clinical practice. In clinical trials of antifungal agents, it is important to distinguish between trials that examine prophylaxis from those that examine preemptive therapy, empirical therapy, or treatment of definitive disease. The presence or absence of invasion of pathogenic organisms and the presence or absence of symptoms differentiates these entities.

For the purposes of clinical trials, one can define “prophylaxis” as administration of antimicrobials to patients at high risk of developing the disease under study, who are not infected at the time of study entry and who are not manifesting symptomatic disease. One can define “infection,” for the purposes of such trials, as invasion of a potentially pathogenic organism measured...
by laboratory testing for the organism (e.g., culture and antigen testing) or for the host response to that organism (serological testing). Infection in this sense is not synonymous with the presence of signs and symptoms, but rather a measurement of invasion of the organism. The goal of prophylaxis is to prevent the development of symptomatic disease, not merely infection.

A clinical trial of prophylaxis examines a specific disease caused by a specific organism or set of organisms in a specific patient population. For example, the clinical trials that resulted in US Food and Drug Administration (FDA) approval of fluconazole for prophylaxis examined the prevention of diseases caused by Candida in the population receiving bone marrow transplantation [1].

Authors have used the term “preemptive therapy” in a variety of ways in the medical literature. For the purposes of clinical trials, one can define preemptive therapy as the administration of antimicrobials to patients already infected, that is, in whom there is evidence of pathogen invasion but who do not yet manifest symptomatic disease. This same definition, however, is synonymous with what authors have termed “secondary prophylaxis” or “preventive therapy.” Such patients have been previously infected and have experienced symptomatic disease but may still harbor organisms and/or remain at risk for further episodes of symptomatic disease. For example, the administration of ganciclovir to asymptomatic solid-organ transplant recipients with positive results of tests for cytomegalovirus antigen or culture of blood for cytomegalovirus has been termed “preemptive” therapy [2]. On the other hand, administration of isoniazid to asymptomatic patients with a positive skin-test result after exposure to Mycobacterium tuberculosis has been termed “preventive therapy” [3]. Administration of fluconazole to a patient after recovery from cryptococcal meningitis has been termed “secondary prophylaxis” [4]. In all of these examples, the goal of the administration of antimicrobials is the same: the prevention of symptomatic disease after the occurrence of documented infection. One then could consider the design of trials for preemptive therapy, secondary prophylaxis, and preventive therapy in a similar way. Authors have used the term “preemptive prophylaxis” to refer to prophylaxis in patients with specific risk factors for infection [5]. Study of a drug in this setting would still seem to be consistent with prophylaxis, rather than preemptive therapy. It would be helpful for investigators, clinicians, and regulators to clarify the terminology of “preemptive” trials.

One can define “empirical therapy” as administration of antimicrobials to patients with signs and symptoms of disease when clinicians interpret those signs and symptoms as indicative of infection by a particular organism or set of organisms. In empirical therapy, there is no microbiologically or histologically definitive proof, at least initially, by culture or other means, of an infectious etiology. There is, however, a high suspicion that infection is the cause of the patient’s symptoms. This situation is not unusual in clinical trials of infectious diseases. For instance, patients with fever, cough, sputum production, and presence of an infiltrate on chest radiography often are enrolled in clinical trials of community-acquired pneumonia, even in the absence of positive blood or sputum culture results [6]. In the case of clinical trials of antifungal drugs, empirical therapy is administered to neutropenic patients who remain febrile after receiving antibacterial therapy for 4–7 days [7]. Clinicians administer empirical antifungal therapy to such patients with the idea that fever represents a presumed fungal infection. The limitations of current diagnostic techniques, however, often preclude making a definitive microbiological diagnosis [8]. Prior autopsy studies demonstrate that these patients may have occult fungal infections [9].

