**DATE:** November 21, 2011

**TO:** FDA Antiviral Drugs Advisory Committee Members/Guests

**FROM:** Division of Antiviral Products (DAVP) Smallpox Review Team

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**TOPIC:** Approaches to Antiviral Drug Development for Treatment of Human Smallpox.

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DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the Advisory Committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought scientific issues and challenges in the development of antiviral products for the treatment of human smallpox disease under the Animal Rule to this Advisory Committee in order to gain the Committee's insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the Advisory Committee. The FDA will not issue a final determination on the issues at hand until input from the Advisory Committee process has been considered. The final determination may be affected by issues not discussed at the Advisory Committee meeting.

## 1.0 Introduction

The purpose of this document is to provide the Antiviral Drugs Advisory Committee with a summary of DAVP's perspective regarding the key scientific issues and challenges in the development of antiviral products for the treatment of human smallpox disease under the Animal Rule. This document also includes a brief summary of some of the regulatory considerations under the Animal Rule, and illustrates some of the scientific challenges involved in designing animal studies to extrapolate the efficacy of an investigational agent using surrogate elements in study design proposals, including but not limited to, surrogate animal hosts, surrogate orthopoxviruses (for studies not using variola virus), and surrogate endpoints (for studies not designed to show an effect on mortality).

The overall goal of the meeting is to obtain advice from the Committee on approaches to antiviral drug development for treatment of human smallpox should an outbreak occur. Because naturally occurring smallpox has been eradicated and it would be unethical to expose humans to any of the remaining smallpox virus, animal models are an important consideration in these drug development programs. However, there are many concerns regarding the adequacy, applicability and reproducibility of the proposed animal models for orthopoxvirus infection.

Our comments for discussion in this document are provided in the context of the unmet medical need for antiviral product(s) for the treatment of human smallpox if an outbreak were to occur.

### 1.1 Role of Advisory Committee Discussion

The objectives of the Advisory Committee meeting are to discuss key scientific issues and challenges in the development of antiviral products for the treatment of human smallpox disease under the Animal Rule, and to obtain input from the Advisory Committee on the best potential paths forward for developing smallpox drugs. Please see section 1.2 for specific questions for the Committee, and section 6 for discussion of the key issues.

Because of the importance of the topic and its unique challenges, we have previously discussed issues in drug development for the treatment of smallpox at an FDA public workshop[i] and have also published a draft Guidance document for public comment.[ii] The intention at this time is to seek advice from the Antiviral Drugs Advisory Committee, including individuals with expertise in a wide variety of medical and public health issues,

[i] http://www.fda.gov/Drugs/NewsEvents/ucm169065.htm

[ii] Guidance for Industry: Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention (2007). available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071179.pdf

on scientific issues that are inherent in the area of drug development for treatment of smallpox. The focus of this discussion is on treatment of established smallpox illness that might occur in humans if there were to be a future hostile re-introduction of variola virus into the human population. Input from these discussions will be considered by the Agency together with other input from variously specialized sources.

### 1.2 Draft Topics for Advisory Committee Discussion

The following draft topics for discussion by the Advisory Committee are presented in this section for ease of reference:

1. Appropriate animal model(s) for extrapolating efficacy of a drug to human smallpox

2. Appropriate primary endpoint for drug efficacy studies in animal models of orthopoxvirus infection

3. Study design issues

4. Role of Human Data from related naturally occurring diseases

## 2. Background

### 2.1 Historical and Clinical Aspects of Smallpox in Humans

#### Smallpox

The history of smallpox is that of an unparalleled source of death, disfigurement, and discomfort. Historic responses to smallpox stimulated historic and far-reaching advances in the development of immunizations and other public health interventions, and culminated in the unprecedented eradication of a major infectious disease as a naturally occurring human pathogen through intensive surveillance, containment, and immunization efforts. In recent years, as concerns arose about the potential for use of possible unacknowledged stocks of stored smallpox virus (variola) as an agent of bioterrorism or biowarfare, a distinct set of challenges also arose with respect to evaluation of possible therapies for a disease that has not been seen for over thirty years and that we all hope will never occur again. What do we know about smallpox from its historic occurrences, from attempts at preventive and therapeutic measures prior to its eradication, and from other diseases, that might assist in the present assessment of new treatments proposed to be available for use if a smallpox outbreak were to occur in the future? And what level of confidence and/or of uncertainty would this historic knowledge support, in making a determination of whether a new therapy can be considered effective for its proposed uses? Review of a few relevant aspects of the available information about smallpox disease may help to set the stage for such inquiries.

The following highlights were selected to illustrate some of the questions that might arise from both scientific and regulatory perspectives.

**Clinical picture and clinical classification of smallpox cases**

The conventional historical picture of smallpox is that of a human-to-human communicable disease[1] characterized by an asymptomatic incubation period (averaging close to two weeks but with substantial variability[2]), an initial period of nonspecific symptoms lasting a few days (fever, headache, back pain, prostration), then evolution of skin manifestations followed by death or by gradual recovery with various degrees of scarring. Skin findings could vary from diffuse purpura, to the most distinctively characteristic picture of centrifugally-distributed macule-to-papule-to-vesicle-to-pustule-to-scab-to-scar evolution (with initial stages of a day or two each, scab evolution and separation over a period of a few weeks, and scarring perhaps evaluable over a few months' time), to a few lesions with less characteristic distribution and morphology or no recognizable lesions at all[3]. Within the spectrum of the focally evolving smallpox lesions, differences were also seen in overall appearance, texture, and rapidity and completeness of progression. Mucosal involvement could be extensive and severely limit nutrition and hydration during the acute illness; both direct involvement and secondary superinfection of the respiratory tract were reported in some cases; other organ system involvement and bacterial superinfections were variably described; encephalitis was an occasional severe complication and delirium was common although some of the sickest patients were reported to retain clear consciousness throughout. In addition to prominent facial scarring, more occasional sequelae in survivors included blindness and skeletal deformities[4]. Experts in the management and control of smallpox tended to classify smallpox cases using a combination of descriptors including number and density of skin lesions, nature and evolution of skin lesions, and clinical assessment of the patient's toxic appearance ("toxemia") and severity of illness.

Generally speaking, within a given smallpox outbreak multiple clinical types and subtypes would appear. Most of the clinical descriptions are based on variola major, the more serious form that was also more prevalent (at least more widely recognized) throughout most of the history of the disease, and that is also the focus of concerns regarding potential biothreat uses of variola virus. A virologically distinguishable variant, variola minor (also called alastrim[5]), was recognized during the last few centuries as a much less lethal disease with extensive clinical overlap: although most variola minor patients fell within the less serious clinical categories of smallpox, the rash could be extensive, occasional patients died, and the physician examining an individual patient could not distinguish between variola major and variola minor without knowledge of the epidemiologic context. Except where otherwise specified, the terms "smallpox" and "variola" in this background document should be understood as referring to variola major.

Because the classifications of smallpox illness were considered to have prognostic significance but were not necessarily apparent at the time of clinical presentation or disease recognition,[6] and were not defined uniformly by different sources, illustrating a

few of the different classification schemata may be useful. Historically, the number and density of skin lesions might have served as a convenient form of classification, but many experts' assessments suggested that more complex classification systems were more relevant to their areas of concern in clinical management or epidemiology and control. For example, a 1962 textbook on smallpox listed nine clinical types; a 1972 monograph listed four principal varieties each containing two to four subtypes for a total of twelve categories; and the 1988 World Health Organization history of the smallpox eradication program listed five types of which two were subdivided into two or three subtypes for a total of eight possible categories.

