Guidance for Industry

Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention

DRAFT GUIDANCE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)

November 2007
Clinical Antimicrobial

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic.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 provides recommendations to potential sponsors (including industry, academic, and government) on the development of drugs to treat or prevent infection caused by variola virus, the etiological agent of smallpox.[2] The guidance focuses mainly on drugs that are expected to act by inhibiting variola virus replication; however, sponsors of drugs proposed to act against smallpox by other mechanisms are encouraged to consult this guidance for relevant content, as well as to discuss questions and proposals directly with the appropriate review division at the Food and Drug Administration (FDA). Most sponsors consulting this guidance will wish to develop and file an investigational new drug application (IND) with the FDA, with the eventual goal of submitting a new drug application (NDA) for these indications. Because of the unique and challenging issues arising in this development area, we strongly encourage beginning with pre-investigational new drug application (pre-IND) consultations between sponsors and the FDA addressing the sequence and content of nonclinical and clinical study proposals

This guidance does not address the following types of development:

- Drug development for the treatment of bacterial complications of smallpox
- Development of biological therapies such as vaccines or antisera to treat or prevent variola

[1] This guidance has been prepared by the Office of Counter-Terrorism and Emergency Coordination and the Division of Antiviral 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.

- Drug development for infections from viruses other than variola

This guidance also does not contain discussion of the 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* and *E9 Statistical Principles for Clinical Trials*.[3] This guidance focuses on drug development and trial design issues that are specific to the study of smallpox (variola) infection.

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

For centuries, smallpox affected human populations. The most severe form, variola major, had reported mortality ranging from 5 percent to 50 percent in different outbreak situations (Fenner and Henderson et al. 1988). This form is the principal source of concern regarding potential bioterrorist uses of smallpox and, therefore, the most relevant to this guidance. A less severe variant, variola minor, caused a similar rash but generally less than 2 percent mortality. Worldwide efforts at case identification, containment, and vaccination improved smallpox control, and eventually clinical smallpox was declared eradicated in 1980 by the World Health Organization. Retention of variola virus stocks was limited by international agreement to two sites, one in Russia and the other at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia. However, concerns exist that variola virus could be used as a weapon of bioterrorism.

The first line of defense against smallpox infection is vaccination with vaccinia virus.[4] Vaccination before exposure to variola provides substantial immunity against smallpox and it is thought likely to prevent or reduce the symptoms of smallpox if given a few days after exposure to variola (CDC 2001; CDC 2003a). Substantial protection generally is thought to last for at least a few years; information about any longer-term benefit is incomplete and controversial. Expectations about the usefulness of vaccination in a biothreat situation would depend upon the ability to vaccinate exposed and at-risk persons, and on assumptions about whether and to what extent immunity produced by vaccinia vaccine might be able to protect against a variola virus strain used in a terrorist attack.

[3] 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.

[4] See the Smallpox Response Plan and Guidelines on the CDC Web site at http://www.bt.cdc.gov/agent/smallpox/response-plan/index.asp.

Routine smallpox vaccination in the United States was discontinued in the 1970s. Currently, mass smallpox vaccination for civilians is not recommended, although some designated smallpox response team health care and public health workers and members of the military have received vaccinations. In addition, there are specific recommendations against nonemergency vaccine use for certain segments of the population at elevated risk of adverse events (CDC 2003b; CDC 2003c). The discontinuation of mass vaccination, along with a lack of natural disease exposure, means that most of the population is immunologically naïve to smallpox.[5]

Historically, treatment for smallpox was supportive (Dixon 1962). It is not known what effect technologically advanced supportive care might have on mortality and morbidity. Generally, the mode of death in fatal cases was considered unclear, and could have been multifactorial. Superinfections may have accounted for some fatalities. A variety of hypotheses have been proposed for other contributing factors. Antigen-antibody complex formation, fluid and electrolyte imbalance, and direct cytopathic effects of replicating virus (in organs such as kidney, liver, and lung) have been suggested. Terms such as *toxemia*, *sepsis*, and *cytokine storm* have been invoked as potential contributors and might reflect combinations of such factors (see for example Fenner and Henderson et al. 1988, Dixon 1962, and Jahrling and Hensley et al. 2004).

Antiviral drugs might prove to be a valuable adjunct for exposure situations in which vaccination was not feasible or had failed to provide adequate protection if suitable evidence of drug benefit can be established. Before the eradication of naturally occurring clinical smallpox, several drugs were studied for therapy of established illness and postexposure prophylaxis of patient contacts. Some of these drugs were reported to show effects in a variety of animal systems using orthopoxviruses, and there were occasional reports of some reduction of human disease in smallpox contacts, but none were found to provide reproducible protection of contacts or to be reliably effective in humans with established smallpox illness (Fenner and Henderson et al. 1988). In addition, toxicity profiles of these drugs were limiting.

The approach to development programs to support the efficacy of drugs to treat smallpox is affected by numerous distinctive features of smallpox and its history, including:

- The absence of smallpox cases for decades because of the success of the eradication program
- Ethical issues that clearly preclude human challenge studies
- Restriction of variola virus to two designated maximum containment facilities with stringent procedures to prevent any potential release
- The exceptionally narrow host range of variola virus

[5] A submission for review of a new cell-culture-derived smallpox vaccine was discussed at an FDA advisory committee meeting on May 17, 2007. If new vaccine preparations come into use, recommendations for populations to be vaccinated could change. Updated FDA and CDC documents should be consulted where appropriate for current recommendations.

- Disease differences between humans and nonhuman primates[6] and lack of pathogenicity for other host species after variola exposure

- The lack of any previously recognized effective drug, which severely limits any conclusions that might be drawn regarding relationships between in vitro activity, blood levels of the drug, and clinical effect from comparison of new agents against existing ones

- The possibility of antiviral drug interference with effects of the live-virus vaccine

- The absence of detailed information on the pathophysiology of human smallpox itself, including the mode of death

- The limited amount of additional information that potentially can be derived from existing records

- The lack of readily encountered human diseases that can be considered as closely analogous for purposes of preliminary investigation of potential treatments

- The differences between variola and other orthopoxviruses in disease characteristics, drug susceptibility, and host range

Taken together, these characteristics affect drug development strategies and differentiate the investigation of drugs for smallpox not only from assessment of drugs for common infectious diseases, but also from study of vaccines against smallpox and study of drugs for other potential agents of bioterrorism or biowarfare. For example, new smallpox vaccine candidates are likely to be related to, and might be compared against, an existing vaccine with a long history of successful use and a substantial amount of information on immunologic responses and protective effects across host and viral species; the availability of this comparator, and of immunogenicity measurements in human volunteers, might support development and licensure without use of a variola-virus animal model (sponsors should contact the Office of Vaccine Research and Review in the Center for Biologics Evaluation and Research (CBER) for information). As another example, drug development for some bacterial biothreat agents can use accepted effective comparator drugs, as well as animal models in which disease caused by the biothreat organism is more similar to the human disease than has been observed with variola virus, and may be able to relate results to human dose-response measurements in other bacterial infections. Therefore, although based on common principles of drug development, many specifics of the approach to drug development for smallpox are likely to differ even from the approaches to other situations involving rare and life-threatening diseases.

[6] Although a number of primate species can be infected, the typical illness in most nonhuman primates is mild relative to historical experience with human disease (Brinckerhoff and Tyzzer 1905; Hahon 1961), and mortality has been rare except for a few reports with added immunosuppression (Rao and Savithri Sukumar et al. 1968) or with intravenous injection of an extremely high viral inoculum (Jahrling and Hensley et al. 2004) suggesting major differences in host-pathogen interactions.

III. REGULATORY APPROACH REGARDING EARLY DRUG DEVELOPMENT

A. Selected Issues with Distinctive Impact

As summarized in section II., Background, the unique characteristics of smallpox illness and variola virus call for distinctive approaches to some aspects of drug development compared to other viral diseases. For example, sponsors of potential antivariola drugs should prepare appropriately for increased emphasis on the following issues: importance and extent of pre-IND interactions with the FDA to facilitate the development process, special attention to procedures for facilitating access to investigational drugs if an emergency situation were to occur during development, and careful consideration of preliminary investigations using other related viruses and discussion of their relevance to variola. The following sections briefly outline these issues so that they can be revisited as appropriate in subsequent sections of this guidance.

In each topic area that follows, the amount and timing of the information recommended relative to other steps in the development sequence may vary. Initial discussions with the FDA are encouraged to address priorities and timelines for each proposed development plan.

1. Pre-IND Consultations

Before preparation of a protocol for human use of a drug under an IND, pre-IND consultations with the FDA provide an opportunity to discuss the design and conduct of nonclinical studies and approaches to development of human studies, when appropriate, based on nonclinical study results. Some candidate antiviral drugs for smallpox may warrant repeated pre-IND consultations as initial nonclinical data become available for review and contribute to the discussion of additional studies. Pre-IND consultations might involve written responses to sponsor submissions, telephone communications, and/or face-to-face meetings between sponsor and FDA staff, as warranted and appropriate for review of preliminary proposals and data and for efficient transmission of advice.

Plans for any human use of a drug directed against variola might depend in part on animal studies greater in number and extent than is usual for drugs developed for other diseases. Discussions of the design and use of such studies should take place at the pre-IND consultation stage and early in the IND process. These issues highlight the importance of early interactions with the FDA through pre-IND mechanisms, and pre-IND consultations and discussions might be far more extensive than in many other areas of drug development. Potential studies to be discussed in pre-IND and early IND phases might include studies of in vitro and in vivo activity against a variety of poxviruses, animal toxicology studies, animal model pharmacokinetic studies, human safety and pharmacokinetic studies, and consideration as to how human and nonhuman pharmacokinetic data might be linked if an animal model is contemplated. Preliminary studies of drug efficacy and safety in human patients with other diseases (that might be performed under other INDs for other indications in some instances) also might be considered to provide supportive information. Each of these components of preparation for an IND are discussed further in subsequent sections of this guidance. As outlined in the following sections, the selection and design of studies to obtain preliminary data will warrant interdisciplinary assessment of a range of in vitro systems, animal models, and any available human data (e.g.,

from use in nonsmallpox illnesses or in volunteer safety or pharmacokinetic studies) that might prove to have indirect relevance to the use of a candidate drug if a smallpox outbreak were to occur.