It is important to distinguish that clinical trials of empirical therapy are not designed to examine prevention of disease. This is important since the inclusion and exclusion criteria as well as the end points of prevention trials differ from those of treatment trials. It is logical that one cannot prevent a disease that a patient has already acquired. If the signs and symptoms experienced by the patient are not sufficiently specific for selecting patients with the disease, then whether those patients require empirical therapy is called into question. More specific selection criteria may help address this problem. Alternatively, better diagnostic testing may obviate the need for empirical therapy.

**GENERAL CONSIDERATIONS IN ANTIFungal PROPHYLaxis TRIALS**

Clinical trials of prophylaxis of infectious diseases differ in several important ways from trials that examine treatment of established disease. Most important, the evaluation of the risks and benefits of administering an antimicrobial agent are different in prophylaxis versus treatment. In a treatment trial, presumably all patients in the trial have the disease under study or at least are suspected of having the disease. In a prophylaxis trial, patients are asymptomatic but at risk of developing the disease. In a prophylaxis trial, it is accepted that many patients will not develop the disease under study. Thus, many patients in the trial will not obtain benefit from administration of the antimicrobial but remain at risk for drug-related adverse events. Administration of antimicrobials to patients who do not develop disease may also result in selection pressure for colonization with organisms resistant to the prophylactic agent [10]. Colonization with resistant organisms may place these patients at risk for infections with these organisms. Also, these patients may become a reservoir for spread of resistant organisms to others.

Given these considerations, clinical trials of prophylaxis of infectious diseases should carefully select the diseases most likely to warrant prophylaxis and the patients most likely to benefit

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from administration of prophylactic agents. Infectious diseases that are likely targets for prophylaxis should be relatively common in the patient population under study [11]. Patients are most likely to benefit from prophylaxis when established disease is difficult to treat either because there are few therapeutic alternatives or because available drugs have significant toxicity. The risk-to-benefit ratio of prophylaxis is most favorable when established disease is associated with significant morbidity and mortality.

Several examples illustrate these concepts. Oropharyngeal candidiasis is relatively common in patients who have AIDS and low CD4+ cell counts. However, oropharyngeal candidiasis is easily diagnosed and treated and is not associated with significant morbidity or mortality. For these reasons, experts do not recommend routine prophylaxis for oropharyngeal candidiasis [12]. Cryptococcal meningitis in patients with AIDS is associated with significant morbidity and mortality. However, the disease is relatively uncommon, even in patients with low CD4+ cell counts; therefore, experts do not recommend routine prophylaxis. Pneumocystis carinii pneumonia is relatively common in patients with AIDS who have low CD4+ cell counts. P. carinii pneumonia is associated with significant morbidity and mortality. The high doses of trimethoprim-sulfamethoxazole required for treatment can be associated with toxicity. For these reasons, experts do recommend routine prophylaxis for P. carinii pneumonia in patients with AIDS and CD4+ cell counts of <200 cells/mm³ [12].

For regulatory approval of an antifungal drug for a given disease, the US FDA usually requires 2 adequate and well-controlled trials demonstrating that the drug is safe and effective for the disease under study, because of the natural variability in clinical trials and the desire to show that the results of one successful trial are reproducible. One trial may be acceptable in certain situations in which there are strong supportive data. These supportive data are usually in the form of results from other clinical trials showing the efficacy of the drug in treatment of diseases caused by the same organisms against which prophylaxis is intended. These data are most easily applicable if the dose in the supportive trials is similar to that proposed in the prophylaxis trial. Whereas in vitro and animal models are helpful in assessment of drug efficacy, they are not substitutes for clinical information. Supportive information may differ, depending on the characteristics of the drug under study. In the case of fungal disease, a nonabsorbable antifungal agent may show efficacy against mucosal disease. A systemically absorbed antifungal drug may show efficacy against various forms of invasive disease. In a study that attempts to show that a new antifungal drug is similar in efficacy to some approved control drug (noninferiority trial) in the prophylaxis of fungal disease, the need for some demonstration of antifungal efficacy in the treatment of established disease is even greater. This need is due to the possibility that the absence of documented fungal infections in both arms of a noninferiority prophylaxis trial may mean that both drugs were effective at preventing infections or neither was needed for prophylaxis if the population was at low risk.