Table 1. Examples of clinical smallpox classifications[7]

| Ricketts 1908[8] | Dixon 1962[9] | Rao 1972[10] | Fenner 1988[11] |
|---|---|---|---|
| **Rash**<br>• Hemorrhagic<br>• Confluent<br>• Discrete<br><br>**Fever**<br>• Toxemic<br>• Eruptive<br>• Suppurative<br><br>Various combinations and modifications | 1 Fulminating<br>2 Malignant confluent<br>3 Malignant semi-confluent<br>4 Benign confluent<br>5 Benign semi-confluent<br>6 Discrete<br>7 Mild<br>8 Abortive<br>9 Variola sine eruptione | **Haemorrhagic**<br>• early<br>• late<br>**Flat**<br>• confluent<br>• semiconfluent<br>• discrete<br>**Ordinary**<br>• confluent<br>• semiconfluent<br>• discrete<br>**Modified**<br>• confluent<br>• semiconfluent<br>• discrete<br>• variola sine eruptione | **Ordinary**<br>• confluent<br>• semiconfluent<br>• discrete<br>**Modified**<br>**Variola sine eruptione**<br>**Flat**<br>**Haemorrhagic**<br>• early<br>• late |

## Outcomes of principal concern in smallpox treatment

The most feared consequence of smallpox historically was, of course, its propensity to cause death; and reduction in mortality was the principal objective of attempts to develop treatments for established illness during the pre-eradication era. Mortality in variola major is commonly cited as about 30% but was reported to vary widely among outbreaks,[12] from as little as 5% (compared to 2% or less in variola minor) to 40% or more. The most rapidly progressive forms were almost invariably lethal but did not account for most deaths in outbreak reports. For example, among 497 patients in North Africa, Dixon (1948)[13] reported 4 "purpura variolosa" (or "fulminating") cases all fatal, but 80 "malignant confluent" cases with 55 deaths, and 75 "malignant semiconfluent" cases with 18 deaths (even "benign semiconfluent", 43 cases with 5 deaths, accounted for more deaths than "purpura"). Among 3544 unvaccinated patients in Madras, Rao (1972) reported 85 "hemorrhagic" with 96% mortality and 236 "flat" cases with 96% mortality, but also 3147 "ordinary" cases with 30% mortality: again, when the "ordinary" cases were divided by lesion density, even "ordinary semiconfluent" accounted for several fold more deaths than "early hemorrhagic" and "late hemorrhagic" combined.

Facial scarring was a common and feared outcome in survivors. Blindness was commonly described as being less common than previously described,[14] such that an actual incidence is difficult to derive: malnutrition and trachoma were cited as potential contributing or determining factors. Discomfort and incapacitation could be very severe over a period of weeks. Disruption of personal and societal functioning could be further exacerbated by the requirement to remain in isolation until all scabs had separated.

## Predictors and determinants of smallpox outcomes

The pathogenesis, pathophysiology, determinants, and predictors of smallpox outcomes were topics of much investigation and debate in the pre-eradication era and in subsequent retrospective discussions related to potential countermeasure development.

-- *Source (viral strain and clinical type of source case)*
Grouping of viral strains into variola major and variola minor clearly was related to greater or lesser mortality at the epidemiologic level, although variola minor patients could exhibit dramatic rashes.[15] Distinctions between variola major strains were sought, but no definite relationship to mortality was proven.[16] Suspicions persisted that endemic strains in some areas were more virulent than others, but were difficult to confirm[17] especially given the potential for confounding by other factors such as socioeconomic conditions, levels of care available, and nutritional reserve. Within an outbreak, exposure to a specific clinical case type was not found to be a good predictor of contact case type. Contacts exposed to a hemorrhagic smallpox case could develop ordinary or mild smallpox; while contacts exposed to mild variola-inoculation cases could develop full-blown life-threatening smallpox.[18] Likelihood of transmission (as distinct from the type of case produced by that transmission) was believed to vary by case type but this was also controversial and could be confounded by characteristics other than viral shedding: for example, the sickest patients were likely to have fewer but closer contacts because of their prostration, immobility, and need for care; less severe cases might move about more extensively after symptoms and shedding began but before the disease was definitively recognized (which might not occur until some days of rash even when experienced observers were available); and patients with hemorrhagic or otherwise nonspecific rash might have additional delays in recognition and institution of precautions against spread.[19]

-- *Host factors*
Certain population groups were recognized as having increased risk of the most severe forms and fatal outcomes of smallpox: the most widely recognized risk factor was pregnancy.[20] Immunosuppression was mentioned as a risk factor but with less documentation; presence of antibody early in disease course was reported to have a relationship to disease severity, but not a straightforward one.[21] Extremes of age could affect the likelihood of adverse outcomes but interactions between age and vaccination status were difficult to analyze precisely.[22] Vaccination was widely recognized as having the potential to attenuate disease severity in those who became ill despite having been vaccinated at various time points in the past; however, the duration of partial or complete

protection was debated over time, and "modified" smallpox similar to that seen in vaccinated patients was also reported in unvaccinated patients, some authors emphasizing that "modified" had inappropriately been interpreted as "vaccine-modified" and should be evaluated strictly on clinical grounds without respect to availability of vaccine history.

-- *Route and size of inoculum*

Exposure to variola virus was most typically believed to take place through the upper respiratory tract (although no primary entry lesions could be identified) by exposure to virus shed from the upper respiratory tract of a patient with mucosal lesions in the early period of rash evolution. Percutaneous exposure, by deliberate variolation in the pre-vaccine era[23] or accidentally during patient care, could result in a primary local lesion with focal satellite lesions, fever and usually a much milder rash and systemic illness (though with occasional fatalities) than usual exposures: this observation was the basis of efforts to immunize against smallpox before discovery of less virulent cross-protecting vaccine viruses. Variolation to induce immunity was also practiced in some localities by nasal insufflation of stored ground-up smallpox scabs[24]: as this practice resulted in respiratory tract exposure, production of less severe disease than natural exposure has been hypothesized to result from inactivation during storage,[25] virus trapping in the scab matrix, and/or differences in inoculum size (which could not be measured).[26] The viral inoculum required to produce disease was a subject of speculation – as little as one virion was suggested, but acknowledged not to be based on direct evidence.[27] Outbreaks with cases attributable to very brief or distant indirect contact also suggested small inoculum sizes and potential for airborne as well as droplet transmission.[28] Attempts at quantitative assessments of shedding were made using inoculation of specimens into embryonated egg chorioallantoic membranes.[29] Exposures considered likely to be more intensive (such as exposure to patients for longer periods, in closer quarters, during periods of presumed peak viral shedding) did appear to increase the likelihood of infection but the relationship to severity of the contact case was not consistent.[30] No information is available regarding the likely initial clinical manifestations of a deliberate focused aerosol exposure as postulated in some biodefense scenarios[31]; however, subsequent generations of contact cases could potentially be similar to those seen in the pre-eradication era.

