Guidance for Industry

Malaria: Developing Drug and Nonvaccine Biological Products for Treatment and Prophylaxis

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

June 2007
Clinical/Medical

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance is one in a series of documents developed by the Office of Antimicrobial Products in the Center for Drug Evaluation and Research at the Food and Drug Administration (FDA) to assist pharmaceutical manufacturers and clinical sponsors in developing antimicrobial drug and nonvaccine biological products.[2] The purpose of this guidance is to assist sponsors in the clinical development of drugs for the treatment and/or prophylaxis of malaria. Specifically, this guidance addresses the FDA's current thinking regarding development programs for antimalarial drugs and the design of the clinical trials to be conducted in these programs. It is the intention of this guidance to serve as a focus for continued discussions among the Division of Special Pathogens and Transplant Products (DSPTP), pharmaceutical sponsors, the academic community, and the public.[3]

This guidance does not address vaccine development, which is regulated by the Center for Biologics Evaluation and Research. This guidance also does not discuss general issues of clinical trial design or statistical analysis. Those topics are addressed in the ICH guidances for industry *E8 General Considerations for Clinical Trials*, *E9 Statistical Principles for Clinical Trials*, and *E10 Choice of Control Group and Related Issues in Clinical Trials.*[4] This guidance

[1] This guidance has been prepared by the Division of Special Pathogens and Transplant Products in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

[2] For the purposes of this guidance, all references to *drugs* include both human drugs and therapeutic biological products unless otherwise specified.

[3] In addition to consulting guidances, sponsors are encouraged to contact the DSPTP to discuss issues that arise during antimalarial drug development and to schedule meetings with the FDA as needed.

[4] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

focuses on drug development and clinical trial design issues that are unique to the study of malaria. This guidance may be revised as new scientific information accumulates regarding malaria and its treatment or prevention.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Use of Foreign Studies

Malaria is a global problem with the greatest burden of disease and mortality occurring in developing countries. Although cases of malaria are uncommon in the United States, antimalarial drugs have significant public health importance in the United States: antimalarial prophylaxis is used extensively by U.S. travelers and by U.S. citizens residing in or deployed to endemic areas (e.g., military personnel). Since malaria is uncommon in the United States, drugs or nonvaccine biological products developed for the treatment of malaria can be eligible for orphan drug designation.

Because malaria is not endemic in the United States, clinical data used to support an application for a new antimalarial therapy (or regimen) probably will be obtained from studies conducted abroad. FDA regulations permit studies performed in foreign countries to be used for drug approval when these studies meet FDA standards for the conduct and design of clinical trials (21 CFR 314.106).

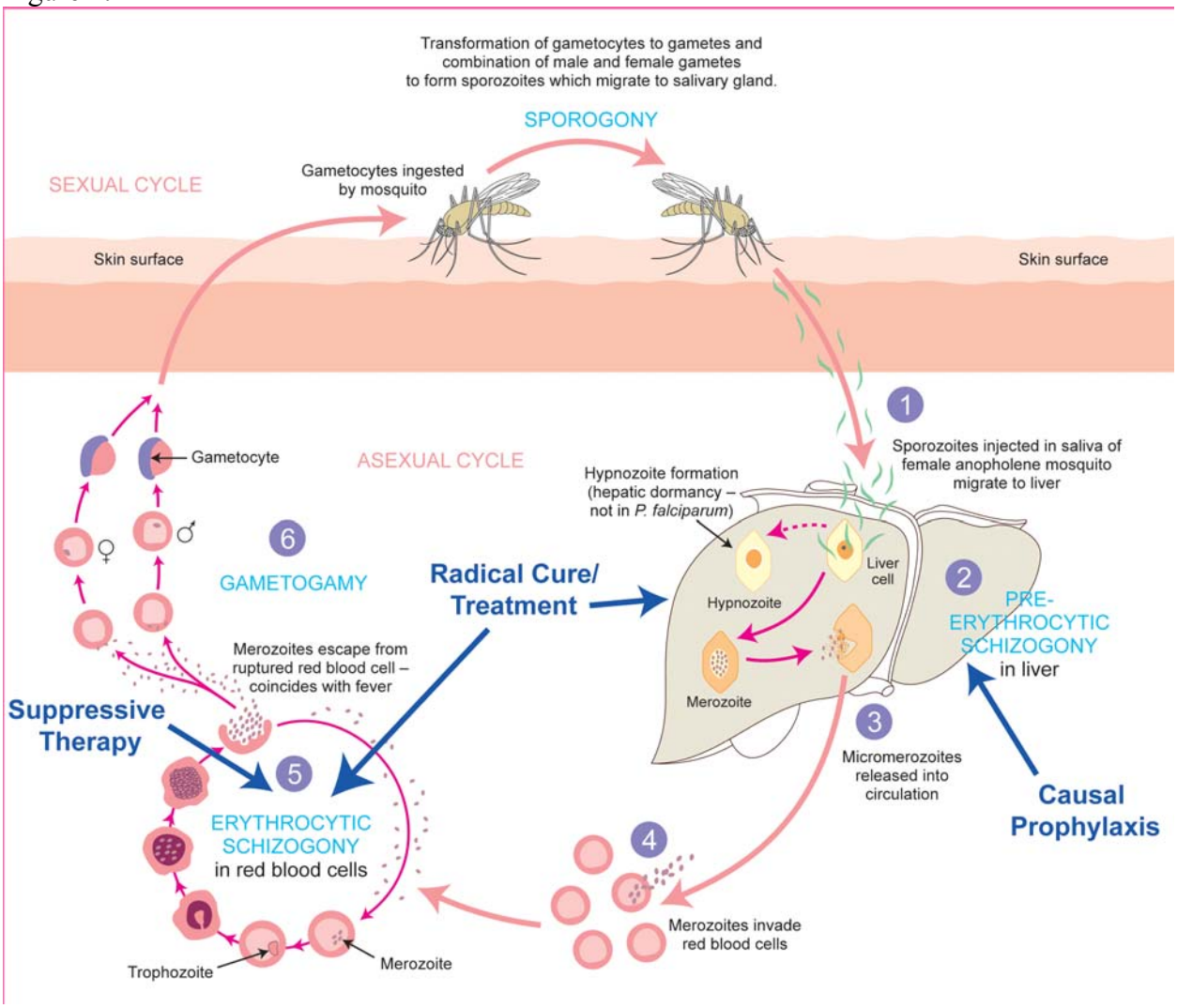
The FDA recognizes the challenges involved in performing studies abroad, and the need to reconcile regulatory requirements with local laws and practices in countries where studies are done. However, complete and comprehensive data for efficacy and safety evaluation are important for drug approval: technical or financial constraints at foreign sites should be addressed by the sponsor during drug development to ensure that FDA regulations regarding clinical trials and good clinical practice are followed.[5] Foreign sites also should be prepared to allow FDA auditing of the site, if requested.

B. Biology of Malaria Parasite

The unique life cycle of plasmodial species (malaria parasite) has specific implications for antimalarial drug development. Following the inoculation of sporozoites by the mosquito, plasmodia undergo initial replication in hepatocytes (hepatic or exoerythrocytic phase) followed by cycles of replication in the peripheral blood (hematogenous or erythrocytic phase), as shown in Figure 1.

[5] See http://www.fda.gov/oc/gcp/default.htm.

Figure 1.[1]



[1] Reproduced with modification by permission of Health Protection Agency, United Kingdom (http://www.hpa.org.uk/infections/toolkit/mosquito.htm).