There may be considerable variability in the incidence of fungal infections in prophylaxis trials [13], such that it may be hard to predict the expected rate of fungal infections in a given population. In a recent prophylaxis trial comparing itraconazole with fluconazole as prophylaxis among bone marrow transplant recipients, the rate of fungal infection in the fluconazole arm was 25%, compared with 2.4% in a trial comparing fluconazole with micafungin in a similar population. Three recent descriptions of the rate of fungal infections in small numbers of patients with nonmyeloablative transplants varied from 0% to 33% [14–16].

INCLUSION AND EXCLUSION CRITERIA

The inclusion and exclusion criteria of a prophylaxis trial select the patient population most at risk for developing the disease under study. The selected patient population may have a large effect on the ability to demonstrate the efficacy of a prophylactic drug compared with placebo. For example, clinical trials of fluconazole for prophylaxis demonstrated a benefit in decreasing invasive Candida infections in hematopoietic stem cell transplant recipients [1] but not in patients with acute leukemia [17].

The risk factors for acquiring a given infection are determined from prior studies in that patient population. The risk of disease in that population should be high enough in a placebo group to detect a difference between the placebo and the prophylactic drug when “reasonable” numbers of patients participate. Large sample sizes can make a clinical trial impractical from both economic and clinical points of view. However, issues related to sample size are different in clinical trials examining treatment compared with those examining prophylaxis. It may be difficult to study patients with an established disease that is relatively rare, but this does not mean that studying treatments for rare diseases is not an important public health goal. On the other hand, because many patients who will be exposed to a prophylactic agent may not develop the disease while still being exposed to adverse effects or resistant pathogens, the public health equation is different for prophylaxis trials. If thousands of patients must be studied to show only a small benefit for a prophylactic agent over placebo, then one must question whether that disease or that patient population warrants prophylaxis. A statistically significant difference determined with a very large number of patients may not be clinically meaningful.

In determining the inclusion and exclusion criteria for a prophylaxis trial, it is most helpful to select a patient population that is at risk for a finite period of time. The efficacy of drugs administered for prophylaxis may decrease over time as patients
become colonized with organisms resistant to the prophylactic agent [10]. For instance, patients who have neutropenia after receiving cancer chemotherapy are at risk during the period of neutropenia, but patients with spontaneous bacterial peritonitis remain at risk as long as they have liver dysfunction, which may be indefinitely [18]. In the latter case, it is difficult to pick a meaningful time for an end point, because the patients are at continuous risk for developing the disease under study, even after completion of the trial.

The methods for selection of the patient population in the trial should be clear and reproducible so that clinicians can use them in practice. For example, in a recent single-center trial of antifungal prophylaxis in a surgical intensive care unit, patients were enrolled in the trial if they had an expected stay in the unit of at least 3 days as determined by the principal investigator [19]. In a multicenter trial, these criteria would need to be spelled out in the trial protocol so that other investigators could use the same criteria in discerning the expected duration of stay. These criteria then could be put into prescription drug labeling so that clinicians in practice could use the same criteria in selecting patients who may benefit from prophylaxis.

Patients who are not colonized with Candida appear to be at lower risk for developing Candida infections [11]. Ideally, patients who are not colonized with Candida may be excluded from studies of prophylaxis of Candida infections. Unfortunately, with current diagnostic techniques, it takes some time to determine whether patients harbor Candida. This delay may make the performance of screening cultures for Candida before enrollment impractical. However, in the future, new diagnostic techniques may allow a more timely determination of fungal colonization and may be useful in determining entry criteria for prophylaxis clinical trials. Other technical issues, however, complicate the use of colonization as a screening tool. There are no accepted standards for determining the sites from which to obtain culture samples, the number of sites from which to obtain samples, and what quantity of organisms, if any, define colonization.