--*Characteristics of prodrome or pre-eruptive symptoms*

Symptoms were nonspecific in the few days at the end of the incubation period before focal rash appearance. Neither height of initial fever (which could be as high as 103-105º F across a wide variety of patients) nor severity and duration of pre-eruptive symptoms appears to have been reliably related to severity and outcome of disease.[32] Nonspecific skin findings could appear during this period, and could be mistaken for measles, drug eruptions, or other illnesses. Sometimes these rashes then developed into various types of smallpox rash; in other instances, apparently they resolved before onset of focal pox lesions. Fever patterns were described differently in different smallpox types but remission of fever at the end of the pre-eruptive period was common across disease classifications; subsequent "secondary fever" was often associated with evolution of skin lesions and "tertiary fever" with superimposed complications. Thus, prolonged or

recurrent fever after lesion appearance could be associated with poor outcome, but more as a marker of a complicated course than as a prognostic or predictive factor.

-- *Timing of lesion appearance*
Patients with the most rapid and fulminant forms of smallpox might die before any characteristic skin lesions appeared. In cases developing characteristic pox lesions, reportedly the first few lesions might not be noticed initially such that a precise time of onset could be difficult to determine, but then remaining lesions could appear quickly within a day or so,[33] possibly with a few additional lesions in subsequent days but without an extended series of "crops"– hence the observation that most lesions in one skin area would be synchronous, and the overall rash nearly synchronous, during the remainder of its evolution. In other instances, a nonspecific diffuse rash could reportedly become vesicular without countable initial lesions. Additional "cropping" or ongoing lesion development over several days, as well as more rapid development through early lesion stages, was reported as occurring in some very mild or "modified" cases[34] and hence not an indicator of greater severity. Conversely, delayed slowly-developing rash was reported in some "virulent, toxic (hemorrhagic), or malignant" cases.[35] The smallpox diagnosis might not be clear until some days after the first appearance of rash. Overall, by the time disease was clearly identifiable, it might be questionable whether lesion numbers (further discussed below) could better be considered an outcome or a baseline characteristic, as the preponderance of lesions might appear before diagnosis although they might then evolve over a period of days to weeks.

-- *Number of skin lesions*
Though lesion counts or scores were convenient for classifying, they were not considered to be the principal determinant of disease course and outcome. The prognostic significance of lesion number could be affected by viral source (variola minor patients with numerous lesions could be much less ill than variola major patients with fewer lesions); lesion morphology and evolution ("flat semiconfluent" or even "flat discrete" was reported as more fatal than "ordinary confluent")[36]; toxic course ("malignant semiconfluent" was reported as more fatal than "benign confluent")[37]; and vaccination status (patients with vaccination scar reportedly had much lower case fatality rate for the same extent and intensity of rash compared to those without a vaccination scar)[38].

-- *Measurements of viral burden*
Many attempts were made to measure virus in the time period between introduction of the first virologic methods and the achievement of smallpox eradication. Typically, as was often noted, cases were rare where the most advanced virologic methods were available, and laboratory resources were rudimentary or absent in geographic areas with the greatest smallpox burden. However, clinicians and investigators made great attempts to better understand the location and replication of the virus. Its behavior during the incubation period was largely unknown in humans[39] and hypothesized on the basis of animal models with variable resemblance to smallpox; however, given the presence of species-specific molecular mimics and inhibitors of host immunologic processes in variola (and with variations in other poxviruses[40]), the incubation period might have been a virologically and immunologically active phase with major implications for the

subsequent course of disease[41]. Throat cultures sometimes yielded virus in asymptomatic contacts – who did not necessarily go on to develop disease.[42] Throat cultures could often be positive during active disease, especially in the period of rash development when mucous membrane lesions were evident, though the relation to illness severity might vary.[43]

Viremia was assumed to occur soon after infection and again at the beginning of symptomatic illness, based largely on extrapolation from animal studies with other viruses.[44] Virus could sometimes be detected in blood, but most patients developing characteristic smallpox rash no longer had detectible viremia by the time of rash appearance. Patients with the most fulminant disease had more virus detectible in blood, but investigators were uncertain whether the high viremia was the pathogenetic factor or was a marker for host failure to control the infection. In some of the rare instances in which serial samples were assayed, diminishing titers over time were reported even in fatal cases.[45] Early antibody response was reported to be related both to amount of circulating virus and to disease evolution and outcome, though the relationship was not uniform across published reports. Detection of soluble viral antigen was also reported, and considered by some authors to be a worse prognostic factor than isolation of virus.

Substantial variability in circulating virus was reported, including virus detected in blood after several days of illness in patients who recovered,[46] and one report of virus isolation from blood 38 days after illness onset and 14-15 days after shedding of scabs.[47] Virus was abundant in scabs from skin lesions of recovering patients, but was considered to be tightly bound in the scab material and expert opinions differed as to whether it had any clinical or epidemiologic importance at that point, beyond the nuisance of keeping patients in isolation until all scabs separated; the quantity of virus shed in scabs was reported not to correlate with the stage or severity of disease.[48] Overall, while there were strong suggestions that high titers of circulating virus at the time of clinical presentation could be a poor prognostic factor, information on viral burden is insufficient to determine timing or magnitude of effects over time on any of these measurements that might be reliably predictive of differences in clinical outcome.[49]

-- *Pathophysiology of terminal events*

Although mortality was the most important outcome of smallpox, events leading to death were not well understood, and were often described as heterogeneous.[50] Patients might be described as toxic or toxemic but no toxin was identified. Specific pathologic findings were principally cutaneous but were not considered to adequately explain fatality in many cases.[51] Skin lesions appeared variably related to prognosis but most patients were not believed to have died from the skin lesions themselves. There was substantial agreement that the pathogen directly invaded and caused lesions in the skin and some areas of mucous membrane; and that multiple organ systems could display abnormalities whose relationship to the primary pathogen was less clear, with relative contributions to disease outcome varying in different patients and possibly in different geographic and temporal settings.[52] Explanations proposed for smallpox deaths included heart failure, pulmonary edema, general "toxemia," or effects of circulating immune complexes containing antibody bound to soluble antigen (although early antibody formation was considered

favorable).[53] In more recent years appearance of toxemia and sepsis has been interpreted as possible cytokine storm[54]; on the other hand, a modern-era pathology review has suggested that direct cytopathic effect of the virus on vital organs was more important to mortality than was appreciated by experts in the pre-eradication era[55]. Some patients were reported to die from respiratory compromise, including upper respiratory obstruction from laryngeal and tracheal involvement as well as pneumonia; fluid and electrolyte imbalances were postulated but usually not precisely measured. Progression of rash could produce a degree of skin damage and vulnerability to complications such as fluid imbalance and superinfection comparable to burn injury; as noted above, it was not clear why some patients' skin lesions evolved to this level of severity while others with large numbers of lesions escaped similar complications. Some deaths were attributed to "exhaustion." Severe thrombocytopenia and clotting abnormalities including prolonged prothrombin time (as well as markedly abnormal blood smears, sometimes confused with leukemia) were reported in patients diagnosed with hemorrhagic smallpox, and clinically important hemorrhage was believed to contribute to death in some cases, although patients with non-hemorrhagic forms of the disease could also have thrombocytopenia.[56] Occasionally encephalitis or encephalopathy was reported as contributing to fatality. Bacterial superinfections were reported, and were considered common by some authors, but their incidence and importance continue to be debated.[57]