2. Procedures for Facilitating Access to Investigational Drugs in Emergency Situations

One of the most important features distinguishing development of smallpox drugs from other types of drug development is that the effect of the drug in humans infected with variola would not be possible to assess unless cases of smallpox were to occur, but if even one case were to occur, it would be responded to as a unique public health emergency. Therefore, it is particularly important to develop a background of preliminary data providing evidence of safety and potential benefit of the candidate drug to support development of protocols that might be used to treat humans in such a public health emergency situation. Such a protocol might be planned and reviewed as a controlled or uncontrolled study under a standard IND, or as a treatment IND, depending on the circumstances and supporting information. Single-patient emergency IND (EIND) proposals also might be considered if a case were to occur for which an investigational drug would be considered potentially beneficial but no appropriate protocol was ready for use; however, we prefer that sponsors give early attention to preparation of protocols for possible outbreak situations, as such preparation should minimize the need for EIND consideration.

If drugs targeting smallpox are at appropriate more-advanced development stages with sufficient data available at the time that a smallpox emergency occurs, consideration might be given to using EUA provisions of the Project BioShield Act (P.L. 108-276). This authorization, issued by the FDA Commissioner under section 564(b) of the Federal Food, Drug, and Cosmetic Act (the Act), allows the introduction into interstate commerce of a drug, device, or biological product intended for use in an actual or potential emergency during the effective period of an emergency declaration. EUA candidates include products and uses that are not approved under the Act or the Public Health Service Act. An EUA may be issued for a specific product if the totality of available scientific evidence indicates that it may be effective for diagnosing, preventing, or treating a serious or life-threatening condition that is caused by the agent that is the subject of the emergency declaration; in addition, the known or potential benefits of the product must outweigh its known or potential risks. Finally, there cannot be an adequate, approved, and available alternative to the product for diagnosing, preventing, or treating the relevant serious or life-threatening disease or condition.[7,8]

The precise requirements for the issuance of an EUA are not as extensive as the requirements for full approval, and the requirements for a given countermeasure cannot be determined in the absence of the actual emergency because of the need for a risk-benefit assessment. However, unapproved or unlicensed countermeasures in advanced stages of development that are expected to have sufficiently promising risk-benefit information might be considered by the Strategic National Stockpile and might be evaluated for investigational use under an IND or potentially

[7] See 21 U.S.C. 564.

[8] See the guidance for industry *Emergency Use Authorization of Medical Products* at http://www.fda.gov/oc/guidance/emergencyuse.html.

under an EUA if a situation of sufficient magnitude arises in which criteria for such use are met. In general, drugs proposed for consideration of potential use under an EUA should have substantially more data available than usually required to support initial administration to patients under an IND protocol, and should have evidence of sustained progress toward an NDA, so that appropriate risk-benefit evaluations could be made to decide whether the interim use of an EUA would be justified in a potential emergency situation. Sponsors who wish to propose their drugs as potentially appropriate for use under an EUA are encouraged to discuss their proposal with the review division as early as possible. To do so, we recommend sponsors provide as much information as possible to the pre-IND or IND, as appropriate. Sponsors also should provide frequent updates during the course of the development, proceeding toward fulfillment of requirements for an NDA, while compiling summary information that might support use under an IND or EUA, as appropriate, should an emergency arise before the development process is complete.

3. Use of Different Poxviruses and Assessment of Potential Relevance

In the preliminary development of candidate antiviral drugs for potential use against variola, initial studies of in vitro and in vivo antiviral activity should rely heavily on use of other related viruses, principally the nonvariola orthopoxviruses. Although data obtained through the study of nonvariola orthopoxviruses cannot directly substitute for studies using variola virus, they should provide useful ancillary information about the safety and activity of experimental antivariola compounds. Such studies are likely to be particularly important because of the restrictions on the use of variola virus. Nonvariola orthopoxviruses can be used for initial investigations of in vitro activity of candidate drugs, and for development and characterization of animal models for preliminary assessment of in vivo activity. In addition, some nonvariola orthopoxviruses can cause infections in humans (naturally or as complications of vaccination) in which therapeutic investigations might contribute to supporting information for investigational treatment of smallpox. Results from studies of nonvariola orthopoxviruses are not known to directly predict activity or clinical benefit in treatment of smallpox, but accumulation of such data should be important in evaluating the overall evidence base for drugs that potentially might be used in human smallpox.

We recommend that candidate drugs that appear sufficiently promising to pursue development be tested against several orthopoxvirus species, as no single virus has been identified as a best approximation for variola in terms of specific prediction of drug effects. Viruses suitable for preliminary studies can include a range of related nonvariola orthopoxviruses, with emphasis on vaccinia and with additional consideration of other orthopoxviruses that have been reported as causes of human disease (examples include monkeypox and cowpox) and viruses that can cause virulent outbreaks in animal hosts (examples include ectromelia, rabbitpox, and camelpox). The list of viruses to be studied should prominently include vaccinia because it has been more extensively studied and characterized in the past than other orthopoxviruses. In addition, vaccinia is in a group of orthopoxviruses closely related to variola, and studies of vaccinia also might be relevant to the development of drugs to treat complications of vaccination. An important factor in the evaluation of new drugs should be their ability to show substantial effects consistently across different poxviruses and animal models.

Evidence of activity against similar targets in multiple different poxviruses over a wide range of drug doses and viral inoculum challenges should add some preliminary support to the likelihood of activity against smallpox and also can contribute to other therapeutic goals. For example, a drug that successfully treats vaccinia in animals might be studied to treat the complications of smallpox vaccination,[9] and studies of monkeypox might contribute to improving the treatment of human monkeypox.

When undertaking studies of drug activity against nonvariola orthopoxviruses, sponsors should provide evidence that the drug targets studied in the other orthopoxviruses are relevant to variola. We recommend that even with such evidence regarding mechanism of action, extrapolations of pathophysiological studies or treatment results across viral and host species be limited and cautious, because fundamental characteristics of variola-related viruses can differ significantly from those of variola. For example, although vaccinia is structurally similar enough to variola to confer immunity through vaccination, drugs reported to be active against vaccinia in some animal studies were not found to be useful against human smallpox (Fenner and Henderson et al. 1988). Similarly, although camelpox is virulent in camels and may have a closer genetic relationship to variola than other poxviruses (Gubser and Smith 2002), it has not been reported as a major human health problem in areas with substantial contact between humans and camels.

Evaluations of antiviral activity in the course of drug development typically should begin with exploration of in vitro data, followed by animal data. Because smallpox is a potentially serious threat but does not occur naturally (so clinical trials cannot be performed in field situations) and human challenge studies would be unethical, animal models may provide important information for the evaluation of treatment effect and may contribute directly to drug approval per 21 CFR part 314, subpart I (the Animal Rule)[10] if a suitable approach is agreed upon (see additional discussion under section IV., Animal Models). It is important to obtain evidence of a therapeutic effect using several species of animal models. The sponsor should make an effort to develop animal models that resemble a range of variola-associated disease manifestations seen historically in humans, and to generate evidence relevant to prediction of treatment responses in humans. In addition to exploring antiviral activity, animal model data on pharmacokinetics and pharmacodynamics can contribute toward selection of a preliminary drug dose range that might be used to explore safety in humans, to facilitate exploration of treatment of other viral diseases that might provide preliminary supporting information, and, if possible, to permit prediction of an estimated dose range for optimal in vivo antiviral activity.

Human data from study of nonsmallpox illnesses also should play an important role in attempts to develop a drug that might be useful against smallpox. Clinical studies might demonstrate

[9] See the draft guidance for industry *Vaccinia Virus — Developing Drugs to Mitigate Complications from Smallpox Vaccination*. When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

[10] In some cases, therapeutic proteins or monoclonal antibodies may be evaluated using processes similar to antiviral drugs, but considered under the biologics version of the Animal Rule under 21 CFR part 601, subpart H. For ease of reference, to reflect regulations governing standard antiviral drug development, and because the primary focus of this guidance is on antiviral drugs, 21 CFR part 314 (and 312 where appropriate) citations are used throughout this guidance. Sponsors interested in developing therapeutic proteins or monoclonal antibodies for use against smallpox are encouraged to discuss their approach with the review division as early as possible in development.

whether the drug is efficacious in any studies that can be performed in naturally occurring infections with nonvariola orthopoxviruses such as vaccinia or monkeypox, or less closely related poxviruses, such as molluscum contagiosum. Drug safety data should be evaluated using the same types of human safety studies typically employed in other types of drug development, as well as human clinical trials for other illnesses where investigation of the candidate drug may be warranted. Sponsors should discuss with the FDA their plans and proposals for obtaining safety data from sufficiently large and diverse study populations to support each successive development stage.

The preliminary study of nonvariola orthopoxviruses is likely to warrant some differences in approach, but should nevertheless be relevant, for development of products that are hypothesized to act through mechanisms other than direct inhibition of viral replication. If a candidate drug is proposed that is not considered to have an antiviral mechanism of action, the sponsor should provide an adequate explanation of the mechanism of the drug's potential for utility in persons who may be exposed to, or infected with, variola. The sponsor also should provide proposals for early discussion to identify any differences in study approach that may be appropriate. Sponsors of any such drug candidates should still provide data from evaluation of the effect of the drug on viral replication, as part of the confirmation of the proposed mechanism of action and to assess for any deleterious effects that might occur.

Sponsors should ensure that all studies and procedures incorporate adequate precautions to avoid transmission of pathogenic virus or generation of novel biological hazards, including containment measures and vaccination of study staff, as appropriate. Even beyond the precautions warranted for other pathogens, it is critically important that the risk and benefit of any investigation involving variola virus be carefully weighed, and that sponsors stringently adhere to all measures to avoid any release or any increase in hazard associated with the virus. Sponsors should give careful attention to observing all provisions of the Select Agent Rule (42 CFR part 73; also see http://www.cdc.gov/od/sap/sitemap.htm) and other applicable governmental and institutional biosafety and biosecurity provisions.

B. Interactions Among Industry, Academic, and Government Sponsors

Because developing drugs for variola represents a unique situation, early and frequent collaboration with government agencies is strongly encouraged, when appropriate to enhance development in areas of unmet medical need or to facilitate suitable prioritization of access to restricted resources such as containment facilities. As discussed earlier, substantial preliminary data on the activity of candidate drugs for orthopoxvirus infections should be generated using nonvariola orthopoxviruses. Early in the course of development, sponsors of candidate drugs may find it useful to contact the National Institute of Allergy and Infectious Diseases, National Institutes of Health, to identify sources of funding (e.g., grants and contracts) and to learn more about collaborative programs where aspects of drug screening and development may be under way. If, on the basis of study results with other orthopoxviruses, it appears appropriate to consider studies using variola virus, pre-IND communications might address whether sponsors should approach investigators at the CDC to explore potential collaborations with those who work with variola virus and who are familiar with the biosafety level 4 (BSL-4) laboratories and extensive precautions that are necessary for virus handling.