CHARACTERISTICS OF STUDY DRUG AND CONTROLS

An adequate safety profile is one of the important characteristics of a drug under study for prophylaxis. As stated above, the risk-benefit equation for a prophylaxis trial is different from that for a treatment trial. In a treatment trial, one may be willing to accept some toxicity of a drug if there are few alternatives for treatment and established disease carries significant morbidity and mortality. In a prophylaxis trial, however, one may be less accepting of drug toxicity, because patients are asymptomatic at enrollment and many patients will not develop symptomatic disease.

The preclinical data on the study drug should support the planned duration for use in the prophylaxis trial. For instance, if the preclinical animal safety studies of a drug show data for up to 6 weeks of administration but the planned duration of dosing in a prophylaxis trial is 12 weeks, additional animal safety studies may need to be done. These studies help to demonstrate that there are no potential safety problems before this duration of treatment is initiated in clinical trials of humans.

The preclinical in vitro and animal data also should provide an adequate rationale for the proposed dose of study drug in a clinical trial. If there are other clinical trials of the study drug’s use against established fungal diseases, these may be helpful in providing such rationale. This extrapolation of dose selection in a clinical trial for prophylaxis is easiest when the dose selected for prophylaxis is the same as that in trials of established disease. For instance, if a drug sponsor studies a given dose to treat esophageal candidiasis, it is most straightforward to select that same dose for prophylaxis because that dose has demonstrable antifungal activity. There is precedent for use of a different dose or schedule of administration for prophylaxis than that used for treatment of established disease. Examples include azithromycin in the prophylaxis of infections due to Mycobacterium avium-intracellulare complex and trimethoprim-sulfamethoxazole to prevent P. carinii pneumonia [12]. Although, in theory, the amount of drug necessary to prevent a disease may be less than that necessary to treat established disease, one still needs to show that the lower dose is indeed effective as an antimicrobial agent, especially in the setting of a noninferiority trial. There is less concern if the planned prophylaxis trial is designed to show superiority, rather than noninferiority, of a lower dose of the study drug to placebo or another antimicrobial agent, because superiority directly demonstrates antimicrobial efficacy.

One could consider a placebo control, an active control, a dose response, or a historical controlled trial to examine prophylaxis. Active controlled clinical trials used for registration of a drug product usually use a US FDA-approved control, because the safety and efficacy of the approved agent is known. At present, the only systemically active US FDA—approved drug for prophylaxis of fungal infections is fluconazole for Candida infections in patients receiving bone marrow transplantation. Trials can use unapproved comparators if the trial is designed to show superiority of the test drug over the unapproved control [20]. This ensures that the test drug has some activity greater than that of placebo. Alternatively, one could design the study to show similarity of the test drug to the unapproved control. These trials are termed “noninferiority” trials, because, technically, they attempt to show that the test drug is no worse than the control drug by some amount, rather than to show true equivalence. One can design a noninferiority trial with a test drug compared with an unapproved control by providing adequate data from the medical literature and elsewhere showing the benefit of the unapproved control over...
placebo and the magnitude of such benefit in the population and the disease under study.

When there is no approved drug for prophylaxis for a given disease or population, it may be most efficient to study a test drug in comparison with placebo. A relatively high rate of infection in the population under study is the reason for performing a prophylaxis trial but does not allow the conclusion that the test drug is safe and effective before the results of the trial are obtained. Finally, one could question the ethics of giving a drug to large numbers of patients, many of whom may obtain no benefit from the prophylactic drug, in the absence of data on the safety or efficacy of the drug in prophylaxis.