-- *Pathophysiology of scarring*

Smallpox scarring was variable in both nature and severity, and relationship to severity of the initial disease appears to have been complex. For example, the rare patients who survived high-mortality "flat" smallpox were noted to have much more superficial scars than those who survived "ordinary" smallpox and developed the well-known deeply pitted scarring.[58] This latter form of scarring was also reported not to be a simple function of the extent of initial skin lesions (for example, scarring was more prominent on the face than the arms even after abundant arm lesions), and long-term appearance was not necessarily obvious[59] at the time the patient was clinically recovering from the initial disease (when the most prominent feature might be pigmentary disturbances that would gradually resolve, while the pitted scars developed more gradually). Presence and number of scars was reported to vary with age and vaccination status.[60] Variola minor was reportedly associated with fewer and/or shallower, less persistent scars.[61] Varicella was reported as occasionally causing scars indistinguishable from those of smallpox though fewer in number.[62] Scarring was variously attributed to dermal involvement, bacterial superinfection,[63] or collapse of the sebaceous glands,[64] with the latter theory gaining ground toward the end of the pre-eradication era[65] (with the qualification that bacterial superinfection was still considered to have the capacity to worsen scarring).

### 2.1.1 Therapeutic attempts in the pre-eradication era

Multiple attempts at both symptomatic/supportive and specific therapy were made throughout history. Supportive care in various settings included analgesics, skin hygiene, and attempts at fluid, nutrition, and electrolyte support.[66] Transfusions, oxygen, and digitalis were used in some of the twentieth-century treatment attempts. Corticosteroids

and/or ACTH were also mentioned anecdotally.[67] Because of the historical observations of bacterial invasion, as antibacterial drugs became available they were used in attempts to improve smallpox outcomes.[68] Generally, results were not considered encouraging and the effect on case-fatality rates and on scarring were considered to be small. Systematic randomized controlled trials were lacking. Some experts did report at least small effects on outcomes and continued to use antibacterials routinely.[69] Antibacterial agents used in treatment of smallpox included sulfa drugs, penicillin, chloramphenical, streptomycin, and tetracyclines. Convalescent serum was also tried though less widely.[70]

The era of smallpox eradication coincided with the infancy of antiviral therapies. Numerous attempts were made to develop and study antivirals for treatment of smallpox, starting as early as the mid-20th century.[71] Entry of compounds into clinical trials was generally based on limited data on viral susceptibility in the laboratory, either in chick embryo cultures or in early cell culture experiments, together with limited animal data, using rough measures of activity against variola in suckling mice, or against related viruses such as vaccinia that could more readily be studied in animals. Given the long-established role of vaccination in pre-exposure and to some extent in post-exposure protection, there were hopes that antivirals could fill an important gap by improving outcome in those who nevertheless developed smallpox illness. However, no antiviral was established as filling this role. The following table gives several examples of antiviral drugs that were studied in the treatment setting in the pre-eradication era.

Table 2. Examples of antiviral supporting information and human clinical treatment trial reports[72]

| Drug | Examples/highlights of data supporting clinical treatment trial | Examples/highlights of clinical treatment trial results |
|---|---|---|
| N-methylisatin-thiosemicarbazone (Marboran)[73] | Reported activity against vaccinia in cell culture and variola in infant mice (not against monkeypox in monkeys)<br>Variable reports of protective effect as post-exposure prophylaxis of human smallpox contacts<br>Variable reports of effect on vaccine response in humans | Controlled trial, 208 active, 215 placebo (excluded hemorrhagic cases and those already scabbing)<br>o overall mortality 21.6% active, 18.6% placebo<br>o in vaccinated, 3.6% and 6.5%<br>o unvaccinated, 33.3% and 28% |
| Ara-A (adenine arabinoside)[74] | Reported protection or rescue of vaccinia-infected mice, suppression of vaccinia and Shope fibroma virus in rabbits | Randomized clinical trial in human smallpox<br>o 4/11 placebo, 5/9 active died<br>o Mean fever duration in survivors 7.7 and 7.3 days<br>o Mean days to scabbing confounded by treatment onset imbalance<br>o No clear-cut difference in viral shedding detected |
| M&B7714 (a thiosemicarbazone, same drug class as methisazone/Marboran)[75] | Reported mortality decrease rabbitpox/rabbits, variola/baby mice, "mice infected intracerebrally with neurovaccinia"<br>o Dose-response, late single dose partly protective (3 d delay; median control survival 4.8 d), survivors protected against | Treatment trial<br>o Mortality 140/601 (23.3%) placebo, 155/692 (22.4%) active<br>o Authors concluded "for all practical purposes…compound has no role"<br>Household prophylaxis<br>o Smallpox incidence 29.9% placebo (CFR 20%), 20.4% active (17.5% |

| | | |
|---|---|---|
| | virus | CFR)<br>o Earlier intervention had lower attack rate in both groups (vaccination program concurrent; no effect on take rate reported)<br>o Authors concluded "Because of the toxic effects…and relatively small reduction of case incidence…not recommended in the routine prophylaxis of smallpox" |
| Ara-C (cytosine arabinoside)[76] | Reported variably active in cell culture, equivocal in mice | Case series<br>o 9 smallpox patients<br>o Reported lower mortality, rapid reduction in severity<br>o Compared treated cases to untreated patients in same locality and to family members<br>2 Controlled trials<br>o 9-10 active, 9-11 placebo<br>o One trial reported "no important differences" on multiple measures of disease course despite preliminary in vitro susceptibility; other reported increased mortality (9/9 vs. 4/11) on ara-C |
| 6-azauridine[77] | Reported to inhibit variola replication on chorioallantoic membrane and to have effects on vaccinia virus in cell culture, mice, and rabbits | One controlled trial (67 per treatment arm) reported reduced mortality in "ordinary," 100% mortality in hemorrhagic or flat variety; stated "Further trials are warranted with this drug and they are in progress." |
| CG662 ("urea derivative of diphenyl sulphone")[78] | "some antiviral activity against vaccinia" | One controlled trial, 341 active, 343 placebo, case fatality 15.8% active and 18.1% control |

### 2.1.2 Comparisons to other diseases and interventions

The concepts addressed under this heading are intended to place available information about smallpox in the context of other diseases encountered in clinical practice and also in the context of other current concerns regarding chemical and biological threat agents.

--*Diseases that could be confused with smallpox*
During the pre-eradication era, the differential diagnosis of smallpox was addressed differently according to both stage of disease and clinical category. Symptoms prior to rash appearance were nonspecific and could be confused with a wide variety of febrile illnesses (or "influenza-like illnesses"). In addition to symptoms such as fever, chills, headache, and back pain, some patients had prominent nausea and vomiting, and some had abdominal pain that could be confused with surgical acute abdomen.[79] After skin manifestations appeared, fulminant or hemorrhagic cases could be confused with sepsis syndromes such as meningococcemia, or other disorders such as acute leukemia that could present with fever and bleeding diathesis:[80] several outbreaks in countries where

smallpox was no longer endemic were attributed to such missed diagnoses of individual cases. Disease with papulovesiculopustular lesion progression could be confused with chickenpox, sometimes even by experienced experts, despite the distinctions (centrifugal versus centripetal rash distribution, synchronous versus heterogeneous rash evolution) taught to generations of medical students: a number of suspected smallpox cases in the post-eradication period were subsequently attributed to varicella.[81] Intrinsically milder forms of smallpox itself, variola minor cases could have rashes indistinguishable from variola major at the individual-case level, with variola minor outbreaks identifiable only by knowledge of the epidemiologic setting (with very low mortality rate) and virologic assessment. Another recurrent source of confusion was the possibility of confounding of diagnosis by intercurrent effects of medical interventions: for example, drug rash and fever secondary to treatment of the initial symptoms, or vaccinia skin lesions and fever as responses to vaccination during the incubation period[82].