The type of antimalarial activity that drugs demonstrate may depend on the stage of plasmodial replication that they target (i.e., exoerythrocytic forms (including hypnozoites) or erythrocytic forms (including gametocytes)). Depending on the target, antimalarials can be suitable for radical treatment (elimination of erythrocytic and exoerythrocytic forms), suppressive therapy (suppression of erythrocytic forms following exposure to prevent symptomatic malaria, with no effect on exoerythrocytic forms), causal prophylaxis (eradication of exoerythrocytic forms during prophylaxis), and radical cure (eradication of hypnozoites in relapsing malaria). These terms should be used as appropriate in the development of clinical protocols.

III. SPECIFIC INDICATIONS

The treatment and prophylaxis of malaria include the following specific FDA-recognized indications:

- **Treatment of malaria caused by:**
  - *Plasmodium falciparum infection*
  - *Plasmodium vivax*, *ovale*, or *malariae infection*

  Qualifiers of a treatment indication include:[6]
  - Uncomplicated malaria
  - Severe or complicated malaria
  - Radical cure of relapsing malaria
  - Chloroquine-resistant malaria
  - Multidrug-resistant malaria[7]

- **Prophylaxis of malaria caused by:**
  - *Plasmodium falciparum*
  - *Plasmodium vivax*, *ovale*, *or malariae*

  Qualifiers of a prophylaxis indication include:
  - Suppressive therapy
  - Causal prophylaxis
  - Prophylaxis of chloroquine-resistant malaria

The safety and efficacy of new drugs for the treatment of malaria can be most clearly established in patients with uncomplicated malaria. Effective therapies should have high clinical and parasitological cure rates. In uncomplicated malaria, rescue treatment can be provided promptly to patients who do not respond to study drugs if clinical deterioration occurs, and observations of drug adverse effects are not obscured by the signs and symptoms of severe or complicated malaria. In contrast, study of new drugs for severe or complicated malaria may be difficult to interpret in the face of high mortality rates from complications that are often independent of the parasite load; accordingly, proposals for studies in severe or complicated malaria should be discussed with the DSPTP.

To demonstrate radical cure of relapsing malaria, studies should include adequate numbers of patients with *P. vivax* or *P. ovale* infection to evaluate the eradication of hypnozoites. Patients should be followed for a sufficient duration of time to exclude relapse. The drug under study for the radical cure of malaria should be compared to a drug recognized to be effective against hypnozoites; or should demonstrate a statistically significant reduction in relapse rate when compared to a drug without activity against hypnozoites.

[6] These terms are defined in the following text and in the Glossary.

[7] Clinical development of antimalarial therapy should address regional variation in malarial resistance. This is discussed in the following sections.

The activity of antimalarial drugs against chloroquine-resistant malaria (for treatment or prophylaxis) can be inferred when studies are performed in regions with known high rates of chloroquine resistance. Activity against more broadly resistant malarial isolates (i.e., multidrug-resistant strains), can be supported by a combination of clinical, epidemiological, and microbiological data (see section IV.A.).

IV. DEVELOPMENT PROGRAM

A. General Considerations

1. Preclinical Microbiology

Drugs for the treatment and/or prophylaxis of malaria should be tested in vitro and in animal models before submission of an initial investigational new drug application (IND). Pre-investigational new drug application (pre-IND) guidance regarding the choice of appropriate preclinical models is available from the FDA.[8] The following sections describe preclinical microbiology assessments that should be considered by sponsors as components of the drug development program.

a. Mechanism of action

The mechanism by which the drug exhibits antiplasmodial activity should be investigated, if possible. These studies should include an evaluation of the biochemical and molecular effect of the drug on the different stages of the parasite.

b. Activity in vitro

In vitro activity of an antimalarial drug can be measured against the erythrocytic and exoerythrocytic stages of the *Plasmodium* species using an appropriate model. The results can be expressed as an effect on growth and/or morphology by microscopic examination, or the uptake of radio-labeled hypoxanthine. Other methods may be appropriate, but should be discussed with the DSPTP.

Testing should include laboratory strains of *Plasmodium* species with known patterns of resistance to currently approved antimalarials, and at least 100 clinical isolates from different geographical areas such as Africa or Southeast Asia. Isolates from the regions where clinical trials are planned also should be tested. Appropriate positive controls (e.g., currently approved antimalarial drugs) and negative controls (e.g., drug vehicle) should be included in the study. Different concentrations of the drug under development should be tested in vitro to determine the:

- Optimal concentration effective for inhibiting growth and/or killing of the organism
- Effect of drug on different stages of the parasite in synchronous cultures

[8] See http://www.fda.gov/cder/ode4/preind/default.htm.

There should be an effort to optimize the in vitro testing conditions. This can involve assessing the effects of:

- Using culture-adapted versus fresh isolates
- Using synchronous versus asynchronous cultures
- Having different inoculum sizes
- Using different incubation periods

If optimal testing conditions have been previously established, then the references supporting the testing conditions used should be included in the IND or pre-IND submission. Attempts also should be made to identify and designate a quality control strain during testing.

c. Activity in vivo

Appropriate animal models should be identified to measure the activity of the drug when administered for either prophylaxis or treatment. Considerations when choosing an appropriate model and experimental design include selecting *Plasmodium* species relevant to human infection, the similarity of the course of infection and disease in animals and humans, and the ability to obtain reproducible parasitemia. Endpoints should include:

- Survival
- Reduction in parasitemia
- Effect on erythrocytic and exoerythrocytic stages
- Time to parasite clearance and relapse or recrudescence

In animal studies, parasitological counts and other laboratory measurements should be done at baseline, at regular intervals after the initiation of therapy, and post-treatment. Post-treatment counts and assessments should include evaluations after animals are aparasitemic. Evaluation of the effect of host splenectomy can be useful for determining if a curative effect is sustained. Similar to in vitro studies, appropriate positive and negative controls should be included in each animal study.

Sampling for drug concentrations and pharmacokinetic assessments is strongly encouraged in animal studies, and should be included whenever possible.

The progression of disease in the animal model selected for the study should mimic the disease in humans. Some of the parameters that should be measured include:

- Prepatent period
- Peak parasitemia
- Duration of parasitemia
- Presence or absence of different developmental forms in the blood and liver (including hypnozoites)
- Infectivity of gametocytes

If such parameters were previously established in an animal model (*Plasmodium* species/host animal used), supporting references should be included in the IND or pre-IND submission. In addition, efforts should be made to optimize the testing conditions such as inoculum size or the time therapy is initiated if not already known.

d. Activity of metabolites

The activity of any drug metabolite, identified in humans, should be determined in appropriate in vitro and/or animal models of infection.

e. Drug resistance and cross-resistance

The ability of *Plasmodium* strains to develop resistance when subjected to drug pressure should be examined in appropriate in vitro and/or in vivo models; this examination should include evaluating the potential for cross-resistance to drugs in the same class or in other classes. If resistance is demonstrated, it is important to identify the mechanism of resistance. Attempts should be made to evaluate the clinical significance of any changes in phenotype (e.g., in vitro susceptibility to the drug) or genotype observed in preclinical studies by correlating such changes with clinical outcome.

f. Drug combinations

Preclinical evaluations can be valuable for examining whether there is a potential advantage of combination treatment relative to individual drugs. The following situations should be studied if combination regimens are being considered for study in humans:

- In vitro activity of the combination versus individual drugs against laboratory strains and clinical isolates
- Activity in appropriate animal models of infection
- Activity in vitro and in animal studies against resistant isolates or strains, including those from the geographical areas where the drug is intended to be used
- Characterization of the mechanism by which the drugs exhibit additive or synergistic microbiological effects
- The potential for development of resistance in vitro and in vivo

There are other possible reasons for using combination therapy that may not be reflected in preclinical models (e.g., reducing drug toxicity or convenience of the regimen). However, for combinations that are proposed on the basis of superior antimalarial activity, this effect should be demonstrated in preclinical models before clinical studies are initiated. (For information regarding preclinical safety evaluation of combination therapy, see the guidance for industry *Nonclinical Safety Evaluation of Drug or Biologic Combinations*.)