Two other alternatives are dose-response and historical controlled trials. Dose-response trials test several doses of a test drug. Superior efficacy of higher doses compared with lower doses of a drug provides inferential evidence that the drug has efficacy greater than that of placebo. Historical controlled (external controlled) trials compare the results of the efficacy and safety of the test drug with data external to the trial. These data may be obtained concurrently. However, control data are usually gathered before initiation of the trial, hence the term “historical” control. Historical controlled trials have several unique forms of bias [20]. Determination of the percentage of breakthrough fungal infections in a given population may depend on changes in the practice of medicine, supportive care, and host effects. As noted above, the rate of fungal infections in various studies, even in the same population, may vary widely [14–16]. These variations may be explained in part by selection criteria for the patients and by prior antifungal therapy. However, this example demonstrates the potential biases in comparing the results of a test drug with those of an external control. Historical controlled trials also tend to underestimate the efficacy in the control group. Finally, the acquisition of safety data in the trial of the test drug and the external control may differ, which may make safety comparisons between drugs difficult in the historical controlled trial.

END POINTS

Interpretation of the results of a clinical trial is highly dependent on the questions asked before initiation of the trial. Perhaps this is most evident in the selection of end points. End points for trials should be clinically relevant to patients and clinicians. Patients are most concerned about the signs and symptoms of disease. Therefore, resolution of these same signs and symptoms are important end points for trials. In the case of prophylaxis, however, patients are asymptomatic at the beginning of the trial, so the relevant end point is the occurrence of signs and symptoms. Patients who develop microbiologically documented breakthrough fungal infections in association with signs and symptoms should be considered to have experienced failure of prophylaxis. However, the diagnostic accuracy of current tests for invasive fungal disease is not optimal. Therefore, patients who develop fever and other symptoms that clinicians interpret as sufficient evidence of a fungal infection to initiate empirical antifungal therapy may have occult invasive fungal disease as well, even in the absence of microbiological documentation of disease. This introduces some degree of subjectivity into the trial, because clinicians may differ in what they consider evidence of an occult fungal infection. This is most problematic if there is an imbalance between the arms of the trial in the way clinicians prescribed empirical therapy. To minimize this potential bias, clinical trials should standardize the criteria for administration of empirical therapy. The strongest evidence of efficacy is provided when a prophylactic drug can show a decrease in both microbiologically documented breakthrough infections and the need to initiate empirical antifungal therapy.

The testing used by investigators to determine the presence of breakthrough infections is an important consideration in clinical trials. Different centers may have various standards and various degrees of rigor for evaluating the presence of baseline and breakthrough infections. Standardization of the methods used to evaluate patients for these infections in clinical trial protocols would help minimize this potential source of bias. Likewise, protocols also should standardize the criteria for ruling out a baseline fungal infection in patients before enrollment in prophylaxis trials.

An important question in prophylaxis trials is whether the surrogate marker of colonization, rather than actual breakthrough infections, is a useful end point. Because far more patients may become colonized than infected, colonization is a surrogate end point and not a true end point of prophylaxis. Although it seems intuitive that decreasing colonization would result in a decrease in infections, several issues make this association less clear. First, the actual number of breakthrough infections in colonized patients affects the clinical relevance of prophylaxis. If one needs to administer prophylaxis to large numbers of colonized patients to prevent few documented fungal infections, this calls into question the clinical relevance of prophylaxis in that setting. There are also technical issues with the measurement of colonization, including which sites to obtain culture samples from, the number of sites to obtain samples from, the quantitation of how many colonies represent colonization, and the reproducibility of culture results. When cultures are done as clinically indicated, rather than prescribed by protocol, then the more ill patients will have more cultures done and a better chance for detection of colonization. These issues become more complicated as one considers the impact of nonabsorbable drugs versus systemic agents. One would not expect nonabsorbable, orally administered drugs to decrease colonization at extraintestinal sites, yet colonization at these sites may affect the incidence of breakthrough infections. Finally, US FDA review of previous prophylaxis trials evaluating
colonization as a secondary end point found that the compliance rate of obtaining cultures at multiple sites and at multiple time points was less than optimal.