If development of a candidate drug during pre-IND and early IND review processes yields sufficiently promising results, we suggest that sponsors develop a protocol that would provide for investigational use of the drug if a smallpox release were to occur. Discussions with the FDA are strongly encouraged when developing a protocol to facilitate drug use by federal, state, and local public health agencies in the event of a smallpox outbreak. Such communications can contribute to ensuring that proposals for protocol sections (e.g., drug availability and data collection) are adequate, and that investigator brochures contain all relevant material.

Because collaborative opportunities change over time, sponsors are encouraged to contact the review division early during the pre-IND stage of drug development to obtain current information regarding potential collaborative contacts.

C. Drugs with Previous or Concurrent Studies for Other Indications

Because smallpox is no longer a naturally occurring disease, data from studies of a candidate drug in other human illnesses might play a more important role than usual in the preliminary evaluation of both activity and safety. Useful information might be obtained either from studies that have been used to support another indication, or from investigational study information available to the sponsor.

If data concerning the use of the candidate drug for other diseases do not exist, sponsors should consider whether the drug shows promise for treating other diseases, warranting pursuit of a parallel line of development. This approach might provide safety and efficacy information relevant to those other diseases, and safety information that might contribute to support of investigational use if a smallpox emergency were to occur. In addition, if the other areas of development include study of viral infections related to variola, such studies might provide ancillary activity information to support investigational treatment of smallpox.

Some drug safety data should already exist if the drug under evaluation has previously undergone substantial development, is currently or will be under study for other indications, or has had approval sought for a nonvariola indication (whether orthopoxvirus-related or not), even if it has not been previously approved by the FDA. In this case, depending on the extent of already available safety information, the sponsor may not need to collect as much additional information to complete the initial safety database.

In addition to safety data from healthy human volunteers, it can be particularly important to have safety data from studies for other indications involving treatment of patients who are acutely and severely ill. If a terrorist event involving smallpox were to occur, it is likely that a significant proportion of patients would be severely ill, with organ system dysfunction and imbalances of physiology that could increase the possibility of drug side effects. Safety data from previous studies of the candidate drug used for treatment of any other disease should be provided to the FDA, if available.

Information on drug safety, pharmacokinetics, and pharmacodynamics in special populations (including studies in the pediatric population, the geriatric population, pregnant women, lactating

women, and persons with renal and hepatic impairment) should be provided to the FDA, if available. The sponsor should document the adequacy of the available data to support the safety of a proposed clinical protocol. If the sponsor does not own the supporting safety data, and if those data are not in the public domain, it is the sponsor's responsibility to obtain letters of authorization allowing the FDA to refer to those studies in its evaluation of the proposed IND.

If the drug under evaluation has already been approved for other indications, the sponsor can either obtain a right of reference to the safety data or rely on the FDA's previous finding of safety of that drug. The sponsor also should provide any additional data that may be appropriate to support the proposed investigational use (examples would include information sufficient to support a different dose or patient population as compared with the approved use). However, if the sponsor relies on the FDA's previous finding of safety, any future submission of an NDA would be subject to the provisions of 21 CFR 314.54, Procedure for Submission of an Application Requiring Investigations for Approval of a New Indication for, or other Change from, a Listed Drug.

Early discussion with the FDA can help to identify planning strategies that can lead to the most efficient design of overlapping development plans. For those drugs that are new chemical entities, refer to section III.E., Nonclinical Toxicology, for information regarding the recommended safety studies.

D. Chemistry, Manufacturing, and Controls

We recommend that the sponsor provide CMC information as described in the guidances for industry *Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products* and *INDs for Phase 2 and Phase 3 Studies, Chemistry, Manufacturing, and Controls Information*. We recommend that sponsors consult other relevant guidances and discuss plans and questions with the review division.

E. Nonclinical Toxicology

A sponsor must supply information about the pharmacological and toxicological studies of a drug performed in vitro or in animal studies adequate to support the safety of proposed clinical investigations (21 CFR 312.23(a)(8)). The dose, duration, route, and overall design of animal and other studies that should be submitted varies with the duration and nature of the proposed clinical investigations. FDA guidances recommend how such requirements can be met. These guidances are referenced in the following sections. Many of the elements listed as necessary in IND submissions (see citations in the following paragraphs) also may be desirable in a pre-IND submission to the extent that appropriate information is available.

The information submitted must include the identification and qualifications of the individuals who evaluated the results of these studies and concluded that it is reasonably safe to begin the proposed clinical investigations (§ 312.23(a)(8)). In addition, the sponsor must include a statement detailing where the investigations were conducted and where the records are available

for inspection (§ 312.23(a)(8)). As drug development proceeds, the sponsor should submit nonclinical and clinical safety information as amendments to the IND or pre-IND.

Under § 312.23(a)(8), the sponsor must submit an integrated summary of the toxicological effects of the drug in vitro and in animals. Depending on the nature of the drug and the phase of the investigation, the summary should include the results of acute, subacute, and chronic toxicity tests, safety pharmacology tests, tests of the drug's effects on reproduction and the developing fetus, tests of the drug's genetic toxicity, any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology), and any in vitro studies intended to evaluate drug toxicity. We also prefer that animal studies describing the pharmacological effects and mechanisms of action of the drug and information on the absorption, distribution, metabolism, and excretion of the drug be submitted. For each toxicology study that is intended to support the safety of the proposed clinical investigation, a full tabulation of data suitable for detailed review must be submitted (§ 312.23(a)(8)(ii)(b)).

Under § 312.23(a)(9)(i) and (iii), the sponsor must submit a summary of previous human experience with the investigational drug. Detailed safety data as well as information relevant to the rationale of drug development for any investigational drug marketed in the United States or abroad should be submitted. A list of countries in which the drug has been marketed or withdrawn from marketing for reasons related to its safety or efficacy also must be submitted. Additionally, if the drug has been studied in controlled clinical trials, relevant data regarding the drug's effectiveness for the proposed investigational trial should be submitted. Published material relevant to the safety or effectiveness of the drug or clinical investigation must be provided, whereas less-relevant published material should be provided as a bibliography.

Regulatory and pharmaceutical industry representatives from the United States, Europe, and Japan (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)) have written guidances for many of the nonclinical requirements for safety studies. These guidances recommend international standards for, and promote harmonization of, the nonclinical safety studies appropriate for supporting human clinical trials of a given scope and duration.

1. Timing of Nonclinical Studies to Support the Conduct of Human Clinical Trials

Usually, once a drug has been shown in nonclinical studies to be sufficiently safe for clinical trials to begin, such trials are conducted to demonstrate the drug's safety and efficacy in humans. Phase 1 trials evaluate the safety and pharmacokinetic profile of the drug. These trials start with relatively low drug exposure in a small number of subjects, often using healthy volunteers. The pharmacokinetic data, together with activity data in vitro, should ideally demonstrate that a high inhibitory quotient (IQ) can be expected at doses that are safe for the administration of the drug (see section III.F.1.d., Inhibitory quotient). Efficacy evaluations generally are carried out in trials of longer duration; therefore, phase 1 trials are usually followed by clinical trials in which drug exposure increases by dose, duration, and/or size of the exposed patient population.

In trials of candidate drugs designed for potential future use against variola, studies to assess the safety of the drug in humans typically can be conducted first in healthy volunteers. Thus,

sufficient nonclinical studies usually would be carried out to support the safety of administration of the drug for at least 2 weeks, or until pharmacokinetic measurements have demonstrated that the drug has reached steady state in the healthy volunteers. In general, toxicology studies of 2 weeks duration in a rodent and a nonrodent species should support submission of protocols for review for phase 1 clinical trials of up to 2 weeks. Upon the completion of studies to support a dosing duration of up to 2 weeks, a 1-month (or longer) study, again in healthy volunteers, might be appropriate to consider. However, to support the dosing of humans in clinical trials for a period longer than 2 weeks, nonclinical toxicology studies of a longer duration should be performed.[11] The clinical manifestations of variola infection suggest that some cases may require treatment for longer than 2 weeks; therefore, we recommend that initial toxicology and safety studies take this possibility into account.

2. Acute and Subacute Toxicity Studies

Acute toxicity studies are commonly the first studies carried out on a drug intended for humans and use a single dose or multiple doses administered for no longer than a 24-hour period. Subacute studies by definition are longer than acute studies, and are generally multiple-dose studies carried out for no longer than 6 months. Most commonly, an acute study with drug administration by the proposed clinical route of administration as well as a parenteral route (usually intravenous) in a rodent and a nonrodent species is performed to set the doses for longer term nonclinical studies and to evaluate the immediate toxicity profile of the drug. If the proposed clinical route of administration will be intravenous, intravenous evaluations alone will usually suffice. We recommend that observational evaluations, as well as clinical chemistry and histopathologic evaluations, be performed at the end of 2 weeks for the acute studies.

3. Safety Pharmacology Studies

Safety pharmacology studies evaluate the interaction of the drug with organ systems such as the central nervous system, cardiovascular system, and respiratory system. In some cases, the sponsor can incorporate some safety pharmacology evaluations in animals into the design of toxicology, kinetic, and clinical studies, whereas in other cases these endpoints are best evaluated in specific safety pharmacology studies. Although the adverse effects of a substance might be detectable at exposures that fall within the therapeutic range in appropriately designed safety pharmacology studies, such effects may not be evident from observations and measurements used to detect toxicity in conventional animal toxicity studies.[12]

4. Genetic Toxicity

We recommend that the sponsor perform a comprehensive assessment of a new drug's genotoxic potential before its administration into humans. Since no single test is capable of detecting all relevant genotoxic agents, the most common approach is to carry out a battery of in vitro and in vivo tests for genetic toxicity. A standard test battery of studies has been selected under ICH to

[11] See the ICH guidance for industry *M3(R1) Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* (http://www.ich.org/cache/compo/276-254-1.html).

[12] See the ICH guidance for industry *S7A Safety Pharmacology Studies for Human Pharmaceuticals*.

evaluate a new drug for its ability to cause genetic toxicity. In general, two of the in vitro tests should be completed before the initial submission of an IND. The remainder of the battery should be completed before phase 2 studies.[13]

Detection of genetic toxicity can cause an ethical dilemma. Generally, no more than one dose of a genetically toxic drug should be administered to a healthy volunteer. It is considered unethical to subject a healthy volunteer, who does not stand to benefit from drug administration, to a drug that might cause cancer. It is possible that a drug with potential for efficacy against variola also can be a genetic toxin. We recommend that the sponsor confer with the review division regarding such an issue as soon as possible.

5. Reproductive Toxicity

Reproductive toxicity studies assess the effect a drug can have on mammalian reproduction from premating (adult male and female reproductive function) to sexual maturity of the offspring. ICH guidances address the design of reproductive toxicity studies and offer a number of choices for carrying out reproductive toxicity studies.[14] The reproductive toxicity studies vary from indication to indication, but they should be submitted before phase 3 trials. In studies of poxvirus infections, risks that women entering the trials might be pregnant, and potential toxicity to male and female fertility, are concerns. A study of fertility from conception to implantation and at least one organogenesis study should be completed before the early studies in healthy volunteers, and the full complement of studies preferably should be completed before the administration of the drug in patients. The informed consent form should outline the potential hazards associated with drug administration.