During the smallpox eradication campaign, it became evident that another orthopoxvirus caused skin lesions similar to common forms of variola infection in some parts of Africa. These illnesses were ascribed to human infection with monkeypox (discussed further in next section). In this setting also, skin lesions could not be distinguished from smallpox lesions during individual case examination, but clinical presentation often included prominent lymphadenopathy not generally encountered in smallpox.[83] At the community level, typically the mortality and transmissibility were lower than for variola and the more serious or fulminant presentations associated with smallpox were not reported.

--*Diseases virologically related to smallpox*

The various species of orthopoxviruses had long been identified as having substantial cross-immunogenicity and to some extent similar propensity for causing pock-like skin lesions, but different host ranges and different pathogenicity for their respective hosts. Variola, ectromelia (mousepox), and camelpox, for example, ranged from very to extremely host-specific. Cowpox, monkeypox, and vaccinia (including rabbitpox and buffalopox) appeared capable of infecting and transmitting in a wide range of mammalian hosts, and the tendency to name them after the first recognized host was typically acknowledged to be misleading as more information became available. Genomic similarity was not a good predictor of either host range or pathogenicity; and genomic variations between species have been considered to involve a number of genes related to host range and virulence factors such as immunomodulation and immunomimicry, but have not led to direct predictions of disease relationships.[84]. For example, within the orthopoxvirus genus monkeypox appeared to have gene sequences more distant from variola than some vaccinia and cowpox strains but caused systemic disease in contrast to the typically localized lesions of vaccinia and cowpox; camelpox appeared closer to variola than any of these, but has rarely been reported to cause any human disease and the few case reports have involved localized lesions rather than generalized smallpox-type presentations.[85] Several species of poxviruses outside the orthopoxvirus genus (such as several zoonotic parapoxviruses and human-specific molluscum contagiosum) could also cause skin lesions but without the same potential for diagnostic confusion.[86] Chickenpox, of course, was eventually recognized not to be a poxvirus at all, but differentiation of the diseases was reported as a recent phenomenon even into the twentieth century.

Variola appeared to be extremely human-specific. Other animals were not noted to acquire disease naturally except for occasional transmission to nonhuman primates (either deliberately in the laboratory or occasionally as a "curiosity" due to contact exposure), which generally produced much milder disease than in humans (again, with the exception of a few great-ape case reports, or under the influence of immunosuppressants or very massive inocula), and did not appear to contribute to natural circulation of the disease.[87] In addition to the potential for experimental infection of monkeys and chick embryos, variola virus could be made to replicate in suckling mice and in rabbit skin, but did not cause characteristic disease in these hosts.[88] Indeed, this host-specificity was considered critical to smallpox eradication.

Monkeypox in humans has been difficult to assess in detail because of its sporadic appearance principally in geographic areas with few clinical, scientific, and infrastructural resources.[89] The virus has appeared to infect not only humans and monkeys but also a variety of small mammals considered more likely to be the natural reservoir, unlike variola which was determined to have no nonhuman reservoir for persistence based on extensive investigations during the smallpox eradication program. Descriptions of clinical and pathologic details were more recent but sparser than for smallpox. Major similarities to smallpox included papulovesiculopustular skin lesions and fever; from the initially recognized outbreaks, little histologic information was available but one report describing a skin biopsy noted similarities to smallpox and tanapox lesions.[90]. Differences included striking lymphadenopathy, which might suggest differences in host-pathogen interactions; typically lower mortality and transmissibility (with most cases considered attributable to zoonotic exposure rather than human-to-human contact); and eventually, division into clades of differing pathogenicity after a US outbreak[91] characterized by relatively mild disease associated with exposure to prairie dogs infected by rodents imported from Africa. Encephalitis, corneal involvement, gastrointestinal and respiratory symptoms have been reported; given the smaller numbers of cases assessed, different historical times of most assessments, incompleteness of ascertainment, and evidence of clade differences, direct comparison of complication frequencies between monkeypox and variola is uncertain. In keeping with the typical cross-antigenicity of orthopoxviruses, smallpox vaccine has been considered to have some protective effect against monkeypox illness (though not absolute or permanent). Diagnostic confusion with chickenpox, and sometimes concomitant circulation of monkeypox and varicella viruses in the same populations, has added to the challenges of case ascertainment.[92] Recent research has reported increases in identification of human monkeypox cases since the cessation of smallpox vaccination programs[93]; ascertainment of actual incidence, spectrum of clinical and epidemiologic characteristics, and need and potential for intervention has posed major challenges in the resource-poor setting of most recognized illness[94]. Like all other orthopoxviruses studied in any detail, monkeypox has been reported to encode a number of proteins expected to have immunomodulatory effects on the host in addition to host range factors and virulence factors: differences between monkeypox and variola have been reported in these regions of the genome.[95]

Cowpox (with a broader host range than many orthopoxviruses, one of the largest genomes, and enough within-species diversity to prompt suggestions that it should be considered as several species[96]) continues to be sporadically reported in humans, though recent cases are more likely to be associated with cats or rats than with cows, and rodents are believed to be the most likely natural reservoir.[97] Several European case reports have been associated with pet rats. Clinical presentation might not always be typical pox-like lesions, but has sometimes been associated with focal necrotic skin lesions and potential uncertainty in diagnosis and management[98].

The original source of vaccinia virus, and the date at which it replaced cowpox as the usual protectant against smallpox, are both unknown. However, strains of vaccinia and closely related viruses appear to be capable of infecting a wide variety of small and large animals; and natural transmission of such strains, some of which are believed to be possibly escaped vaccine strains, has been reported as a public health problem among domestic livestock and humans in several geographic regions.[99]

--*Comparisons of smallpox vaccine and antiviral evidence base*
Although both are of ongoing interest for preparedness if any future smallpox event should occur, the history and evidence base for smallpox vaccines and antiviral therapies differ in many respects. Use of orthopoxviruses for protective immunity against one another – starting with variola itself, then cowpox, and subsequently several different strains of vaccinia in different vaccine preparations – has a centuries-long history that must be considered successful, and is supported both by compelling evidence of benefit in many different human populations with different vaccine preparations and by numerous animal investigations showing results similar to available human data, despite the lack of randomized placebo-controlled trials in humans. Minor virologic differences between vaccines were not found to compromise outbreak control, although some differences in adverse events were reported. Currently licensed vaccine is derived from and very closely related to vaccine used during smallpox eradication activities, and can be used as a comparator in study of newer candidate vaccines which are also related (though more distantly) to existing and previously used products. The distinctive skin reaction called a "take" was accepted as a surrogate indicator of a protective immunologic response to smallpox vaccine and has been used in vaccine approval decisions; even for novel vaccines that do not produce a "take," other immunologic markers are available that are less well established but can make some potential contribution to assessment of vaccine response in human volunteers to be considered together with animal data, as well as providing a positive control in animal studies.[100]

In contrast, attempts to develop antivirals for treatment of established smallpox illness met with scant success in the pre-eradication era even when initial laboratory and animal data were believed to be promising. No established effective comparator is available to help in design and interpretation of antiviral drug studies. No biomarker comparable to vaccine "take" or even to more exploratory immunologic measurements is available for antiviral drug assessment. In addition, antiviral drugs for therapy of established disease would be used at a very different disease stage from vaccines, in much sicker patients with well-established complex host-pathogen interactions and complications in progress;

and orthopoxviruses, like other pathogens, have the capacity to develop resistance mutations that could arise much more quickly and give rise to much higher-level resistance than has been reported for vaccines, both because of high levels of viral replication and because of low genetic barriers to small-molecule drug resistance.