2. Drug Development Population

Ethnically diverse male and female subjects of all ages should be included in drug development programs for malaria.[9] Since children living in endemic areas are at particular risk for complications from malaria because of the absence of immunity, appropriate pediatric formulations and dose recommendations should be established early in the drug development program so that children can be included in phase 3 studies.

3. Efficacy Considerations

Similar to drug development in other therapeutic areas, two or more adequate and well-controlled studies generally are appropriate for approval of an indication for the treatment of malaria. The Indications and Usage section of the labeling for antimalarial drugs should restrict indications to the specific plasmodial species studied and found to be effectively eradicated in clinical trials.

Although parasitemia is a direct measure of antimalarial drug activity, and an important endpoint in clinical studies, the evaluation of parasitemia can be complicated by variability in the sensitivity and specificity of malaria smears. This is of particular concern for prophylaxis studies where laboratory methods should maximize sensitivity for the detection of breakthrough parasitemia. In treatment studies, parasitological and clinical endpoints generally should be combined into a composite study endpoint, recognizing that fatal complications of malaria may occur after parasites have been effectively eliminated or that asymptomatic parasitemia may exist.

The development of drugs to treat infections caused by resistant plasmodial species represents an important public health need at the present time. The FDA will consider a combination of the following types of data used to support a claim that an investigational antimalarial drug is active against plasmodia species resistant to another approved antimalarial drug:

- Evidence of superior efficacy when the investigational antimalarial drug is compared with another approved antimalarial drug to which resistance is encountered.
- Epidemiological evidence of clinical drug resistance to another approved antimalarial drug in the area where the study is to be performed. High clinical failure rates provide the strongest evidence for antimalarial drug resistance in a given region.
- Evidence of clinical response in patients who have failed alternative treatments because of drug resistance.
- In vitro evidence of activity against isolates with genetic markers of resistance to other antimalarial drugs.
- In vitro evidence of activity against isolates resistant to other approved antimalarial drugs in drug sensitivity assays.

[9] See the guidance for industry *Collection of Race and Ethnicity Data in Clinical Trials* and the ICH guidance for industry *E5 Ethnic Factors in the Acceptability of Foreign Clinical Data* (http://www.fda.gov/cder/guidance/index.htm).

4. Safety Considerations

A safety database of at least 1,000 subjects in phase 1, 2, and 3 studies exposed to the proposed dose and for the proposed duration of treatment should be included in an application for an antimalarial indication. Safety populations should include males and females spanning all ages (i.e., including pediatric and geriatric subjects). The safety population also should sufficiently represent the diverse racial groups likely to be exposed to the drug if it is approved. Drug interaction studies for the drug under development also should be included, as appropriate.

5. Labeling Considerations

The Indications and Usage section should reflect the specific indications and plasmodial species studied. Any important limitations to use also should be included.

B. Treatment Studies

1. Study Design

Clinical trials for a treatment indication should be randomized and double-blinded unless blinding is precluded by technical aspects of the study. If a study cannot be fully blinded, attempts should be made to blind as many study personnel as possible (e.g., study microbiologists interpreting malarial smears). Studies should be conducted in different geographical regions to address variations in the susceptibility of isolates to existing antimalarial therapy, as well as to reflect differences in population host factors.

Antimalarial therapy can take the form of a single antimalarial drug, a combination of drugs, or more than one drug used sequentially. The following sections include specific concerns regarding the development of a combination or a sequential regimen.[10]

a. Combination regimens

Under 21 CFR 300.50, data are required to demonstrate that each component of a fixed-dose combination contributes a measurable advantage over the individual components (e.g., increased efficacy, reduced emergence of resistance, fewer (or less severe) adverse events, or a simplified treatment regimen). Development of a combination regimen for the sole purpose of reducing the emergence of resistance should be discussed with the DSPTP before initiating studies as this endpoint may be difficult to demonstrate even in large clinical trials.

b. Sequential regimens

Several existing treatment regimens employ a short-acting antimalarial drug together with, or followed by, a long-acting drug to prevent recrudescence. Ideally, the comparator and investigational regimens would differ only by the drug used for the corresponding phase of treatment so that differences in outcome can be clearly attributed to the investigational drug.

[10] This is primarily when two active antimalarial drugs are used. Considerations may differ in other circumstances (e.g., when drugs can be combined to improve the pharmacokinetics of one part of a combination regimen).

When this is not possible, additional strategies should be used to demonstrate the contribution of each component of a sequential regimen.

2. Study Population

Although most clinical studies for treatment are carried out in symptomatic patients with documented malaria, initial *proof of concept* studies can be performed in patients with asymptomatic parasitemia to minimize the risk and consequences of treatment failure.

We prefer studies of malaria treatment to be conducted with subjects monitored in a hospital setting so that adverse events can be assessed and treated, and possible treatment failure can be expeditiously addressed. At a minimum, subjects should remain in a monitored setting until resolution of clinical and parasitological abnormalities. In some situations it may be appropriate for subjects to remain in a controlled, monitored setting for the duration of the study to prevent re-infection, thereby permitting a more accurate assessment of cure and recrudescence rates.

Host responses to malaria vary depending on several factors, including immune status (e.g., those living in endemic areas for many years may experience low levels of parasitemia with no ill effect), blood type (e.g., Duffy negative blood types are resistant to infection with *P. vivax*), pregnancy, and age (e.g., pregnant patients and infants are particularly susceptible to complicated malaria). Study designs should take these factors into account. Both immune and nonimmune subjects should be studied, and unless contraindicated, pregnant women and children should be included either in large studies or in specific studies of these subpopulations.

The pharmacokinetics of the drug under development should be characterized in the populations where the drug will be used. This should include study across all age ranges (i.e., pediatric and geriatric subjects), pregnant women, and members of different ethnic groups.

Pharmacogenomic differences between study populations may be a particular concern in malaria studies, and may affect the tolerability or efficacy of antimalarial therapy (e.g., G6PD deficiency resulting in hemolysis following the use of certain antimalarial drugs). Pharmacogenomic concerns should be addressed in the clinical development plan.

3. Entry Criteria

The following general entry criteria are recommended for malaria treatment studies:

- Both adult men and women should be enrolled at all stages of drug development, barring specific sex-related concerns.
- Pregnant subjects should be included when preclinical and human safety data indicate that benefit from use outweighs risk since pregnant women are a population at particular risk for malarial morbidity.
- Children can be included in efficacy trials if preliminary data on adult safety and efficacy are available from earlier studies, and sufficient information is available for determining appropriate pediatric dosing. Though not routinely expected, toxicology studies in juvenile animals should be considered if concerns emerge indicating potential increased

sensitivity in children.[11] Pharmacokinetic studies in children should be conducted early in drug development so that information to guide pediatric dosing is available at the time larger efficacy studies are initiated.