6. Carcinogenicity Studies

In general, we do not anticipate that carcinogenicity studies are likely to be necessary for drugs that might be used only to treat established variola illness since the administration of such drugs will not, in most cases, exceed 6 months. However, decisions regarding the performance of carcinogenicity studies should be made on a case-by-case basis and depend upon the mutagenic potential and/or possible structure-activity relationship of the test drug with other known carcinogens.[15] Further discussions also should take place if there is a possibility of longer-term (and possibly recurrent) prophylactic use.

F. Microbiology

This section discusses important issues for consideration in the microbiological evaluation of candidate drugs. Some components may change as more investigations take place in this field

[13] See the ICH guidances for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals* and *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals.*

[14] See the ICH guidance for industry *S5(R2) Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility* (http://www.ich.org/cache/compo/276-254-1.html).

[15] See the ICH guidances for industry *S1A The Need for Carcinogenicity Studies of Pharmaceuticals* and *S1B Testing for Carcinogenicity of Pharmaceuticals* (http://www.ich.org/cache/compo/276-254-1.html).

(e.g., increased opportunities to study cross-resistance or interactions with other antivariola virus drugs). The sponsor should make available for review adequate information on sample collection, assays performed, and on validation approaches for these assays. Use of a specific procedure, method, or test system in an investigational protocol for a nonclinical laboratory study, or as laboratory procedures supporting a clinical trial, does not constitute FDA endorsement of that procedure, method, or test system, or FDA approval for clinical laboratory use. This guidance addresses these points further in the following descriptions, and sponsors are encouraged to discuss questions with the review division early in the drug development process. Additional information on virology studies in some of the principal areas of antiviral drug development can be found in the guidance for industry *Antiviral Product Development — Conducting and Submitting Virology Studies to the Agency*. If a diagnostic assay proposed for use in a clinical trial has not been previously cleared by the FDA but eventually may be developed for commercial distribution, the sponsor should consider early discussions with the Center for Devices and Radiological Health as well as the Center for Drug Evaluation and Research (CDER) to facilitate collaborative or consultative review and comment as appropriate.

1. Components of Nonclinical Virology Studies and Reports

Nonclinical virology studies are an important component in the review process of a candidate antivariola virus drug. These studies contribute to the evaluation of the antiviral activity and safety of a candidate drug before its use in humans. Submitted study reports should identify the mechanism of action, establish specific antiviral activity of the compound in cell culture and animal models, and provide data on the development of viral resistance (or reduced susceptibility of the virus) to the candidate drug. Distinctive factors affecting the generation of virology data related to smallpox include limited access to the two approved smallpox laboratory facilities, lack of an adequate animal model for smallpox (or animal host comparable to human disease), and the critical importance of risk-benefit assessment and prioritization of resources for any consideration of studies involving variola virus; therefore, information from related but more common and less pathogenic viruses should be carefully compiled and analyzed, to a greater extent than for the development of other antiviral products, before discussing potential applicability to variola virus or actual study of variola virus.

Although data from other orthopoxviruses should not be considered definitive evidence of antivariola activity, exploratory studies with such viruses can provide important adjunctive information to the extent that these studies are safe and feasible to perform. Sponsors are encouraged to assess activity of the candidate drug against several orthopoxviruses including vaccinia virus. These nonclinical studies should be well advanced or completed before the introduction of the candidate drug into humans. Pre-IND submissions should be used as an opportunity for discussion of initial data obtained with nonvariola poxviruses and for identification of additional studies that may be desirable with such viruses. They are also an opportunity to discuss how and when it may be possible to generate data more directly applicable to variola virus while maintaining experimental safety and appropriate prioritization of studies.

a. Mechanism of action

A candidate drug might act directly by targeting a specific viral-encoded function (e.g., an enzyme inhibitor) or act indirectly (e.g., interferon induction of the host cell response). Reports of nonclinical virology studies should include background information describing the rationale and data showing the mechanism of action of the candidate drug, and the sponsor should provide complete publication copies of all key cited references. The sponsor also should provide biochemical, structural, cellular, or genetic data to support the proposed mechanism of action. Examples include data demonstrating receptor binding, inhibition of enzymatic activity, X-ray crystallographic structure determination of bound inhibitor complex, and characterization of resistance mutations in the gene encoding the target. The sponsor should demonstrate the specificity of the candidate drug for the viral target over host proteins, especially when a viral enzyme has a cellular counterpart. For example, if the candidate drug is designed to target the variola DNA polymerase, specificity against the polymerase from related orthopoxviruses should be shown in comparison with host DNA and RNA polymerases. If studies with polymerases from more common and less pathogenic poxviruses are promising, applicable regulations or guidances of relevant public health agencies at the time of drug development should be consulted to determine whether assessment of specificity against recombinant variola polymerase is appropriate. For nucleoside or nucleotide analogs, the intracellular half-life ($t_{1/2}$) of the triphosphate form of the active drug moiety should be determined.

Immunomodulatory drugs might have unintended effects on the immune system that result in activation of viral replication or in progression of clinical disease. Therefore, studies that only show general immune stimulation by a candidate immunomodulatory drug are likely to be of limited value, and sponsors should design studies to demonstrate whether an antiviral effect on appropriate orthopoxviruses can be achieved.

b. In vitro antiviral activity

Cell culture systems and surrogate virus/animal models (e.g., vaccinia virus infection of mice with congenital or induced immune compromise) should be used to show the candidate drug has specific, quantifiable antiviral activity against an appropriate range of orthopoxviruses. The FDA and organizations such as the Clinical and Laboratory Standards Institute (formerly the National Committee on Clinical Laboratory Standards or NCCLS) do not recognize or recommend a specific test system for assessing antiviral activity. Sponsors can consult published work[16] or present additional proposals for review.

We recommend that sponsors consider including vaccinia vaccine strains as well as other laboratory strains (including any strains expected to be used in animal models) in microbiological testing, not only as part of a broad-based orthopoxvirus testing strategy to screen for potential relevance to variola, but also to assess the potential of the candidate drug for use in clinical trials to treat vaccine complications.[17] As outlined below, investigation of the treatment of vaccination complications offers the opportunity to test the candidate variola drug in a human

[16] Examples include Smee and Sidwell et al. 2002 and Kern and Hartline et al. 2002.

[17] See note 9, supra.

illness caused by a related virus (though the illnesses are not similar and extrapolation from one to the other is likely to be limited), in addition to the intrinsic benefit that might arise from development of treatments for vaccine complications. In addition, if a candidate drug has suitable safety and in vitro activity profiles, studies of treatment effects in humans infected with monkeypox virus also might offer useful preliminary information relevant to variola therapy as well as possible direct benefit for future monkeypox treatments. We recommend that information on antiviral activity also be generated for other related poxviruses, including any nonvaccinia poxviruses that can be studied in animal models (e.g., cowpox, ectromelia, monkeypox) to provide ancillary information on the effectiveness of the candidate drug. Ultimately, the sponsor should explore the potential appropriateness of testing the antiviral activity of the candidate drug against variola isolates if other data are sufficiently promising to proceed to this stage.

We recommend that specific antiviral activity be determined using a quantitative assay to measure virus replication in the absence and presence of increasing drug concentrations. The drug concentration at which virus replication is inhibited 50 percent is the effective concentration (EC_{50}) (also referred to as the inhibitory concentration (IC_{50})). We also recommend that the sponsor document the sources of viruses (e.g., blood, plasma, defined laboratory strains, clinical isolates), the method of isolation and the characterization, storage and stability, and cell culture procedures and materials. Sponsors are encouraged to consult FDA and ICH guidances for definitions on assay validation.[18] For any assay developed or used for showing antiviral activity, or other investigational assay used in the nonclinical and clinical studies, the sponsor should provide sufficient information about the assay to assess the appropriateness of its use in the specified study setting. Assays should be well-documented, and should adequately meet requirements of 21 CFR part 58, Good Laboratory Practice for Nonclinical Laboratory Studies. The test system should be standardized with well-defined control strains. The sponsor should discuss with the FDA the specific information to be provided.

It is important to consider whether the inhibitory concentration is consistent with data supporting the mechanism of action, such as a K_i or binding data. A candidate drug that inhibits virus replication at a concentration much lower than is expected from the biochemical data supporting the proposed mechanism suggests that another target may be affected or another mechanism of inhibition may be operating.

c. In vitro antiviral activity in the presence of serum proteins

Serum proteins bind and sequester many drugs and might interfere with the antiviral activity of a drug. Therefore, we recommend that the in vitro antiviral activity of a candidate drug be analyzed both in the presence and absence of serum proteins. The effects of human serum (45 to 50 percent) and human plasma plus α-acidic glycoprotein on the in vitro antiviral activity of the candidate drug should be evaluated by determining a median serum adjusted EC_{50} value and an EC_{50} value in the presence of 2 mg/mL of α-acidic glycoprotein. For several well-defined strains of orthopoxviruses appropriate for study, the sponsor should evaluate the effects of human serum

[18] See the ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and Methodology* (http://www.ich.org/cache/compo/276-254-1.html) and the guidance for industry *Bioanalytical Method Validation.*

(40 to 50 percent) and/or human plasma plus 2 mg/mL of α-acidic glycoprotein on the in vitro antiviral activity of the candidate drug and determine a median serum adjusted EC_{50} value.

d. Inhibitory quotient

Drug concentrations are an important factor in the response to viral therapy. Therefore, we recommend that the sponsor determine an inhibitory quotient, IQ = C_{min}/serum adjusted EC_{50}. An IQ integrates plasma drug concentrations and resistance testing. A high IQ indicates the potential that a drug concentration might be achieved in a patient that might effectively inhibit the virus and minimize the development of drug resistance. A high IQ can help to identify promising drugs for additional studies, and those additional studies in turn might make it possible to obtain additional information on the relationship between IQ and outcome.

e. Cytotoxicity and therapeutic index

After drug exposure in a cell culture model, host cell death might be misinterpreted as antiviral activity. Cytotoxicity tests use a series of increasing concentrations of the candidate drug to determine what concentration results in the death of 50 percent of the host cells. This value is referred to as the *median cellular cytotoxicity concentration* (CC_{50} or $CCIC_{50}$). The relative effectiveness of the candidate drug in inhibiting viral replication compared to inducing cell death is referred to as the therapeutic index (i.e., CC_{50}/EC_{50}) or as the *selectivity index*. A high therapeutic index is desired, as it represents maximum antiviral activity with minimal cell toxicity. We recommend that the CC_{50} be assessed both in stationary and dividing cells from multiple human cell types and tissues for potential cell cycle, cell type, or tissue specific toxicities. We also recommend that the effects of the candidate drug on mitochondrial toxicity in cell culture be monitored by examining measures such as mitochondrial morphology, glucose utilization, lactic acid production, and mitochondrial DNA content. These studies might reveal the potential for toxicity in vivo.