--*Comparisons of anthrax PEP and smallpox antiviral evidence base*
Drug development for treatment of smallpox also differs in many respects from the most prominent example of antimicrobial development for a bacterial biothreat, the approval of two fluoroquinolones for post-exposure prophylaxis of inhalational anthrax using a nonhuman primate model together with other sources of supporting information relevant to both efficacy and safety.[101] For the anthrax PEP review processes, extensive human efficacy and safety information were available from use of the same and similar drugs for a wide range of serious bacterial infections with many points of resemblance to inhalational anthrax, and the products under consideration had already been found effective and approved for a wide range of bacterial infections. Extensive information was also available regarding the toxins mediating clinical manifestations of anthrax illness, the latent period before germination of inhaled spores and production of toxins, and the clinical and pathological resemblance between humans and monkeys exposed to the same bacterial species. Existing products with anthrax indications could be studied in the animal model in parallel with the newer products, and documented to have effects consistent with expectations. Relationships between antimicrobial pharmacokinetics and human disease outcomes in bacterial infections were also understood better than for most antiviral interventions. This extent of supporting information has not been available for any product proposed for antiviral development targeting treatment of smallpox.

--*Comparisons of chemical protectant/antidote and smallpox antiviral evidence base*
Animal model data have contributed to approval of products for potential chemical threats. In contrast to many infectious diseases and especially smallpox, chemical intoxications are understood to be mediated by mechanisms defined at a molecular level and comparable across many species with respect to the specific molecular mechanism and its consequences. In common with anthrax studies but in contrast to the majority of smallpox-related studies, the same toxic substance has been used in animal studies of chemical agents as the toxic substance for which the human use indication is intended, and that substance is similarly toxic for humans and experimental animals. Typically for such approvals some relevant human experience has been available as supporting information: even if less in quantity or more indirect in relevance compared to the anthrax PEP approvals, this experience has generally been more informative than human use information currently available for antivirals targeting smallpox.[102]

## 3.0 Animal Models of Orthopoxvirus Infection

### 3.1 Overview

The Animal Rule requires that benefit be demonstrated in more than one animal species expected to react with a response predictive of humans, unless demonstrated in one species that represents a sufficiently well-characterized animal model for predicting response in humans exposed to the toxic substance (agent). The toxic substance is variola virus for smallpox.

Key elements of animal models of smallpox are the virus, the host animal, route of viral inoculation and size of the viral inoculum. Table 3 shows some orthopoxvirus animal models. This list is not meant to be exhaustive but rather to identify models that have been used in some ongoing development programs. The following discussion of animal models does not describe any of these models in detail but rather highlights challenges and areas of concern. Specific details of models relevant to each sponsor will be addressed by the sponsors. Relating the disease course in the various animal models to humans is especially challenging and clinical aspects of these challenges are discussed elsewhere in this document.

Table 3. Orthopoxvirus Animal Models

**MICE**

| Animal | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 [a] | MTD [b] |
|---|---|---|---|---|---|---|---|
| A/NCr, C57Bl/6, SKH-1 | Ectromelia | Moscow | aerosol, footpad (ID), intranasal | 0.6-10,000 pfu | mortality | <1 pfu in some strains | 6-12 days |
| A/NCr | Ectromelia | ECTV-11KM-IL-4 | intranasal | 20-600 pfu | mortality | | |
| BALB/c | Vaccinia | WR, IHD | intranasal | 10,000-30,000 pfu | mortality | 2000 pfu | 6-8 days |
| NMRI | Vaccinia | | intravenous: tail vein | 4,000 pfu | lesions at Day 8 | n/a | n/a |
| BALB/c | Cowpox | Brighton | intranasal | 33,000 pfu | mortality | 2000 pfu | 8-10 days |
| dormice | Monkeypox | Zaire 79/CDC V79-I-005 | intranasal | 100-1,000 pfu | mortality | 12 pfu | 7-12 days |

**IMMUNODEFICIENT MICE**

| Animal | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 | MTD |
|---|---|---|---|---|---|---|---|
| nude mice (7 weeks old) | Vaccinia | WR | intranasal | $10^3$-$10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 7-13 days |
| SCID mice (7 weeks old) | Vaccinia | WR | intranasal | $10^3$-$10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 7-14 days |
| BALB/c CD4+ T cell depleted (5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |
| BALB/c CD8+ T cell depleted (5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |
| $J_H$ (mature B cell deficient, 5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $J_H$ CD4+ T cell depleted (5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |
| $J_H$ CD8+ T cell depleted (5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |
| $J_H$ CD4+ and CD8+ T cell depleted (5-7 weeks old) | Vaccinia | WR | intranasal | 2.5 x $10^5$ pfu | mortality | 2.5 x $10^4$ pfu | 6-8 days |
| athymic (*nu*/*nu*) | Vaccinia | Lister | intradermal: lumbosacral scarification | 500,000 pfu | lesion score on Day 22 | n/a | n/a |
| STAT-1 knockout | Monkeypox | Zaire 79/CDC V79-I-005 | intranasal | 100-1,000 pfu | mortality | 47 ♂/213♀ | 7-12 days |
| CAST/EiJ (5-6 week old ♀, also MOLF/EiJ and PERA/EiJ) | Monkeypox | Zaire 79/CDC V79-CB2 | intranasal | $10^2$ pfu, $10^3$ pfu, $10^4$ pfu, $10^5$ pfu, and $10^6$ pfu | mortality | 680 pfu | 5-7 days at >= $10^4$ pfu |
| CAST/EiJ (5-6 week old ♀, also MOLF/EiJ and PERA/EiJ) | Monkeypox | Zaire 79/CDC V79-CB2 | intraperitoneal | 1 pfu, 10 pfu, 33 pfu, 100 pfu, 1000 pfu | mortality | 14 pfu | 8-10 days |

## RABBITS

| Animal | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 | MTD |
|---|---|---|---|---|---|---|---|
| New Zealand White | Rabbitpox | Ultrecht | intradermal | 200-1,000 pfu (intradermal) | mortality | 36 pfu | 7 days |
| New Zealand White | Rabbitpox | Ultrecht | aerosol | 200-1,000 pfu (intradermal) | mortality | | |

## GROUND SQUIRRELS

| | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 | MTD |
|---|---|---|---|---|---|---|---|
| | Monkeypox | Zaire 79/CDC V79-I-005 | intranasal | 100 pfu | mortality | 0.5 pfu | 6-9 days |
| | Monkeypox | Zaire 79/CDC V79-I-005 | intraperitoneally | 100 pfu | mortality | 0.5 pfu | 6-9 days |
| | Monkeypox | Zaire 79/CDC V79-I-005 | subcutaneously | 100 pfu | mortality | 0.5 pfu | 6-9 days |