- Patients should have fever at entry, or patients afebrile at enrollment should have fever documented within 24 hours of entry.
- In general, patient symptoms should include shivering, chills, malaise, headache, and loss of appetite in adults, and also include irritability, lethargy, and anorexia in children.
- The infecting *Plasmodium* species should be identified, and entry parasitemia should be limited to values between 1,000/μl and 200,000/μl (0.25 percent to 4 percent).[12] Proposals to study parasitemia outside of this range should be discussed with the DSPTP before protocol submission.
- Patients with mixed plasmodial infections can be included in *P. falciparum* treatment studies with the protocol indicating how these patients will be evaluated.
- Patients with severe or complicated malaria usually should be excluded from studies to evaluate an investigational drug's efficacy and safety. It may be difficult to demonstrate the effect of the drug on these patients because in advanced disease, even active drug therapy may not be able to reverse the progression to a fatal outcome. However, research study of these patients may be appropriate in certain circumstances and/or after the drug has been successfully studied in patients with uncomplicated malaria.
- Patients with prior antimalarial therapy for the current episode should be excluded unless the new drug is under development for patients failing treatment with other drugs.
- Patients with concurrent febrile illnesses (e.g., typhoid fever) should be excluded.

4. Randomization, Stratification, and Blinding

All studies should be double-blinded and randomized. If subject and/or investigator blinding is not possible, it is highly desirable to blind other study personnel (e.g., study microbiologists during evaluation of parasitemia in blood samples).

In areas where the human immunodeficiency virus (HIV) is prevalent, subjects should be stratified by the presence or absence of HIV at enrollment. HIV status should be confirmed after enrollment, if possible, and CD4 cell counts measured as appropriate, although we recognize that protocol-mandated HIV testing may be problematic in certain areas.

5. Special Populations

All age ranges should be studied in malaria treatment studies, including pediatric and geriatric subjects. It is particularly important to study pregnant women and children during drug development as these populations are at greatest risk of morbidity from malaria.

The need to study other special populations (e.g., patients with hepatic or renal failure) should be based on the characteristics of the specific drug under development. For example, targeted study

[11] See the guidance for industry *Nonclinical Safety Evaluation of Pediatric Drug Products* (http://www.fda.gov/cder/guidance/index.htm).

[12] Based on a normal red blood cell (RBC) count of 5 x 10^6 RBCs per μl blood.

of subjects with renal insufficiency may not be necessary for a drug that has complete hepatic metabolism and no renal excretion. These considerations usually should be addressed after completion of the initial absorption, disposition, metabolism, and excretion studies of the new drug and should be addressed during drug development. Studies in special populations should include pharmacokinetic evaluation; in some circumstances, population pharmacokinetic assessments may be nested within larger treatment studies.

6. Choice of Comparators

We strongly recommend that clinical studies compare treatment with the new drug to treatment with a regimen containing FDA-approved antimalarial drugs. Although the use of unapproved comparators generally is discouraged, unapproved comparators may be appropriate if they represent the local standard of care. If a sponsor wants to use an unapproved comparator, we strongly recommend that the sponsor discuss this with the DSPTP at the time of protocol development. Unapproved drugs that are being considered for use as comparator drugs should have satisfactory evidence of safety and efficacy (e.g., an efficacy rate greater than 95 percent in a large randomized clinical trial) and this information should be provided to the FDA at the time of protocol development. Such data may be less critical if the study goal is to demonstrate that the new drug is superior to the control drug.

We anticipate that, within the application, at least some, if not all, of the controlled clinical studies will include an FDA-approved drug as a control.

7. Efficacy Endpoints

The primary endpoints that should be used in malaria treatment trials are defined as follows:

- **Cure —** The complete resolution of clinical signs and symptoms, malaria-related laboratory abnormalities, and elimination of asexual parasites by day 7, with no recurrence up to day 28 (+/- 2 days). This definition also includes that a study assessment 48 hours after initiation of therapy demonstrate a decrease in the level of parasitemia to less than 25 percent of baseline with no clinical deterioration. For drugs with long half-lives, a follow-up visit at 42 days or longer may be warranted.

  Recurrent parasitemia may represent a new infection rather than a true recrudescence. Attempts should be made to characterize and differentiate the isolate collected at the time of recurrent parasitemia from baseline. This can involve samples being obtained at baseline and at the time of recurrence, and storing these samples under conditions appropriate to enable further characterization of the parasite, such as by genetic methods (e.g., polymerase chain reaction (PCR)) and/or phenotypic methods (see Appendix A). Both crude cure rates and rates adjusted by genotypic and phenotypic information should be reported. Methods to be used for adjusting cure rates should be included in the clinical protocol.

- **Radical cure (for *P. vivax* and *P. ovale*) —** The absence of parasitemia, clinical signs and symptoms, and laboratory abnormalities by day 7 without relapse for at least 6

months after completion of treatment. Relapses of *P. vivax* and *P. ovale* generally occur within the first 6 months of infection, but temperate strains may take more than 1 year to relapse. Whether 6 or 12 months of follow-up is necessary should be discussed with the DSPTP before protocol submission. As the duration of follow-up is extended, genetic and phenotypic comparison of baseline isolates to later isolates becomes increasingly important as a possible means to distinguish relapse from re-infection (see Appendix A).

The secondary endpoints that should be used in malaria treatment trials are defined as follows:

- **Parasite clearance time —** Time in hours from the initiation of therapy until the first of two successive parasite-negative smears are obtained.

- **Fever clearance time —** Time in hours from the initiation of therapy until disappearance of fever for at least 24 hours.

For both *P. falciparum* and *P. vivax /P. ovale* infections, baseline blood samples should be retained to allow comparison with the original strain should parasitemia recur. Appropriate techniques may distinguish recrudescence, relapse, and re-infection (see the Glossary and Appendix A).

Treatment failures can be classified as early treatment failure, late treatment failure, or late parasitological failure, as follows:

- **Early treatment failure**
  - Development of severe malaria on day 1, 2, or 3 of treatment in the presence of parasitemia
  - Parasitemia on day 2 greater than day 0 irrespective of axillary temperature
  - Parasitemia on day 3 with axillary temperature greater than or equal to 37.5 degrees Celsius
  - Parasitemia on day 3 greater than or equal to 25 percent of count on day 0

- **Late treatment failure**
  - Development of severe malaria after day 3 in the presence of parasitemia without previously meeting any of the factors of early treatment failure
  - Parasitemia any day from day 4 to 14 (intense transmission areas) or day 4 to 28 (low to moderate transmission areas) with axillary temperature greater than or equal to 37.5 degrees Celsius without previously meeting any of the factors of early treatment failure
  - Any patients receiving additional antimalarial therapy not specified in the study protocol

- **Late parasitological failure**
  - Parasitemia on day 14 (intense transmission areas) or any day from day 7 to 28 (low to moderate transmission areas) and axillary temperature less than 37.5 degrees Celsius.

8. Study Procedures and Timing of Assessments

The following assessments should be included in a malaria treatment study protocol:

- **At study entry**
  - History and physical examination, including history of prior malaria episodes, prior treatment history, and documentation of splenomegaly.
  - Laboratory studies for parasite count, chemistry and glucose, complete blood count (CBC), and liver function tests. A specimen should be archived for genetic and/or phenotypic studies were recurrent parasitemia to occur.

- **During study**
  - Laboratory testing as clinically relevant for the specific trial or drug under study (e.g., testing for hypoglycemia, anemia, thrombocytopenia, or renal dysfunction).
  - Temperature and vital signs monitoring every 6 hours until resolution of fever, defined as being afebrile for 24 hours.
  - Repeat malaria smears every 6 to 12 hours until parasitemia has been eradicated, defined as two successive parasite-negative smears.
  - Daily recording of signs and symptoms until all have resolved.
  - If parasitological eradication has occurred, subsequent malaria smears on days 7, 14, 21, and 28 of study to document that parasitemia is absent. When a late follow-up visit is included (see below), additional smears should be obtained on days 35 and 42.
  - Malaria smears for patients presenting at any time with fever or other signs or symptoms suggestive of malaria.
  - Specimens obtained to perform genetic and phenotypic comparisons with baseline samples if recurrent parasitemia is detected in either symptomatic or asymptomatic individuals.
  - Samples for drug level assays at the time an early treatment failure is documented.