f. In vitro combination activity analysis

Administration of multiple antiviral drugs might be more effective in inhibiting virus replication than a single drug. Future treatments for variola virus might use combinations of drugs. However, drug interactions are complex to study and interpret, and can result in antagonistic, additive, or synergistic effects with respect to antiviral activity. For this reason, it is important to test the in vitro antiviral activity of candidate drugs in combination with other drugs approved for the same indication. We recommend in vitro drug combination activity studies be performed with any investigational or approved drugs expected to be used with the candidate study drug to treat variola infection at the time that a new candidate drug is entered into development. If other drugs are approved for other poxvirus indications, we recommend in vitro combination activity studies with those drugs as well. In vitro drug combination interactions can be evaluated using analyses based on published work.[19]

[19] Examples include Chou and Talalay 1984 and Prichard et al. 1993.

g. Selection of resistant virus in vitro

The sponsor should assess the risk that variola virus might develop resistance to the candidate drug. *Resistance*, as it is used herein, is a relative, not absolute, term. Because of the unique hazards associated with variola virus, we recommend that the potential for emergence of resistance be carefully explored using vaccinia virus and a variety of other nonvariola orthopoxviruses. The evidence for applicability of these data to variola should be assessed and presented to the FDA for further discussion. The sponsor should be prepared for how it might assess the emergence of resistance if a smallpox emergency were to occur in which clinical use of the candidate drug might be contemplated.

Two basic methods can be employed to isolate viruses in vitro that have reduced susceptibility to the candidate drug. In the first, the virus is propagated for several passages at a fixed drug concentration, using multiple cultures to test different concentrations. In the second, the virus is passaged in the presence of increasing drug concentration starting at half the EC_{50} value for the parental virus. For both of these methods, virus production is monitored to detect the selection of resistant virus. The former method is particularly useful for identifying drugs for which one or two mutations can confer large shifts in susceptibility.

Selection in cell culture of virus resistant to the candidate drug can provide insight into whether the genetic threshold for resistance development is high (three or more mutations) or low (one or two mutations). The rate of appearance of resistant, mutant viruses depends on the rate of viral replication, the number of virus genomes produced, and the fidelity of the viral replicative machinery. Resistance is also a function of the IQ, as previously mentioned. Consideration of these factors can help design tests to detect the appearance of virus resistant to high concentrations of the drug in vitro. In cases when cell culture systems do not produce sufficient virus titers and multiple mutations are required to develop resistance to high drug concentrations, serial passage of the virus in the presence of increasing concentrations of the candidate drug might lead to the isolation of resistant virus.

Well-characterized genotypic and phenotypic assays are important for detection of the emergence of resistant virus during the development of candidate drugs. Sponsors can choose to do phenotypic and genotypic characterization themselves or send samples to laboratories that are registered under section 510 of the Act and use test systems with standard operating procedures. In the former case, it is important that the investigational assay performance characteristics be provided to the review division, and approved handling procedures for laboratory samples be employed.[20]

- **Genotypes** — Genotypic analysis of selected resistant viruses determines which mutations might contribute to reduced susceptibility to the candidate drug. Identifying resistance mutations can be useful in developing genotypic assays and analyzing their ability to predict clinical outcomes and can provide data supporting the proposed mechanism of action of the candidate drug. Frequently occurring mutations can be

[20] For definitions of assay validation, refer to the ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and Methodology* (http://www.ich.org/cache/compo/276-254-1.html) and the guidance for industry *Bioanalytical Method Validation*.

identified by DNA sequence analysis of the relevant portions of the virus genome. We recommend that the sponsor determine the complete coding sequence of the gene for the target protein, and the pattern of mutations leading to resistance of a candidate drug, and compare that pattern with the mutation pattern of other drugs in the same class. The sponsor should report the details of the genotypic assays used along with the results for controls used to standardize the assays. The report should include definition of the lowest percentage for any one mutation present in a mixed population that the assay can detect.

- **Phenotypes** — Phenotypic analysis determines if mutant viruses have reduced susceptibility to the candidate drug. Once resistance mutations are identified, we recommend evaluating their ability to confer phenotypic resistance in a recombinant virus system (e.g., by using site-directed mutagenesis or polymerase chain reaction amplification of relevant portions of the virus genome to introduce these mutations into a standard laboratory genetic background). Construction of recombinants should use only viral species and strains of suitably low risk to humans and should take place only under adequate biosafety and biosecurity conditions (see section III.A., Selected Issues with Distinctive Impact, and the Select Agent Rule). Then recombinant virus could be tested for drug susceptibility in vitro. The shift in susceptibility, or fold resistant change, for a clinical isolate is measured by determining the EC_{50} values for both the isolate and a reference virus under the same conditions and at the same time. The fold resistant change is calculated as the EC_{50} of isolate/EC_{50} of reference strain. We recommend that a well-characterized wild type laboratory strain grown in cell culture serve as a reference standard.

  The utility of a phenotypic assay depends on its sensitivity (i.e., its ability to measure shifts in susceptibility (fold resistant changes) compared to reference strains or baseline clinical isolates). Calculating the fold resistant change (EC_{50} of isolate/EC_{50} of reference strain) makes comparisons between assays possible.

h. Cross-resistance

In the case of antiviral drugs targeting the same protein, cross-resistance (i.e., mutations leading to reduced susceptibility to one drug resulting in decreased susceptibility to other drugs in the same class) has been observed. Although no drugs are currently approved for the treatment of variola infection, increased opportunities to study cross-resistance with other antivariola virus drugs should emerge as more investigations take place in the field. Cross-resistance is not necessarily reciprocal. For example, if virus X is resistant to drug A and shows cross-resistance to drug B, virus Y, which is resistant to drug B, might still be susceptible to drug A. Cross-resistance analysis can be important in the development of treatment strategies (i.e., establishing the order in which drugs are given). The sponsor should evaluate the activity of the candidate drug against viruses resistant to other approved drugs in the same class and the activity of approved drugs against viruses resistant to the candidate drug.

2. Proposal for Monitoring Resistance Development

Pre-IND and early IND discussions of candidate drugs for variola should consider studies that will support the development of a protocol for investigational therapeutic use of the drug in the event of a smallpox release. We recommend that these studies include evaluation of the in vitro and in vivo antiviral activity using an appropriate range of poxviruses. With any such protocol, the sponsor should include a plan to monitor the development of drug-resistant viruses if a situation occurs in which individuals might be treated for smallpox. Animal studies with nonvariola orthopoxviruses should make an important contribution to drug evaluation (see section IV., Animal Models). Therefore, the sponsor should include proposals for the evaluation of resistance in animal studies. We recommend that the resistance monitoring plan include a description of the assays that will be used to monitor viral shedding and viral burden, methods of sample collection and storage and for sample handling (frozen or ambient), and a description of genotypic and phenotypic assays and the time points that will be analyzed (e.g., baseline, day 1, additional specified on-treatment and post-treatment time points). The proposal should define the parties responsible for each component.

We suggest that genotypic and phenotypic data be provided for baseline isolates from all patients and endpoint isolates of patients who were virologic failures and discontinuations. Proposals for resistance monitoring in animal studies should also give particular attention to changes from baseline associated with clinical and laboratory manifestations of treatment failure. Furthermore, we recommend that definitions of virologic failures and discontinuations be discussed with the review division during protocol development. For example, in the more extensively studied setting of therapy for HIV-1 infection, virologic failure definitions have been based on the course of viral load measurements over time and on investigator evaluations of reasons for discontinuation. We urge that information bases be developed to facilitate the assessment of the relationship between clinical course and virologic findings in orthopoxvirus infections. Sponsors are encouraged to consult with the review division on the preferred format for the submission of resistance data.

3. In Vivo Virology Study Reports (Clinical and/or Animal Studies)

In addition to the nonclinical virology studies and reports discussed in the first part of the Microbiology section, virology study reports from any clinical studies (and studies in animal models where applicable) will be an important component of the overall evaluation of candidate drugs as they reach later stages of development. We prefer that complete virology study reports be extensive and include the raw and analyzed data as well as all the information necessary to evaluate the procedures used to obtain those data. Virology study reports convey information on in vivo antiviral activity of the candidate drug, development of resistance to the candidate drug in treated patients and animal models, and cross-resistance with other drugs in the same drug class. The format of a virology study report should be similar to that of a scientific publication and typically should include the following sections: summary, introduction, materials and methods, results, and discussion. The methods section should describe all the protocols employed and include a description of the statistical analyses used. We recommend that sponsors also provide copies of the publications of key references. For information regarding FDA materials on reporting of virology study results, see the guidance for industry *Antiviral Product Development*

— *Conducting and Submitting Virology Studies to the Agency*. Sponsors should discuss with the FDA which aspects of these materials are applicable to orthopoxvirus studies and what modifications may be warranted to address specific attributes of orthopoxvirus studies.

For some antiviral therapies in other settings, quantification of viral loads has been a good measure of the clinical effectiveness of antiviral drugs and has provided insight into whether these drugs have activity in vivo when the clinical benefit may not be apparent or may be temporary because of the development of resistance. Such candidate drugs might prove useful when studied in combination with other drugs. Development of methods for quantification of viral burden or viral shedding, and evaluation of the relationship between these quantitative measurements and clinical outcomes of disease and treatment, is encouraged for all orthopoxvirus studies performed during the development of a candidate drug. However, it is important to recognize that change in viral burden in the setting of variola infection is a biomarker that may not fully capture the net treatment effect from the antiviral drug.[21]

As previously mentioned, we prefer that the sponsor provide a complete description of the methodology and the quantitative assay performance characteristics, the specimen sources of viruses (e.g., blood, plasma, defined lesion specimens), the storage and stability, and cell culture procedures. We encourage efforts to collect specimens in sufficient quantities to allow reserve amounts to be stored for possible re-evaluation by new or improved assays. Additionally, it is important to examine the relationships between phenotypic and genotypic analyses and clinical outcomes in any such studies, to assess the extent to which these assays may be predictive of the utility of treating an individual with the candidate drug. We recommend using viral load and genotypic and phenotypic assay analyses following the same criteria as described in previous parts of the Microbiology section. Sponsors are encouraged to discuss their assays with the review division. Genotypic analysis of baseline and failure isolates from patients failing to respond to therapy or undergoing viral rebound can help identify mutations that contribute to reduced susceptibility to the candidate drug. It is important that phenotypic analyses of baseline and post-treatment isolates be completed to obtain information on the susceptibility of the candidate drug and cross-resistance with other drugs. We recommend that genotypic and phenotypic analysis of at least a subset of baseline isolates be performed to determine response to therapy based on baseline genotype and baseline phenotypic drug susceptibilities. We encourage sponsors to consult with the review division with respect to electronic submission of resistance data.