## PRAIRIE DOGS

| | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 | MTD |
|---|---|---|---|---|---|---|---|
| | Monkeypox | MPX-2003-ROC-358 | intranasal | 3.8 x $10^5$ pfu | mortality | 3,200 pfu | 11 days |

## MONKEYS

| Animal | Virus | Strain/Isolate | Inoculum Route | Inoculum Size | Endpoint | Estimated LD50 | MTD |
|---|---|---|---|---|---|---|---|
| Cynomolgus macaque | Monkeypox | Zaire 79/CDC V79-I-005 | intravenous | 5 x $10^7$ pfu | mortality | $10^6$-$10^7$ genomes | 9-14 days |
| Cynomolgus macaque | Monkeypox | Zaire 79/CDC V79-I-005 | intratracheally | ? | mortality | $10^6$-$10^7$ genomes | 10-14 days |
| Cynomolgus macaque | Variola | Harper | intravenous | $10^8$ pfu | mortality | $10^8$-$10^9$ pfu (inconsistent) | 4-11 days (33% mortality at $10^8$ pfu, highly variable) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cynomolgus macaque | Monkeypox | Zaire 79/CDC V79-I-005 | aerosol (microsprayer/ bronchoscope) | $3.4 \times 10^6$ pfu, $8.4 \times 10^6$ pfu, $3.5 \times 10^7$ pfu | mortality | | 8-16 days |
| Cynomolgus macaque | Monkeypox | Zaire 79/CDC V79-I-005 | aerosol (nebulizer) | $4 \times 10^4$ pfu, $1 \times 10^5$ pfu, $4 \times 10^5$ pfu, $1 \times 10^6$ pfu | mortality | | 8-11 days |
| Cynomolgus macaque | Monkeypox | ?Zaire 79/CDC V79-I-005 | aerosol (nebulizer) | $2.5 \times 10^4$ pfu, $1.3 \times 10^5$ pfu, $1.9 \times 10^5$ pfu, $3 \times 10^6$ pfu | mortality, lesion counts, viremia | | 8-12 days |
| Cynomolgus macaque | Monkeypox | Zaire 79/CDC V79-I-005 | intranasal | $4 \times 10^7$ pfu, $1.2 \times 10^8$ pfu, $4 \times 10^8$ pfu | mortality? | | |
| Cynomolgus macaque | Cowpox | Brighton Red | intravenous | $5 \times 10^5$ pfu, $5 \times 10^6$ pfu, $5 \times 10^7$ pfu | mortality | ? | 5-12 days |
| Marmosets | Calpox (cowpox) | | intranasal | $5 \times 10^2$ pfu, $8.3 \times 10^2$, $8.3 \times 10^3$ | mortality | $8.3 \times 10^2$ pfu | 12-15 days at 10 $MID_{50}$ |
| Marmosets | Calpox (cowpox) | | intravenous | $1\text{-}1.25 \times 10^7$ pfu | mortality | ? | 4-7 days |
| Marmosets | Calpox (cowpox) | | oropharyngeal | $1 \times 10^7$ pfu | mortality | ? | |
| Marmosets | Monkeypox | ?Zaire 79/CDC V79-I-005 | intravenous | 48 pfu, 510 pfu, $5 \times 10^3$ pfu, $7.8 \times 10^4$ pfu, and $9.5 \times 10^5$ pfu | mortality | ? | 6-15 days |

[a] inoculum which kills 50% of animals
[b] mean time to death
pfu = plaque forming unit

The lack of a non-human reservoir was critical to the eradication of variola virus. However, this creates a challenge in developing a sufficiently well-characterized animal model shown that reacts with a response predictive of humans. While a large body of work has been completed with the above animal models (Table 3), it is challenging to attempt to correlate findings from these models that use a surrogate virus in a surrogate host, as well as high viral inocula administered via routes (e.g. intradermal, footpad, intravenous, intraperitoneal, intrathecal, lumbosacral scarification) that differ from natural respiratory route of infection. Most of the animal model data to date fit this scenario, or at best, improve on 1 of these 4 elements (i.e. virus, host, viral inoculation route, viral inoculum size).

### 3.2 Endpoints in Animal Model Studies of Orthopoxvirus Infections

Mortality is considered the most relevant endpoint for smallpox and many animal studies have used "mortality" as an endpoint. To minimize pain and suffering of animals, specific criteria are used to determine when an animal should be euthanized; and mortality is thus based on these criteria. Acute weight loss (a rapid loss of > 10% body weight) is a common indicator of moribund condition in rodents and is often used for euthanasia decisions in murine models. Similarly, acute weight loss of > 10% or > 15% has been used in rabbit models with rabbitpox. In non-human primate studies of smallpox and monkeypox, persistent prostration for ≥ 4 hours and either unresponsiveness to gentle prodding or rectal temperature < 34° C have been utilized as euthanasia criteria. Some studies have included pox skin lesion count and viral load as euthanasia criteria, and these parameters have also been proposed as endpoints in some

studies. However, these are not validated endpoints and in some cases have been inconsistent with mortality or disease progression.

Many studies have evaluated survival shortly after the mean time to death of controls and there is a concern that a drug may only delay death rather than increase survival. The Division has recommended that mortality be assessed in key studies at 4-5 times the mean time to death of controls, that viral clearance be assessed with a sensitive PCR assay, and that some survivors be rechallenged with a lethal inoculum to determine whether protective immunity has been established. The time of mortality assessment has been based upon adding 1-2 times the mean time to death to the time of death of outlier animals (see for example Table 3 in Jahrling *et al.*, 2004[103]. Additionally, this time of assessment would have captured the mortality when a candidate drug delayed the time of death (Grosenbach *et al.*, 2010)[104].

Many of the studies that report increased survival have initiated dosing prior to the onset of clinical manifestations of established illness; and these study designs are more relevant to a prophylaxis (or pre-emptive therapy) indication. Evaluation of efficacy for a treatment indication would preferably be done at time-point(s) after the onset of symptoms, rather than when these first appear.

### 3.3 Orthopoxviruses Used in Animal Models

Variola virus, the causative agent of smallpox, can only be used at a site in Russia and at the Centers for Disease Control and Prevention (CDC) in Atlanta. This requirement limits the size, timing and number of studies. Additionally, studies with variola virus require approval by the World Health Organization (WHO). For these reasons, FDA recommended in the draft smallpox drug development guidance document (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071179.pdf) that initial studies be conducted with several orthopoxviruses. This proposal was not intended to identify surrogate viruses to be used for approval (see lines 629-31), but to identify candidate agents with broad activity that would indicate targeting of conserved elements in orthopoxviruses and increase the likelihood that an agent has activity against variola virus.

Previous experience in antiviral drug development for other viruses indicates that variola virus may show differential susceptibility and durability (i.e. long-term suppression without development of resistance) to some candidate drugs compared to other orthopoxvirus and raises serious concerns about fulfillment of Animal Rule criteria using a related orthopoxvirus or viruses. These concerns are not surprising given that a single amino acid substitution frequently leads to resistance, and, it is not uncommon with approved antiviral drugs and with drugs in development to see differences in susceptibility and durability between closely related viruses. For example, non-nucleoside reverse transcriptase inhibitors approved for the treatment of HIV-1 do not have significant activity against HIV-2, pegylated interferon/ribavirin shows differential

activity against HCV genotypes, and some direct-acting antivirals for HCV not only show differential activity between subtypes 1a and 1b, but also within subtypes.