- **At test-of-cure visit**[13]
  - History and physical examination to confirm resolution of malaria symptoms and absence of fever.
  - Laboratory tests for parasitemia and other tests as appropriate for the drug under study. There also should be repeat assessment of any unresolved laboratory abnormalities from previous tests, and laboratory abnormalities should, in general, be followed to resolution.

We recognize that in rare cases recrudescent infection may occur more than 28 days after initial therapy. Inclusion of a late follow-up visit 42 days after initiation of therapy should be considered, particularly when antimalarial drugs with prolonged half-lives are being studied.

The following study evaluations should be included in malaria treatment studies:

[13] Unless otherwise indicated, the test-of-cure visit should occur at 28 days (+/- 2 days) after starting treatment. Cure is defined as negative malarial smears from day 7 through day 28.

- **Evaluation of early treatment failure.** Transient rises in parasitemia can be seen following treatment with certain antimalarial drugs. Rises in parasitemia observed less than 12 hours after the initiation of treatment and not accompanied by any clinical deterioration may allow ongoing administration of the study drug at the investigator's discretion. Sustained rises in parasitemia or clinical deterioration after 12 hours indicate drug failure and salvage therapy should be instituted. Exceptions to this time frame in a proposed study should be discussed with the DSPTP before protocol submission.

- **Evaluation for relapsing malaria.** For the assessment of radical cure for *P. vivax* or *P. ovale* infection, an additional follow-up period of 6 to 12 months after completion of therapy should be included to document the occurrence of either recurrent fever or relapse over this period. Subjects should be instructed to return to study centers for malaria smears and a complete clinical evaluation if symptoms suggestive of malaria occur. Blood samples should be obtained for genetic and phenotypic comparison with the original strain if malaria is confirmed.

A final study visit should be included at the completion of the follow-up period. This visit can be conducted as a telephone interview, during which a history should be obtained confirming absence of malaria symptoms or antimalarial treatment after the completion of therapy.

The efficacy of a drug to prevent relapses may be difficult to determine in patients remaining in endemic areas, particularly so if suitable genetic and phenotypic studies cannot be performed when malaria-like symptoms recur.

9. Parasite Evaluation

Conventional microscopy using blood smears is considered to be the currently established standard method for detection and morphological identification of the malarial parasite, and thus a direct measurement of drug activity (see Appendix A for details). However, newer experimental procedures are available for establishing parasitemia. If newer methods are used in addition to blood smears in a clinical study, the details of those methods and the performance characteristics of the assays used should be included in the clinical protocol. Study procedures for quality control and interobserver reliability of parasite measurements should be described in the clinical protocol.

Newer microbiological methods may allow detection of drug resistance by genotyping and phenotyping, and possibly can differentiate between new infection and relapse or recrudescence. If any of these methods are used in a clinical trial, the details of these methods also should be included in the clinical protocol.

10. Statistical Considerations

The two primary analysis populations for evaluating efficacy and safety treatment studies are defined as follows:

- **Modified intent-to-treat (MITT) —** All randomized patients with parasitologically confirmed malaria who receive at least one dose of study drug. Depending on the specific study design, the intent-to-treat (ITT) population of all subjects enrolled can include subjects enrolled before complete parasitological confirmation but for whom malaria is not subsequently confirmed. These subjects should not be included in the MITT and per-protocol efficacy analyses.

- **Per protocol —** All patients included in the MITT population who have received at least 80 percent of the protocol-defined therapy and are clinically and microbiologically evaluable after 28 days.

All subjects who received at least one dose of study drug should be included in the safety analysis of the study.

Studies should be appropriately powered (at least 80 percent) to achieve the primary study objective. The estimated treatment success rates described in the study protocol should be referenced and based on valid estimation methods. The exact number of subjects necessary for each study will be dependent on the population and specific indication under study.

All statistical tests should be two-sided with a Type I error rate of 0.05. For noninferiority studies, a 95 percent two-sided confidence interval (CI) should be constructed around the difference in outcome rates (experimental regimen-control regimen) with any prespecified adjustments. If the lower bound of the 95 percent CI is greater than a prespecified, scientifically justified noninferiority margin for both MITT and per-protocol study populations, noninferiority of the experimental regimen can be concluded. For a discussion of factors to consider in the selection of an appropriate noninferiority margin, see ICH E10.

For parasite clearance, 95 percent CIs should be constructed around the 24- and 48-hour time points. Parasite clearance time and fever should be analyzed by Kaplan Meier survival methods.

Patients who prematurely discontinue assigned study treatment and/or receive alternative therapy should be treated as failures in all analyses. Patients who discontinue treatment but who are not lost to follow-up and do not receive additional treatment should be evaluated according to their study outcome in the ITT analysis. Patients lost to follow-up should be counted as treatment failures in the ITT analysis. Sample size calculations should take into account subject dropout and loss to follow-up rates.

Demographics and baseline characteristics should be summarized and compared between treatment groups using descriptive statistics.

Clinical and laboratory adverse events information should be summarized and compared between treatment groups using descriptive statistics.

667 *11. Accelerated Approval (Subpart H) Considerations*
668
669 In general, treatment and prophylaxis indications for malaria have been based on adequate and
670 well-controlled trials using clinical and parasitological endpoints. Exceptional cases where a
671 sponsor is seeking approval for treatment based on 21 CFR 314.500, subpart H, regulations
672 should be discussed with the DSPTP as early as possible during the drug development process.
673
674 **C. Prophylaxis Studies**
675
676 *1. Study Design*
677
678 Clinical studies supporting an indication for the prophylaxis of malaria should demonstrate the
679 following:
680
681 • Efficacy for the prevention of infection following documented or presumed malaria
682 exposure.
683 • Safety in the target population for the proposed duration of prophylaxis at the proposed
684 dose. Physiological diversity in patients likely to use the proposed treatment should be
685 addressed.
686 • Efficacy in nonimmune subjects.
687
688 An application for a prophylaxis indication should include at least two adequate and well-
689 controlled clinical studies, with subjects enrolled from two or more distinct geographical regions.
690 Applications for prophylaxis indications also can be significantly strengthened by other studies
691 with the drug demonstrating efficacy for the treatment of established malaria infection.
692
693 The following study designs have been used to support a malaria prophylaxis indication:
694
695 • **Efficacy studies in malaria endemic communities.** Studies in communities with
696 endemic malaria and significant levels of malarial immunity offer the advantage of
697 studying new antimalarial therapy while limiting the potential risk to patients if efficacy
698 is found to be suboptimal. Placebo-controlled studies may be appropriate in this setting
699 (see below). If a study is performed in a malaria-endemic community as support for a
700 regulatory filing, then other studies in the new drug application (NDA) submission
701 should demonstrate drug efficacy in nonimmune subjects as well.
702
703 • **Active-controlled and historical-controlled studies in individuals deployed to**
704 **malaria-endemic areas.** The deployment of military personnel or civilian cohorts to
705 malaria-endemic regions provides an opportunity to study antimalarial prophylaxis in
706 malaria-naive subjects. Since such deployments may last for many months, it is possible
707 to standardize duration of malaria exposure. When placebo-controlled studies cannot be
708 performed, well-characterized epidemiological attack rates can be used to calculate
709 protective efficacy (see section IV.C.9.). See ICH E10 regarding considerations on use
710 of historical controls.
711

- **Active-controlled studies in travelers.** Travelers may be a valuable population in which to study the safety of antimalarial prophylaxis; however, outcome data in these trials may be difficult to interpret if the overall incidence of malaria is below expected rates in all treatment arms. In this situation, it may not be possible to distinguish drug efficacy from low exposure to malaria (e.g., because of the locations visited, the duration of exposure, or the use of ancillary protection such as bed nets or air-conditioning). The design of these studies should be discussed with the DSPTP before submission to ensure that the expected baseline exposure rate in the treatment groups is quantified and well supported.