G. Clinical Pharmacology

We recommend that sponsors study the relationship between in vitro and in vivo pharmacokinetics and activity using animal models before the initiation of studies in humans (see section IV., Animal Models). Sponsors also should consider developing models of drug pharmacokinetics and pharmacodynamics to study drug dosage and drug regimens further, using both in vitro systems and animals. Developing such models can help to expedite the selection of an optimal drug dose regimen for human clinical studies.

[21] See Fleming and DeMets 1996.

Sponsors should provide human pharmacokinetic and pharmacodynamic information as soon as it is available. If the candidate drug can be appropriately studied in any naturally occurring human viral infection, these studies may provide relevant information about the relationship between the drug's pharmacokinetics and a suitable pharmacodynamic endpoint. If no suitable human pharmacodynamic endpoint is available, then any appropriate analyses of the relationship between human pharmacokinetics and measurements of antiviral activity in animal models and in vitro assays should be provided. Although the applicability to any potential occurrence of human smallpox may not be directly assessable, the purpose of obtaining these data is to explore the following issues:

- To demonstrate that the desired systemic drug concentration in humans actually can be achieved after the anticipated dosage regimen is given
- To explore potential relationships between blood drug concentration and pharmacodynamic response
- To select the appropriate dose
- To evaluate the relationship between drug exposure and subsequent development of viral resistance (see section III.F.2., Proposal for Monitoring Resistance Development)

We recommend that sponsors perform exposure-response analyses where appropriate.[22] These analyses can help determine which drug exposure measures (e.g., area under the curve and concentration at the end of the dosing interval) are relevant to a given outcome. For studies conducted with animal models, the dose regimens used in animals to provide systemic exposure comparable to humans may not be the same as the regimen for humans. Therefore, the sponsor should consider what information it can generate and present to support an assumption that the difference in dose regimens does not affect the drug's efficacy and/or safety. Examples of studies that might contribute to this objective might include studies with infected animals across a wide range of drug doses and dosing regimens showing whether the therapeutic effect is regimen-sensitive, and pharmacokinetic-pharmacodynamic and treatment-outcome studies in related human infections with comparable viral drug susceptibility.

A substantial percentage of the U.S. population older than 45 years has received immunization with vaccinia, and it is possible that vaccine-induced residual immunity might confer prolonged protective effect against variola infection, which might affect drug efficacy assessment. Vaccine and drug interactions can be explored to a limited extent in animal models (see section IV., Animal Models). In addition, in data collection protocol plans for patients receiving drug therapy if a smallpox emergency were to occur (see section V., Clinical Data), sponsors might consider the possibility of obtaining samples to determine titers of antibodies against variola and quantify antigen-specific T-cell responses. Such data should then be incorporated into exposure-response analyses.

[22] See the guidance for industry *Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications*.

The sponsor should fully characterize the metabolic profile (in vitro and in vivo) in humans, and provide information comparing the plasma protein binding of the active drug components across the range of expected concentrations in humans.

Recipients of the study drug may receive several medications concurrently. In vitro drug metabolism studies can direct the investigation of potential human drug-drug interactions.[23] The sponsor should provide drug interaction data; however, information regarding drug interactions should not delay the submission of the IND.[24]

IV. ANIMAL MODELS

When sponsors are developing drugs for potential use in treating or preventing smallpox, human data will be important in a number of ways, including (where appropriate) delineation of the drug's safety profile in healthy volunteers and observation of its safety and activity in other diseases (see section V., Clinical Data). No data from the use of the candidate drug in humans infected with variola virus will be available, unless an emergency involving bioterrorism or biowarfare or accidental release of the virus occurs. Under these unique development circumstances, data from animals, and further development and characterization of animal models, have the potential to provide much useful information in the evaluation of drugs to treat and prevent smallpox. Animal models can demonstrate drug activity in vivo (including the preliminary characterization of drug-drug or vaccine-drug interactions), provide exposure-response data to help estimate dosing regimens, and contribute to the design of a proposed protocol that can be available for investigational clinical use of a candidate drug if a smallpox release were to occur.

This section describes some types of animal studies that may be desirable to support investigational human use of a candidate drug, and provides a basis for discussion of what aggregate accumulation of data might lead to approval in the future. Because the availability of well-characterized animal models and the data supporting their use to predict human treatment responses is expected to change over time, sponsors are encouraged to consult with the review division early in the development process to review and discuss the status of existing models, prospects for studying newer models, and proposals for integrated use of animal and human studies. Sponsors should initiate such interactions at the pre-IND stage to discuss optimal use of resources in the initiation of development plans involving such animal studies.

A. Uses and Limitations of Different Orthopoxviruses

Currently, available data do not establish specific preferred, well-characterized animal models for smallpox, and no animal models have been shown to replicate or to predict human responses to therapy for smallpox. The ability of any animal model to predict human responses to therapy

[23] See the guidance for industry *Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro*.

[24] See the guidance for industry *In Vivo Drug Metabolism/Drug Interaction Studies — Study Design, Data Analysis, and Recommendations for Dosing and Labeling*.

for smallpox is difficult to assess, especially given the lack of any effective drugs that could be used to characterize models and to compare new drugs. The differences in both in vitro drug susceptibility and in vivo pathogenicity of different poxviruses for different hosts, and the immunomodulatory properties of the various orthopoxviruses themselves, add to the difficulty of extrapolating results from animal studies. However, using multiple nonvariola orthopoxviruses (see section III., Regulatory Approach Regarding Early Drug Development) in multiple models can provide useful information about the possibility of finding dose ranges that might offer benefit in human investigational use, particularly if a candidate drug is found to be highly active across a wide range of doses and treatment times relative to the disease course in animals.

To explore such possibilities, we recommend that compounds found to be active in vitro be studied in several animal models using multiple different orthopoxviruses initially, one of which should be vaccinia (animal models using vaccinia might provide information relevant to drug development for treatment of vaccine complications,[25] in addition to serving as part of the range of viruses used in exploration of potential applicability to smallpox treatment). The sponsor should discuss results from such studies with the FDA. These discussions also should include evaluation of the current status of various animal models at the time that drug development is ongoing. Based on data from initial studies and availability of suitably characterized models, the next step may be to assess the appropriateness of additional study in an animal model using variola (this step would require CDC collaboration; see section III.B., Interactions Among Industry, Academic, and Government Sponsors).

B. Selection and Development of Animal Models

We encourage using existing animal models to provide preliminary information on drug activity, as well as further development of models that resemble as closely as possible the pathophysiology and clinical manifestations of human smallpox. Detailed evaluation of the natural history of disease in the model and submission of data supporting such evaluation is important for selection of models and design of treatment studies. Because of the limitations of current understanding of human smallpox, sponsors should present the rationale for comparability of their proposed models based on available information about human smallpox, and also should present any information they can add regarding human smallpox (e.g., from written sources or pathology archives; see sections II., Background, and V., Clinical Data).

When considering the further development and characterization of animal models, it might be useful to study host and pathogen combinations including orthopoxviruses that are naturally virulent in the animal host species proposed as a model (e.g., ectromelia infection in mice or rabbitpox in rabbits) to explore the pathophysiological mechanism of toxicity in those models. Useful information also might arise from models that may have been more extensively developed using aggressive viral challenges to ensure reproducible serious disease manifestations, such as cowpox and vaccinia respiratory infection of mice, monkeypox respiratory exposure of nonhuman primates, and infection of immunocompromised animals (which also might have relevance for exploring the possible applicability of animal study results to human special populations likely to be considered for treatment). The sponsor should address the rationale for the route of administration in proposals for model development.

[25] See note 9, supra.

Characterization and use of small animal models with a variety of nonvariola orthopoxviruses and challenge regimens can be especially important as an opportunity to explore the effects of a wide range of drug doses, dosing regimens, and treatment times relative to viral exposure and evolution of disease; differences in viral strain, inoculum, and route of exposure; and other variables. Results of such studies might help both in estimating the possible effect of these variations and in setting priorities for the use of resources (such as nonhuman primates and more pathogenic viruses) that are less readily available or more difficult to work with. We recommend that selection and assessment of nonhuman primate models receive careful consideration in later stages of animal investigations after initial results become available from small animal models. Assessing for similarity of pathologic mechanism and immune response (e.g., the mechanism of virus dissemination throughout the body, virus interactions with the immune system, and the pathologic process that leads to mortality) across different animal species using different orthopoxviruses and different doses and routes of virus inoculation might facilitate the determination of the pathophysiological mechanisms of various orthopoxviruses (Buller and Palumbo 1991; Smith and Kotwal 2002) and facilitate further development of animal models.

C. General Considerations in Study Design

The design of studies using animal models of orthopoxvirus infections should draw upon general principles of human clinical trial design as well as past experience with characterization of animal models and performance of nonclinical natural history and exposure-response studies. Protocols should include detailed clinical observations and laboratory studies in the animals, similar to clinical and laboratory monitoring that might be performed in human clinical trials in drug development programs for other types of serious illnesses. The purpose of such observations is to provide as much information as feasible about the relevance of the animal studies both for design of subsequent human clinical trials and for supplemental information to enhance the interpretability of sparse human clinical data.

In addition to the primary endpoints of mortality or major morbidity, sponsors are encouraged to identify as many secondary endpoints as possible that are associated with or predictive of outcome in the models under development. Other important considerations in refining animal studies include using a range of drug treatment doses, durations, and start times, including treatment started both before and after infection and symptomatology have become clinically established. Investigators should provide evidence that any drug target found is not unique to the virus or animal being studied, but is also applicable to variola and humans. Blinding of observers to treatment assignment may be of greater importance than in standard nonclinical studies.

D. Drug-Vaccine and Drug-Drug Interactions

We recommend that animal models be used to explore the potential effect the drug might have on vaccine efficacy, because vaccination would likely be a predominant part of the response to control any re-emergence of smallpox. Sponsors can propose and discuss the design of studies in which their candidate drug and an effective vaccine would be administered separately or together

for pre-exposure or postexposure protection in a viral challenge animal model to compare the effects of separate and combination administration. Where appropriate, review of such study proposals and results can involve consultative collaboration between reviewers in different parts of the FDA responsible for review of the different products. Separate and combined effects of a candidate drug and passive immunotherapy should similarly be explored where appropriate. Any suspected drug-drug interactions that may have been noted in in vitro studies can be further studied in animal models as well. The sponsor should obtain viral load measurements and virus susceptibilities during animal studies to assess pharmacokinetic and pharmacodynamic relationships and correlates of outcome and to assist in the determination of the emergence of drug resistance. This information also can contribute to the assessment of combinations of antiviral drugs that may be beneficial if drug resistance develops with monotherapy. If there are suitable viral strains identified as resistant to other antiviral drugs or vaccines, the sponsor should also address the potential appropriateness of animal studies using such strains.