The definition of breakthrough infections is an important consideration in prophylaxis trials. Breakthrough infections should be those for which the risk-to-benefit ratio for prophylaxis is most favorable. Various sites of infections may differ in their clinical relevance, possibly because of differences in the severity of disease. For example, superficial fungal infections are easily diagnosed and treated and may not require prophylaxis. Also, there may be varying degrees of diagnostic certainty that positive culture results represent true infection. Cultures of *Candida* from a catheter tip in the absence of a positive blood culture result represent colonization of the catheter and may or may not be indicative of a bloodstream infection. The significance of cultures of *Candida* from bronchoscopic specimens is unclear in the absence of documented pulmonary tissue invasion [21].

Breakthrough infections as the sole end point in prophylaxis trials may lead to misleading conclusions about drug efficacy [22]. It may be most accurate to examine all-cause mortality as well as breakthrough infections as a combined end point for prophylaxis trials. One could question the relevance of preventing breakthrough infections if this does not result in a decrease in mortality for serious diseases. On the other hand, it may be too stringent a criterion to expect a study drug to show a decrease in mortality relative to the control drug, especially in less serious diseases. It is important, however, to examine whether there are differences in the mortality rates between drugs in a trial to at least ensure that the study drug is not associated with worse mortality than the control drug. Given the lack of diagnostic accuracy of current antifungal testing, mortality during therapy may be a manifestation of occult fungal disease. The number of autopsies in clinical trials is usually small, and even with autopsy data it is often difficult to determine the exact cause of death for severely ill patients with multiple comorbidities. With rare exceptions, it is unclear how “fungus-related” mortality is defined compared with other causes of death. Clinicians may differ in their assessments of what constitutes fungus-related mortality, which may introduce a potential bias into the trial. Alternatively, differences in mortality may represent differences in drug-related adverse events. Differences in fungus-related mortality between arms of a trial but no difference in overall mortality means that more patients in one arm must have died of non–fungus-related causes. These deaths may represent occult drug toxicity.

**CONCLUSIONS**

When clinicians examine randomized clinical trial reported in the medical literature, it is usually the results of the trial that are intuitively the most interesting. However, the design of such trials determines how applicable those results are to clinical practice. Elements such as the definition of the disease under study, the selection criteria for patients, and the end points examined in the trial are crucial to determining the internal and external validity of the results. Because these elements often vary between trials involving the same disease, it is sometimes difficult for clinicians to make accurate comparisons of the safety and efficacy among various drugs. Standardization of the methods of clinical trials would allow clinicians to more accurately determine these distinctions. Standardization of the design of clinical trials would also be helpful to investigators and drug sponsors, because it would allow them to design trials most efficiently when seeking licensure of a new drug. Standardizing the important elements for clinical trials will take a combined effort of academicians, clinicians, the pharmaceutical industry, and regulatory agencies.

The design of clinical trials is an evolving and dynamic process, as is medical science itself. As we learn more about the diseases under study, the design of clinical trials should change to accommodate new knowledge. Clinical trials of fungal prophylaxis and therapy would benefit greatly from research on the risk factors for acquiring disease, so that inclusion and exclusion criteria can be designed to select the patients most at risk. With changes in medical practice, these risk factors may change over time. Clinical trials of antifungal agents would benefit greatly from better diagnostic testing. More accurate testing would allow more accurate inclusion criteria for clinical trials and may allow the validation of surrogate end points as well, depending on the characteristics of the diagnostic test.

While awaiting new scientific advances, all interested parties should work together to design trials in a clinically relevant and efficient manner. Clinical trials entail a significant expense, so it is important to design trials efficiently. Perhaps more important, however, if we expose our patients to experimental drugs in a clinical trial, then we have the obligation to those patients to design trials in a way that will give patients and practitioners the best chance of acquiring useful data on the prophylaxis of the disease under study.

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**References**