- **Challenge studies.** Challenge studies ensure a high malaria attack rate in volunteers, while intensive monitoring may ethically permit the use of a placebo arm (i.e., with intervention occurring at the first clinical or laboratory sign of active malaria infection). Generally, challenge studies should be performed with well-characterized strains of chloroquine sensitive *P. falciparum* and should involve 6 weeks of follow-up.

  Since challenge studies generally are limited to one or two laboratory strains, they may not reflect the effect of different strains of malaria or the effect of repeated exposure. Accordingly, challenge studies alone are considered insufficient and should be accompanied by additional studies for a prophylaxis indication.

A specific study can be either placebo-controlled or have an active comparator based on the population being studied.

- **Use of a placebo-control.** In certain circumstances studies enrolling subjects residing in malaria-endemic regions may justify the use of a placebo arm if antimalarial chemoprophylaxis is not the standard of care in the community and there is a high level of preexisting immunity in the study population. It is expected that in this setting the level of immunity present would be sufficient to protect individuals from severe malaria in the absence of prophylaxis. Appropriate approval by local regulatory authorities and individual informed consent are required (21 CFR 50.25). In general, the use of placebo arms should be confined to studies enrolling only adults older than 18 years of age. Since participants entering such trials commonly have asymptomatic or incubating parasitemia, a course of radical treatment typically should be given at study enrollment regardless of the presence of parasitemia.

  Use of a placebo arm has the advantage of directly estimating the malaria attack rate in the study population. Protective efficacy (PE) can then be calculated as 1 - (the incidence of malaria in experimental arm/incidence of malaria in placebo arm).

- **Use of an active-control.** Active-controlled studies do not allow a direct determination of the malaria attack rate in the study population; therefore, a background attack rate should be determined. The risk of infection can be indirectly estimated from local epidemiological data in endemic areas. Ideally, active-controlled studies should be sufficiently large to demonstrate the anticipated breakthrough rate for the comparator, confirming the expected background infection rate. Because breakthrough rates for known prophylactic regimens seldom exceed 1 to 2 percent even in malaria-endemic

regions, large study sample sizes should be used to unequivocally demonstrate efficacy relative to an active-control. This problem is exacerbated in areas with lower background malaria attack rates.

Investigational approaches to this problem by measurement of circumsporozoite antibodies have not yet proven reliable for determining the exposure to malaria and are not recommended at this time.

2. Study Population

Prophylaxis studies should enroll asymptomatic individuals for whom malaria exposure is anticipated and where active or incubating malaria has been either excluded or eradicated. Children can be included in prophylaxis studies after safety in adults, appropriate pharmacology and toxicology data, and appropriate pediatric dosing have been explored. Pregnant women can be included if animal toxicology studies do not indicate a risk to the fetus. When an antimalarial drug is being developed for both treatment and prophylaxis indications, initial safety data in pregnancy should be obtained during treatment rather than prophylaxis since the potential risk-benefit ratio is relatively greater for treatment.

3. Entry Criteria

Entry criteria for field studies and challenge studies are as follows:

- **Field studies**
  - Male or nonpregnant female subjects older than 16 years of age; pregnant subjects can be included after pharmacokinetics in pregnant women have been characterized and reproductive animal toxicology studies have been completed, assessed, and support inclusion of pregnant women. Studies that enroll pregnant women should include targeted assessment of the mother and newborn at the time of delivery and 3 months post-delivery.
  - Subjects younger than 16 can be included if adult safety and pharmacokinetics, and pharmacology and toxicology data, as appropriate, are characterized in prior studies.
  - Mosquito nets and repellants can be used, but subjects should be stratified at enrollment based on anticipated use. This information should be recorded in the case report form. If possible, the study should incorporate the use of subject diaries for the purpose of tracking use of mosquito bed nets and repellants.

- **Challenge studies**
  - Generally, challenge studies should be limited to healthy, nonpregnant adult volunteers. Females of childbearing potential[14] should use appropriate contraception during the study.

[14] Females are considered *females of childbearing potential* if they are older than 10 years of age and if they have not been previously documented to have either a hysterectomy or menopause.

800 *4. Randomization and Blinding*
801
802 All prophylaxis studies should be double-blinded and randomized to minimize potential bias.
803
804 *5. Special Populations*
805
806 Pregnant women should be studied once the prerequisite animal toxicology and human
807 pharmacokinetic studies have been completed and do not show risk to fetus; for children, adult
808 safety also should be characterized before enrollment into studies. Though not routinely
809 expected, toxicology studies in juvenile animals should be considered if concerns emerge
810 indicating potential increased sensitivity in children.[15] Other special populations (e.g., patients
811 with hepatic or renal failure) should be studied when appropriate. For example, a study of
812 subjects with renal insufficiency may be appropriate for a drug with renal excretion but would
813 likely not be appropriate if the drug were hepatically metabolized. Many of these considerations
814 arise after the initial absorption, disposition, metabolism, and excretion studies with the new
815 drug, but should be completed and included in the NDA or biologics license application
816 submission.
817
818 *6. Choice of Comparators*
819
820 When studies with an active comparator are performed, comparator drugs should be selected
821 from FDA-approved drugs that have well-characterized safety and prophylactic efficacy rates.
822 The choice of comparators may involve discussions with regional health authorities to address
823 local public health concerns. The use of unapproved comparators is discouraged as efficacy
824 rates and safety may not be well characterized; if an unapproved comparator is proposed for use
825 in a clinical trial for prophylaxis, this should be discussed with the DSPTP before protocol
826 submission.
827
828 *7. Efficacy Endpoints*
829
830 The following endpoints should be used in malaria prophylaxis trials:
831
832 • **Primary endpoint**
833 – Prophylactic success, defined as the absence of detectable parasitemia during
834 prophylactic drug administration. Negative smears should be demonstrated for 4
835 weeks after completing study drug administration for studies where subjects leave the
836 malaria-endemic area (see Appendix A for details of microbiological evaluation).
837
838 • **Secondary endpoints**
839 – Mean/median time to first slide-proven parasitemia during prophylaxis.
840 – Cumulative incidence of slide-proven parasitemia.
841 – Incidence of slide-proven parasitemia during the follow-up phase for subjects who
842 remain in the malaria-endemic area.

[15] See the guidance for industry *Nonclinical Safety Evaluation of Pediatric Drug Products* (http://www.fda.gov/cder/guidance/index.htm).

8. Study Procedures and Timing of Assessments

Radical treatment to eradicate all active or incubating infections at study onset typically should be included in studies that enroll subjects living in malaria-endemic areas. The following study assessments are recommended during prophylaxis studies:

- **Baseline evaluation/start of prophylaxis**
  - If radical treatment is used, smear confirmation of the absence of asexual forms in the blood within 7 days of starting therapy.
  - Initiation of prophylaxis following completion of radical treatment or on arrival to the malaria-endemic region.
  - Baseline clinical assessment, including documentation of any history of prior malaria and examination for splenomegaly.
  - Laboratory tests including CBC with platelets, chemistry, and liver function tests. Additional studies (e.g., electrocardiograms) may be appropriate based on specific safety concerns for the drugs under study.