E. Sequence and Uses of Studies in Animal Models

The sponsor should discuss initial animal data and plans for further animal studies with the FDA to facilitate priority setting and identification of additional studies that can be useful and feasible. The initial focus should be on accumulation of sufficient in vivo evidence of activity, together with human safety data from early IND studies or from other uses of the candidate drug, to support the development of a protocol that will be available for investigational use if a smallpox emergency were to occur. These discussions can include consideration of studies that may be warranted to support risk-benefit assessment and dosing strategies. If preliminary data are sufficiently promising, the sponsor should present to the FDA for discussion an outline of issues to be considered in moving toward the possibility of submitting an application. In some instances, it may be preferable to pursue approval for other indications that can actually be studied in humans, with refinement of plans for investigational use if a smallpox emergency arises. In other instances, initial discussions might suggest that a sufficient aggregate body of evidence can be assembled to warrant consideration of approval under 21 CFR part 314, subpart I (the Animal Rule)[26] if well-characterized animal models predictive of human treatment responses can be developed. Consideration under 21 CFR part 314, subpart I is limited to drugs used to treat serious or life-threatening conditions that meet the following criteria:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product

- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans

- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity

[26] See note 10, supra.

- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans

If a sponsor believes that a candidate drug can be developed toward potential approval using these criteria, the sponsor should present its rationale to the review division and should also provide supporting data and a proposed development approach, so that identification and design of an appropriate base of studies can be discussed prospectively in pre-IND or early IND interactions and can be suitably revised as initial results become available. Sponsors also should discuss with the FDA any anticipated difficulties in complying with the requirements of the good laboratory practices regulations (21 CFR part 58) where applicable, so that consultation can be provided on how to address these difficulties. If there is a situation in which animal studies are designed and agreed upon as the principal component of efficacy studies for approval, and if results of such studies are then found to be sufficient to support approval under Subpart I, then clinical trials are required to be conducted after such an approval if and when they are feasible as field trials (e.g., after an accidental or hostile exposure), and suitable protocols should be submitted for review during the drug development process. Safety evaluation is not covered under Subpart I but should be conducted under pre-existing requirements for development of new drugs and, therefore, should include appropriate human data.

V. CLINICAL DATA

The approach to acquiring clinical data in drug development for smallpox depends on the unique characteristics of the situation and the intended uses of the drug, as well as on previously established principles of drug assessment. The sponsor should discuss with the FDA during pre-IND communications and early IND processes the prospects for obtaining initial human data and the plans for later stages of development if initial findings are sufficiently promising. We recommend that discussions address generation of initial human safety data, use of the drug for nonsmallpox purposes, and in selected cases, development of plans for use of an investigational drug, such as under an IND or EUA as appropriate to the development stage and the extent of the emergency (see section III., Regulatory Approach Regarding Early Drug Development), if a smallpox event were to occur. We also recommend that the sponsor consider any additional support it can provide for such a clinical approach through examination of human data from existing records of the smallpox era that might contribute to elucidation of the poorly understood pathophysiology of human smallpox. In addition, the sponsor should present plans for study of drug-drug interactions (see section III.G., Clinical Pharmacology). Evaluation of the advisability of, and strategies for, developing clinical protocols typically should involve interdisciplinary assessment of a broad range of initial nonclinical data including in vitro and in vivo studies with nonvariola orthopoxviruses (see section III., Regulatory Approach Regarding Early Drug Development).

Expectations for human data to support investigational use of a drug in the event of a smallpox emergency might differ substantially according to the proposed circumstances of its use. For example, both the likelihood of benefit and the degree of acceptable risk might be different for treatment of established serious illness, for postexposure prophylaxis by persons who have been

exposed to smallpox (before they develop illness or at the first signs of incipient illness) in the hope of preventing or attenuating disease, or for prophylactic use before and throughout a period of exposure risk if vaccine is not available or is believed to be ineffective. Risk-benefit evaluations thus might vary substantially in each of these situations and should be discussed on a case-by-case basis.

If a candidate drug has not been previously studied in humans but has an acceptable risk profile based on nonclinical studies, the initial human protocol for pre-IND discussion and for review in the initial IND submission typically is a phase 1 safety study in healthy volunteers (see sections III.E., Nonclinical Toxicology, and III.G., Clinical Pharmacology). For a candidate drug with greater toxicity (based on nonclinical or any available clinical data), typical phase 1 studies in healthy volunteers may not be appropriate. If despite this greater toxicity a satisfactory risk-benefit balance can be estimated in an existing patient population that might benefit from the treatment (in contrast to healthy volunteers), it may be appropriate to perform early studies in that patient population. Because of the challenges of designing such a drug development program and the greater toxicity of drugs developed using this approach, sponsors are strongly encouraged to take advantage of the opportunity for early consultation with the FDA regarding the design of these studies. This consultation also can include discussion of whether a single IND can be appropriate for the intended range of studies or whether, in some instances, more than one IND may be more suitable. Selection of and supporting data for appropriate dosing, population, and timing of initial human studies can be important to address in later stages of pre-IND consultations. There also might be agents with promising activity data for which, because of greater toxicity or lack of alternative potential uses, it may not be possible to identify a population appropriate for clinical studies to characterize the pharmacokinetics (and also safety) of the candidate drug. In such circumstances, we suggest sponsors discuss potential approaches to drug development with the FDA.

Under some circumstances, development under the IND should include preparation of a clinical protocol that could be available for use if a bioterrorism-associated release of variola were to occur and lead to consideration of using the drug under an IND. Submission and review of such a protocol and its supporting information also can contribute to efficient consideration of a drug for EUA status if warranted. Protocol development for such situations should proceed on a case-by-case basis, taking into consideration such particulars as in vitro and animal activity data and safety data. We recommend that pre-IND discussions be held to address the type of information that should be obtained to justify development of a protocol. The sponsor should provide separate protocols to address the use of the candidate drug for treatment or for prophylaxis if initial discussion suggests these different uses might be appropriate on the basis of preliminary data.

Additional important information regarding the safety and efficacy of the candidate drug can come from studies investigating its use for other indications (see section III.C., Drugs with Previous or Concurrent Studies for Other Indications). If the initial data suggest that the drug can be useful for a nonsmallpox indication, in some instances it can be appropriate to pursue development for such an alternative indication and in the process assemble supporting information for investigational use in the event of a smallpox emergency. In addition to any other indications with previous or concomitant studies, we recommend that investigators seek

out other viral illnesses in humans in which the drug can be appropriately studied. Results of these studies might further contribute to evidence of drug safety and efficacy in the illness studied, and also simultaneously provide ancillary supporting information for smallpox studies. An example of this would be study of the drug for other poxvirus infections such as molluscum contagiosum, vaccinia, or monkeypox. It is particularly important to identify settings in which controlled trials can be conducted appropriately. The IND protocol previously mentioned for use in a smallpox outbreak can be further refined after gathering information from the use of a similar protocol during an outbreak caused by a related virus (see section III., Regulatory Approach Regarding Early Drug Development). We encourage early interaction with the review division to discuss the relevance of any such studies for potential use in variola.

A. Safety Data

The amount and type of safety data available at the time of the initial IND submission depends upon the candidate drug's development history. Candidate drugs that have been developed for other indications may have human safety data available, whereas other candidate drugs may have only nonclinical data available at the time of the initial IND submission. Safety data that have already been acquired during the development of a candidate drug for other indications can help to expedite the development process (see section III.C., Drugs with Previous or Concurrent Studies for Other Indications).

If the candidate drug does not have human safety data from studies in other diseases, the sponsor should propose plans for acquiring initial safety data for discussion through initial studies under the IND. Typically, the initial study can be a single-dose phase 1 study in healthy volunteers. The actual first study in humans will depend on what is known about the candidate drug from nonclinical studies and any available clinical data. An additional noteworthy consideration regarding the candidate drug's safety profile is that the side effect profile can depend in part upon the underlying clinical condition of the study subjects. For example, the side effect profile in patients who are acutely and severely ill may more accurately reflect that of a smallpox-infected individual than a study in healthy volunteers. Efforts to characterize the safety profile as it would be in the target population should be considered and discussed with the FDA.

If a candidate drug were to be used emergently in the setting of a smallpox event, it is likely that persons exposed to the drug also would be recent vaccinees or candidates for vaccination. Historically, there have been expert opinions that replication of vaccinia virus at the vaccination site may be important to development of optimal immunity, especially when vaccination is offered after smallpox exposure (Dixon 1962). Interference with the immunization response is a potentially serious concern, particularly if the candidate drug is under consideration for use to prevent disease rather than only for treating established illness. Therefore, it is important that the sponsor evaluate for the potential and degree of effect the drug may have on vaccine efficacy. We recommend that this potential interaction be addressed to the extent feasible in animal studies before IND submission, and the possibility of further investigations be discussed early in the IND process as appropriate. Depending on review of available information, in some instances such additional investigations can include a human immunogenicity study, similar to those used in evaluating new vaccines, to assess the effect of the antiviral on the immunologic

responses of vaccinated volunteers as well as on viral shedding, with collaborative and consultative review as appropriate from FDA staff in both CDER and CBER.

The amount and type of safety data preferred as support for a protocol for use in humans in an emergency setting depends on the risk-benefit profile of the candidate drug in the context of its potential uses (e.g., for prophylaxis or treatment). If smallpox were to develop in an unvaccinated individual, it might be appropriate to allow the use of a drug with significant toxicity if the drug appears promising for treatment of established variola major smallpox illness, which historically has a high fatality rate. However, the same level of toxicity might be inappropriate for postexposure prophylaxis in a vaccinated individual or an individual with no contraindications to vaccinia vaccination. Therefore, for clinical trial proposals, the use for which the drug is being considered, and the potential toxicity profile (see section III.E., Nonclinical Toxicology) of the drug should be clearly described in the protocol.