- **On-therapy visits**
  - Field studies
    - Blood smears obtained weekly during the period of prophylaxis and for 4 weeks after completion of prophylaxis. Additional protocol-defined study visits should be specified for subjects developing symptoms suggestive of malaria (e.g., fever, rigors, malaise) to include a complete parasitological and clinical evaluation.
    - Recorded use of bed nets, mosquito repellent, and air-conditioning in the case report form. At the time any malarial breakthrough is documented, a blood sample should be obtained for measurement of drug levels.

  - Challenge studies
    - Daily smears from day 6 to 14, then every second day until day 21, then weekly for a total of 6 weeks. Other investigational assays such as PCR have been of supportive value in the early detection of parasitemia.
    - A blood sample obtained for measurement of drug levels at the time any malarial breakthrough is documented.

- **End of therapy**
  - Field studies: the primary endpoint evaluated at the end of therapy, generally after 10 to 12 weeks of prophylaxis, for studies of subjects who remain in malaria-endemic areas. This allows adequate exposure to malaria, and covers the usual anticipated therapeutic duration in travelers. Assessments should include:

    - History and physical examination for signs and symptoms of malaria
    - Blood smear for malaria
    - Other laboratory studies as appropriate for evaluation of safety

For studies of subjects who do not remain in malaria-endemic areas (such as travelers), and effective causal prophylaxis is not anticipated, suppressive therapy typically should be continued for 4 to 6 weeks after leaving the endemic area. The primary endpoint should be determined 4 weeks after completion of therapy.

- Challenge studies (performed 6 weeks after challenge):
  - History and physical examination for signs and symptoms of malaria
  - Blood smear for malaria
  - Other laboratory studies as appropriate for evaluation of safety

- **Post-therapy visits.** Post-therapy assessments are similar for field and challenge study designs; however, post-therapy assessments differ on whether *P. falciparum* or relapsing malarias are the focus of study:

  - *P. falciparum* studies. Among subjects who remain in malaria-endemic areas after completing the study, a post-therapy visit 4 weeks after completion of therapy captures infections incubating at the time prophylaxis is complete. We recognize that it may be difficult to distinguish recrudescence from new infections with increasing time off prophylaxis. Evaluations include:

    - A history and physical examination to confirm the absence of malaria symptoms
    - A malaria smear to confirm the absence of parasitemia

  - Relapsing malaria studies. To document the occurrence of malaria after completion of prophylaxis, an additional follow-up period of 6 to 12 months should be included for subjects who leave the endemic area.

    During the follow-up period, subjects should be instructed to return to study centers for malaria smears and a complete clinical evaluation if symptoms suggestive of relapsing malaria occur.

    A final visit should be included at the completion of the follow-up period. This visit can be conducted as a telephone interview, during which a history should be obtained confirming absence of malaria symptoms or antimalarial treatment after the completion of therapy.

For drugs being tested for causal prophylactic activity against *P. falciparum*, causal prophylaxis can be confirmed in challenge studies where the prophylactic drug is given for a week or less following exposure to malaria.

Field trials in individuals leaving the malaria area after completing prophylaxis also can be assessed for causal prophylactic efficacy. Therapy should be stopped within a week of leaving the endemic area and the test-of-cure visit should occur 4 weeks after completion of therapy. This visit should include:

- A history and physical examination to confirm the absence of malaria symptoms
- A malaria smear to confirm the absence of parasitemia

Appropriate approved regimens for the treatment of breakthrough infections in prophylaxis studies should be described in the study protocols.

9. Statistical Considerations

The two primary analysis populations for prophylaxis studies are defined as follows:

- **Intent-to-treat —** All randomized subjects receiving at least one dose of study drug.

- **Per protocol —** All randomized subjects taking between 80 percent and 120 percent of the dosing regimen who are not lost to follow-up, and who do not prematurely discontinue study drug because of intolerance. Subjects who receive concomitant medication that could influence efficacy findings should be considered failures.

Subjects who prematurely discontinue assigned study treatment because of intolerance and receive alternative therapy should be treated as failures in ITT analyses. Subjects who are lost to follow-up should be counted as treatment failures in the ITT analysis. All subjects who receive at least one dose of study drug should be included in the safety analysis of the study.

All statistical tests should be two-sided with a Type I error rate of 0.05 unless otherwise specified.

a. Primary endpoint evaluation

The proportion of subjects free of detectable parasitemia during prophylaxis (primary endpoint) should be calculated for both the ITT and per-protocol populations. Depending on study design, primary endpoints can be evaluated as follows:

- **Placebo-controlled studies.** The percent PE should be calculated as:

  PE = [1 - (cumulative incidence of parasitemia during prophylaxis in the experimental group/cumulative incidence of parasitemia during prophylaxis in the placebo group)] x 100

  These studies should be designed to show an anticipated PE rate of greater than or equal to 95 percent, with a minimum sample size of 200 subjects per arm.

- **Historical-controlled studies.** PE also should be calculated using the same calculation as for placebo-controlled studies with the cumulative incidence in untreated epidemiological control group substituted for the placebo group incidence. These studies should be designed to demonstrate an anticipated PE rate of greater than or equal to 95 percent, with a minimum sample size of 200 subjects per arm.

The calculation of PE in historical-controlled studies should employ epidemiological attack rates in the study area from at least the past two malaria seasons. Epidemiological attack rates should closely reflect anticipated attack rates in the study population and should be derived from the same geographical area, during the same seasonal period, with similar rainfall and similar subject exposure. Collection and calculation methods should be prospectively defined in the study protocol and statistical analysis plan. Results should be well documented in the final study report.

An active comparator arm should be included as reference to identify problems in the conduct of the study (e.g., errors in laboratory procedures, adherence to therapy), as well as to determine comparative safety.

Sample size calculations should take into account subject dropout and loss to follow-up rates.

b. Secondary endpoint evaluation

For secondary endpoints, the following should be evaluated:

- Incidence (density) rate can be calculated as the number of cases of slide-proven parasitemia divided by the total person-time of follow-up
- Comparative efficacy of time to slide-proven parasitemia can be performed using Kaplan-Meier methods and log rank tests
- Cumulative incidence can be calculated as the proportion of subjects who develop parasitemia during the study

Demographics and baseline characteristics should be summarized and compared between treatment groups using descriptive statistics.

10. Risk-Benefit Considerations

Drugs that are intended for use as prophylaxis should be sufficiently well tolerated to achieve a satisfactory risk-benefit ratio.

11. Labeling Considerations

For antimalarial prophylactic drugs, patient labeling (e.g., a Patient Package Insert or Medguide) should be considered depending on the risk-benefit analysis, with the intention of communicating safety concerns and educating patients about the use of prophylaxis, given that they may not have immediate access to a physician.

GLOSSARY

Causal prophylaxis — Prophylaxis that is effective against hepatic forms of the parasite. Effective causal prophylactics can be discontinued a few days after leaving the region with malaria.

Consolidation regimen — Therapy used together with or after a rapidly acting drug to prevent recrudescence.

Cure — Complete resolution of clinical signs and symptoms, complete resolution of laboratory abnormalities, and elimination of asexual parasites by day 7 with no recurrence up to day 28 (+/- 2 days). This definition also includes that a study assessment 48 hours after initiation of therapy demonstrate a decrease in the level of parasitemia to less than 25 percent of baseline with no clinical deterioration.