Data collection during the clinical use of the drug is crucial, and can help to identify previously unrecognized safety issues relating to the investigational drug. We recommend including a case report form (CRF) as part of a protocol for a specific proposed use that facilitates the collection of safety data. The CRF should provide a tool for efficiently capturing complete safety data, and include specific provisions for ascertaining manifestations of any toxicity the drug may have demonstrated in vitro or in animal studies. The sponsor should provide separate CRFs to address different proposed uses of the candidate drug (e.g., use for treatment versus prophylaxis). We recommend that long-term follow-up also be included, as appropriate, to look for delayed outcomes such as genetic or reproductive toxicity. Some patients with variola infection could receive medications that interact with the candidate drug. Therefore, we also recommend that concomitant medication use be recorded on the CRF. An example of the type of data that should be collected includes, but is not limited to:

- Demographics (patient age, sex, race, and ethnicity)
- History of smallpox vaccination and description of whether there was an adequate take (skin response to the vaccine)
- Patient's past medical history
- Physical examinations
- Serum laboratory tests (e.g., hematology panel, chemistry profile, renal and liver function tests)
- Other therapies specific for smallpox that have been used before or concomitant with the study drug, and outcome
- General supportive therapies that can affect outcome (e.g., fluid replacement)
- Other medications taken concomitantly for unrelated conditions
- Adverse events (including severity, suspected drug relationship, treatment and response)
- Ultimate outcome (principal and subsidiary clinical and laboratory assessments)

Characterization of the metabolic profile for the candidate drug and the potential for drug interactions is important to the evaluation of the safety profile and management of potential risks associated with the candidate drug. Types of studies to address these issues are referenced in section III.G., Clinical Pharmacology.

As more safety data are acquired, the risk-benefit assessment associated with a specific candidate drug can change over time. Therefore, the sponsor should provide for ongoing reassessment through a system such as a data and safety monitoring board during the administration of a protocol. Collaborations between sponsors and public health agencies are encouraged to facilitate optimal ascertainment and use of clinical experiences if a protocol were to be used in an emergency situation (see section III.B., Interactions Among Industry, Academic, and Government Sponsors).

B. Efficacy Data

During the planning stages of smallpox drug development, and during discussion and development of studies of in vitro and in vivo activity and of human safety, we recommend that sponsors also begin to consider how they will prepare to assess efficacy if a human smallpox outbreak were to occur. There are numerous reasons to design protocols for maximal capture of efficacy data as well as safety data, despite the constraints on study design and conduct that can be inherent in such a situation. Advance consideration of the range of possible actions in response to any smallpox event can facilitate both emergency readiness and effective data collection. Data collection will be important for the direct benefit of patients in an emergency situation to guide informed decisions about the continuation or modification of treatment interventions. If a candidate drug were used under IND in such a setting, collection of efficacy data also will be important to support revisions of ongoing protocols and informed development of future protocols, as well as to satisfy requirements for any contemplated application for approval. In addition, even if a drug goes through the development process to the point of approval under 21 CFR part 314, subpart I, Subpart I requires that clinical trials be performed if circumstances arise in which they would be feasible.

The scenarios in which health care professionals or public health officials might consider use of a candidate drug could range across a spectrum of possibilities including, but not limited to, the following:

- A high-mortality mass casualty situation in which vaccine is unavailable or believed ineffective
- A release with substantial initial mortality, after which vaccination and containment measures appear effective in limiting spread
- A limited release with few cases and/or a strain of unexpectedly lower virulence
- A situation in which treatment or prophylaxis may be started on the basis of a preliminary diagnosis that turns out to be inaccurate

It may be unclear, at the time of a decision to activate a prepared protocol, where on this spectrum an outbreak might eventually fall. Factors such as those listed could strongly affect not only the feasibility of systematic data collection and strength of any conclusions drawn from the data, but also the acceptability of risks (including drug toxicity or lack of efficacy) associated with the protocol, effect of perceived risks on drug acceptance by patients, availability of

supportive interventions or any other specific therapies under development at the time, and ability to implement other intervention and control measures such as quarantine and ring vaccination. We recommend that each protocol take into account the range of potential uses as the candidate drug begins to be studied.[27]

We suggest that sponsors consider the possible designs of a protocol to be available for use in the event of an emergency, taking into account both the likely constraints on data collection and the limited interpretability of uncontrolled data. Additional considerations on the approach to clinical studies can be based on published FDA guidance.[28] We also suggest that the range of proposed drug uses, and the effect of each use on appropriate study design, be taken into account. Placebo-controlled trials are unlikely to find acceptance for treatment of established serious smallpox illness unless the candidate drug has safety concerns that are thought to be as important as the preliminary evidence suggesting potential benefit. However, mortality has varied so much among historical outbreaks that comparisons to historical data might well be misleading.

If a proposed drug is shown to have a human safety profile and animal activity results that suggest potential use for prophylaxis or pre-emptive treatment, we recommend that the study design take into account the primary role of vaccine. Even if there are preliminary animal data exploring drug and vaccine interactions, many uncertainties about the uses of drug and vaccine together in an outbreak situation can remain. For example, even a small inhibitory effect of drug on vaccinia virus might be cause for concern if a maximal immunologic stimulus were needed to provide protection by postexposure vaccination; on the other hand, if an outbreak were to occur with a viral strain against which the vaccine was suspected to protect poorly or not at all, adjunctive drug therapy might assume increased importance (although drug effects against such a strain also can be unpredictable). If other drugs have reached similar stages of development, the design of a candidate drug protocol should consider possible comparisons between treatments or combinations of treatments. Comparison of different dosing regimens also should be considered if supported by available risk-benefit information. The sponsor should include all of these issues in IND discussions if development reaches a stage at which development of a protocol for investigational clinical use or use under an EUA appears appropriate.

A key component in the collection of quality data can be a pre-existing protocol that can be rapidly activated in a post-terrorism event setting. We recommend that the protocol include a system for data collection that incorporates appropriate forms to facilitate thorough data

[27] For example, potential smallpox scenarios are most often discussed using the hypothesis of a massive epidemic that could rapidly overwhelm efforts at data collection. Even in this setting, availability of a protocol might be useful to remind health care professionals of dose adjustments and basic safety follow-up that can contribute directly to patient management. The other possible scenarios include the possibility of a few cases receiving intensive medical management, or an unexpectedly mild disease form in which occasional severe drug toxicities have a major effect on future attitudes toward treatment and control measures (the 1976 experience with H1N1 swine influenza vaccination provides a relevant example of the difficulty in predicting evolution of a potential public health threat). Availability of adequate data collection systems can be important to balanced assessment of ongoing and future interventions in the event of a smallpox outbreak anywhere on the spectrum of potential extent and severity.

[28] See the guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products*.

collection. Some of the types of data that should be collected are outlined in section V.A., Safety Data. The design of data forms and data collection systems should take into account the range of circumstances in which they might be used, as previously outlined; the use of electronic rather than paper-based technologies might facilitate collection, quality assurance, and/or analysis of such data.

Study design should allow patients to receive fully situation-appropriate supportive care. Depending on the circumstances of an outbreak, normal medical care processes might generate many of the desired data elements.[29] CRF design should take into account the potential spectrum of supportive care and, where appropriate, provide for selected information to be transcribed after the fact from medical records if such provisions might facilitate the most efficient use of emergency resources.

We recommend that data collection plans provide for appropriate clinical samples that can be useful in evaluating activity of the candidate drug. These samples can include viral load measurements and virus susceptibilities, to assess for activity in vivo and for the emergence of drug resistance. In addition to characterizing the frequency and rapidity of resistance emergence, such information can contribute to identifying combinations of antiviral drugs that might be beneficial if viral resistance occurs using single drug treatment.

Ideally, protocols with strategies to maximize accuracy and completeness of variola drug efficacy data collection should be prepared in advance, as it would be important to have them available if a smallpox event were to occur, not only to assess the outcomes associated with use of an investigational drug but also to facilitate disease assessment, treatment, and monitoring. Clinical and public health expert authorities might recommend standardized patient evaluation and management strategies in an emergency situation. Therefore, sponsors should consider the possibilities for such recommendations and their implications for patient care as well as data collection. Advance discussions between potential sponsors and public health officials can be useful to design investigational protocols and methods for case ascertainment and enrollment for candidate drugs that might be used in such a situation (see section III.B., Interactions Among Industry, Academic, and Government Sponsors).

Because mortality and major morbidity are the greatest concerns when the possibility of a smallpox threat is considered, these outcomes will be the measurements most readily associated with direct demonstration of clinical benefit, and, therefore, should be the most appropriate endpoints in any study of a candidate treatment in the event of a smallpox outbreak. As with the

[29] In the past, supportive care for smallpox patients could be both intensive and extensive, including interventions related to nutrition, hydration, fluid and electrolyte balance, pain control, skin care, attempt to prevent or treat superinfections (both superficial and systemic), and psychosocial support. The potential effects of technological advances in these areas and others such as renal and respiratory support, plus possible attempts to counter the immune modifying properties of the virus, are unknown; experience with severe acute respiratory syndrome shows that supportive modalities continue to have a prominent role, together with outbreak control measures, even when health care workers are confronted with a contagious and potentially fatal disease with no specific treatment or vaccine available. If serious smallpox cases were to occur in a setting permitting intensive management, it is likely that frequent clinical and laboratory assessments would be documented in the hospital record, and drug study protocols and procedures should be designed to reflect appropriate provisions for collection and utilization of such information.

use of animal models, sponsors should try to identify clinical correlates that might be studied to assess whether they are associated with or predictive of clinical outcome. If alternative or surrogate endpoints can be identified that are reasonably likely to predict benefit, we recommend that the possibility of using such markers in clinical trials, if this proves feasible, be discussed with the review division (21 CFR 314.510). A range of secondary endpoints (e.g., skin lesion progression and scarring, measurements of viral burden, duration of illness, and specific organ system involvement) also may be appropriate to assess, depending on the circumstances in which studies might be carried out.[30]

For some drugs, it may be appropriate to discuss preliminary information available from human infections with poxviruses from other genera such as molluscum contagiosum or orf, although applicability of this information to orthopoxviruses cannot be assumed. We recommend that studies involving treatment of human vaccinia complications,[31] or any studies of other human orthopoxvirus infections such as monkeypox, also be considered as potentially contributory. Information available from studies for other indications also may contribute useful supplemental information to overall evaluations of the candidate drug (see section III.C., Drugs with Previous or Concurrent Studies for Other Indications).

VI. SUMMARY

Development of drugs to treat or prevent infection by variola virus presents many challenges that are not common in standard drug development. Sponsors should pursue pre-IND and early IND interactions with the review division to discuss the role of nonvariola orthopoxviruses, animal models, and other aspects of the drug development plan. If drug development progresses to a stage warranting development of a clinical protocol that can be available if a smallpox emergency were to occur, the sponsor should plan for accurate and thorough data collection.

[30] The relationship of an alternative or surrogate endpoint to the primary outcome of interest cannot be assumed a priori (see Fleming and DeMets 1996). One illustrative example is the known historical differences in mortality between variola major and variola minor despite clinically similar nature and extent of skin lesions. Therefore, defining such relationships should be an important element in data collection and analysis.

[31] See note 9, supra.

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