Early treatment failure — Any of the following should be considered early treatment failure:

- Development of danger signs or severe malaria on day 1, 2, or 3 in the presence of parasitemia
- Parasitemia on day 2 greater than day 0 irrespective of axillary temperature
- Parasitemia on day 3 with axillary temperature greater than or equal to 37.5 degrees Celsius
- Parasitemia on day 3 greater than or equal to 25 percent of count on day 0

Failure (of treatment) — Persistent or recrudescent parasitemia regardless of parasite density and/or failure of clinical abnormalities to resolve.

Late parasitological failure — Parasitemia on day 14 (intense transmission areas) or any day from day 7 to 28 (low to moderate transmission areas), with axillary temperature less than 37.5 degrees Celsius.

Late treatment failure — Any of the following should be considered late treatment failure:

- Development of danger signs or severe malaria after day 3 in the presence of parasitemia without previously meeting any of the factors of early treatment failure
- Parasitemia on any day from day 4 to 14 (intense transmission areas) or day 4 to 28 (low to moderate transmission areas) with axillary temperature greater than or equal to 37.5 degrees Celsius without previously meeting any of the factors of early treatment failure
- Patients receiving additional antimalarial therapy not specified in the study protocol

Prepatent period — Interval between inoculation of parasites and detection of erythrocytic forms.

Prophylactic success — The absence of detectable parasitemia during prophylaxis, defined by PE, which is determined by the incidence of breakthrough infections.

Prophylaxis — Prevention of clinical or parasitological malaria infection. Prophylaxis can take the form of suppressive therapy, when medication is administered for a period sufficient to encompass several hematogenous/erythrocytic cycles of replication following which parasitemia does not occur. In general, suppressive therapy is continued for 4 to 6 weeks after leaving areas with malaria. Prophylaxis also can be *causal* when the drug can be shown to eliminate parasites during the hepatic phase before their entry into the blood. Causal prophylactic drugs generally should be continued for a week or less after leaving areas with malaria.

Protective efficacy — PE is calculated as 1 - (the incidence of malaria in experimental arm/incidence of malaria in placebo arm).

Radical cure — Eradication of hypnozoites in the liver of patients with relapsing malaria, and by doing so, elimination of relapses attributable to the original infection.

Radical treatment — Curative treatment employed at the beginning of prophylaxis studies in endemic areas with the goal of eradicating baseline asymptomatic parasitemia and hypnozoites before initiation of prophylaxis.

Recrudescence — Recurrence of the original parasitemia with *P. falciparum*.

Re-infection — Infection with a genetically distinct plasmodial strain after successful treatment of initial infection during enrollment in a clinical trial. When re-infection can be reliably distinguished from recrudescence, re-infection should not be regarded as a treatment failure.

Relapse — Recurrence of original parasitemia attributable to the original *P. vivax* or *P. ovale*.

Severe or complicated malaria — The baseline definition of severe or complicated malaria includes cerebral malaria, severe anemia, renal failure, pulmonary edema, hypoglycemia, circulatory collapse, spontaneous bleeding, repeated generalized seizures, acidemia, macroscopic hemoglobinuria, and in some geographical regions impaired consciousness, prostration hyperparasitemia, jaundice, and hyper pyrexia (Trans R Soc Trop Med Hyg, 1990, 84(2)1-65). This definition can be expanded for use in specific clinical trials. Patients with severe malaria generally have levels of parasitemia greater than 5 percent (greater than 250,000/µl blood). *Moderately severe* disease occasionally has been used in previous treatment studies but is not recommended without prior discussion with the DSPTP.

Suppressive therapy — Prophylaxis that is ineffective against the hepatic forms of the parasite, but if given for an extended period after leaving the region with malaria, will eliminate residual erythrocytic forms (thereby preventing subsequent recrudescence).

Terminal prophylaxis — The addition of a drug at the end of standard prophylaxis to eliminate hypnozoites and prevent relapse.

Treatment — Treatment of patients with a microbiologically confirmed diagnosis of malaria. *Presumptive treatment* has been used to refer to self-administered antimalarial therapy, which is taken before reaching medical care by individuals experiencing malaria symptoms.

Uncomplicated malaria — Symptomatic malaria (e.g., fevers, rigors, malaise, headache) without any of the complications previously listed, and a parasite count of less than 5 percent (less than 250,000/µl blood).

APPENDIX A: MICROBIOLOGICAL EVALUATIONS

Microbiological evaluations within a clinical trial include:

- Detection or identification of the erythrocytic stages of *Plasmodium* species for:
  - Enrollment of patients in the clinical trial (as part of inclusion and exclusion criteria)
  - Measuring drug efficacy
- Measurement of drug resistance (genotyping and phenotyping)
- Differentiating new infection from relapse or recrudescence

Conventional microscopy using blood smears is considered to be the established method for morphological identification of the parasite and measuring drug efficacy. In addition, several experimental procedures are available. The details of the method used for parasitological evaluation should be included in the clinical protocol.

Blood smears

Thin and thick blood smears should be prepared for identification of the species and measuring parasite density. For preparation of blood smears and staining procedures, refer to the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical and Laboratory Standards) guidelines (M15-A, volume 20, number 12) or the World Health Organization (http://mosquito.who.int/cmc_upload/0/000/011/403/malaria_diagnosis.htm). It should be specified whether thin or thick smears were used for measuring parasite count. The quantification of parasitized erythrocytes should be obtained by counting either 200 white blood cells (WBCs) or 1,000 red blood cells (with an oil immersion objective), but should remain consistent within a clinical trial. For example, if the parasite count is obtained by counting 200 WBCs, then the same procedure should be done for all smears collected from all subjects at different time points within a clinical trial. Effort should be made to determine both asexual parasite counts and gametocyte counts.

It should be ensured that:

- The method used is consistent within a given trial.
- Slides are read by two trained microscopists. Discordant readings should be adjudicated by a third microscopist.
- Microscopists are blinded to the treatment.
- Ten percent of the negative and positive slides are reviewed by a third microscopist for the purpose of quality control.
- Morphological speciation is performed on all smears at baseline, and on those obtained at the time of treatment failure.

Experimental procedures

Several experimental procedures such as microhematocrit centrifugation with acridine orange staining, immunochromatographic method, indirect fluorescent antibody tests, enzyme-linked immunosorbent assay, phenotyping (e.g., by determining in vitro susceptibility of clinical isolates to antimalarial drugs), and polymerase chain reaction have been used for:

- Detection of parasites
- Identification of *Plasmodium* species
- Quantification of the parasite
- Measurement of exposure to the parasite in a prophylactic study
- Measurement of drug resistance (relapse or recrudescence)
- Differentiating new infection from relapse or recrudescence

It should be noted that the use of these procedures has not been fully validated in clinical trials for measuring drug efficacy. The use of experimental assays in a clinical trial should be accompanied by the standard blood smear technique. Although the use of experimental methods is encouraged, the performance characteristics of the assays should be carefully and critically evaluated in the laboratory where the actual testing of clinical samples will be done. The clinical study report should address performance characteristics of the assay such as reproducibility, quality controls, sample storage and stability, reagent storage and stability, accuracy of measurement, limit of detection, limit of quantification, cross-reactivity with other relevant pathogens, and positive and negative predictive value of the experimental procedure. Test results should be correlated with clinical outcome. Sponsors are encouraged to contact the DSPTP for more details. It also should be noted that these tests are not approved for in vitro diagnostic use. The sponsor of the test or device is encouraged to contact the Office of In Vitro Diagnostic Devices Evaluation and Safety, Center for Devices and Radiological Health, for approval of the device for marketing.

If there is the intention during a clinical trial to develop a combination of drug or nonvaccine biological product with a new test (i.e., information from a study will be used for approval of a new test that will be used with the drug), then the sponsor of the trials should contact the Office of Combination Products for additional information on developing drug-device combinations.