Another concern with using surrogate orthopoxviruses is that variola virus may have a different tissue tropism than a surrogate virus. Infection of mice by the footpad route results in different cell types infected for vaccinia virus (Norbury *et al.*, 2002)[105] compared to ectromelia virus (Mark Buller, personal communication). Macrophages in the popliteal lymph node are the dominant cell type infected by vaccinia; whereas B cells are the dominant type infected by ectromelia. Different tissue tropism of viruses could lead to false conclusions regarding efficacy if variola virus replicates in an essential tissue in which a surrogate virus does not replicate, and in which drug concentrations are not adequate to suppress variola replication.

### 3.4 Viral Strain

Smallpox outbreaks varied in mortality, with mortality ranging from approximately 5% to < 40% mortality for variola major, and generally less than 2% for variola minor. Recent animal studies with variola virus have employed the India 7124 and Harper strains (Jahrling *et al.*, 2004)[106]. The India 7124 strain was presumably chosen for these non-human primate studies as there was high mortality in this outbreak. Earlier studies employed the Harvey, Brazilian, Pakistani, and Utrecht strains (Noble, 1970[107]; Noble and Rich, 1969[108]). Interestingly, these studies indicated that the Harvey strain may be more virulent, as 10-100 fold lower inocula were used compared to other strains. The Division recommends the use of isolates from lethal human infections, as this provides some means of controlling for variability between isolates (which may impact virulence) from different individuals within the same outbreak. The specific source of and outcome of patients infected with the above isolates has not been reported.

Monkeypox virus has been used in many studies because of some clinical similarities of human monkeypox to smallpox (see clinical discussion). Its natural host is likely a rodent and not a primate, which may explain in part the lower mortality and poor communicability in humans in comparison to the variola virus. Many recent studies have employed the Zaire V79-I-005 monkeypox virus isolate obtained from a nonlethal infection in Central Africa (Breman *et al.*, 1980; case #38[109]). The DAVP considers the virulence of this isolate unclear. This Central African isolate, like variola virus, but unlike other Central African isolates, appears to have a defective gene encoding the IL-1β receptor homolog which is thought to compromise host immune function by blocking the proinflammatory cytokine IL-1β. The IL-1β receptor homolog was identified as a possible virulence factor based on comparisons of Central African monkeypox virus isolates to the less virulent Western African isolates (Likos *et al.*, 2005[110]). Consistent with the IL-1β receptor homolog compromising host immune function are observations with the approved recombinant human monoclonal antibody, canakinumab. This monoclonal antibody, which blocks IL-1β function, has been associated with an increased risk of serious infections, as have other medications designed to inhibit IL-1. As mentioned above, the DAVP recommends the use of isolates from lethal infections as this

provides some means of controlling for isolate to isolate variability that may impact virulence.

Rabbitpox virus was originally identified in a rabbit colony outbreak at Rockefeller University in 1933. The virus is closely related to (or considered a variant of) vaccinia. It is readily transmitted between rabbits by the airborne route. Current studies use the Utrecht strain which was also identified in a colony outbreak in the 1960s.

### 3.5 Viral Inoculum Size

One of the concerns with variola virus as an agent of bioterrorism is that one or only a few infectious units are thought to be necessary for infection of humans. However, to produce serious disease, animal models of smallpox using variola virus have typically needed to employ extremely high inocula, $10^8$ and $10^9$ plaque forming units (pfu) that have been administered intravenously. The need for a large inoculum may imply inefficient replication and may impact interpretation of results, as discussed below. Large inocula may be needed in a less susceptible host to overcome host immune functions which typically are suppressed by orthopoxviruses' genes in natural infections. Inefficient or dysfunctional viral gene products in heterologous hosts may explain the limited host range of some orthopoxviruses. There is a concern that inefficient compromising of host immune functions by the virus in a heterologous host may limit what can be learned about efficacy of a candidate drug as the contribution of host immune functions may help clear the virus. In addition to concerns about the efficiency at which host immune functions are compromised in a heterologous host, a large inoculum could effectively act as a large antigen bolus, stimulating antibody production to a level that helps clear virus in the presence of drug. In fact, the macaque inocula are similar to, or greater than that used in the human nonreplicating modified vaccinia Ankara vaccine inocula.

Other orthopoxviruses have been used in animal studies: ectromelia (mousepox virus; Moscow strain), vaccinia (smallpox vaccine virus), rabbitpox virus (Utrecht strain, related to vaccinia), cowpox virus, and monkeypox virus. Of these, ectromelia and rabbitpox viruses, like variola virus, lethally infect their hosts with a low viral inoculum. The other viruses for the most part have used large inocula to infect the animal host.

### 3.6 Animal Hosts

A major challenge in the development of animal models for smallpox is that humans are the only known permissive host for variola virus, the causative agent of smallpox. Attempts to identify animal hosts that can readily be infected with variola virus by the aerosol route, which has been hypothesized as a possible route of exposure in a terrorist release, have had limited success to date (Noble and Rich, 1969)[111]. Rodents (mice, rabbits, ground squirrels, and prairie dogs) are a logical choice for animal studies using other orthopoxviruses, as rodents are natural reservoirs of orthopoxviruses.

Mice are especially attractive because their size, availability, and cost effectiveness enable a relatively large number of animals to be studied permitting statistical significance to be obtained. They have been used to explore dose size, timing of dose initiation, dose frequency, and dose duration. Importantly, mice offer the only host where the immune system can be readily manipulated to assess the activity of agents in immunocompromised/immunodeficient animals (Grosenbach *et al.*, 2010)[112]. Mice have been infected with many different orthopoxviruses under many different scenarios with respect to route and size of inoculum (Table 3). However, with the exception of ectromelia virus (mousepox virus) or use of immunocompromised/immunodeficient animals, these studies have required large viral inocula. As mentioned above, the use of a large inoculum could, in theory, limit what can be learned about drug effect in some host-pathogen systems, although a combination of models with low inoculum and high host susceptibility, together with other models using very high inoculum sizes, has been suggested as potentially useful for exploratory purposes.

Another model of particular interest is rabbitpox. Rabbitpox can be induced in rabbits with a low viral inoculum via the subcutaneous route and by the inhalation route. In the inhalation model, uninfected animals are housed in adjoining cages with animals infected by the subcutaneous route. The clinical relationship of rabbitpox to smallpox is discussed elsewhere.

Rodent and lagomorph models can be useful for proof-of-concept, initial dose exploration and developing dosing regimens. However, differences in anatomy and physiology complicate comparisons of smallpox pathology with humans raising concerns about direct applicability of these models to humans. Non-human primate hosts provide greater similarity for comparisons of disease pathology and treatment success. Additionally, human and non-human primates have greater homology in some immune effector molecules and might therefore be more likely to have similar responses to poxvirus proteins that target these immune pathways. However, there are limitations in the number of animals that can be evaluated in non-human primate studies at the CDC for variola virus and at other BSL4 containment facilities for monkeypox virus, making it more difficult to obtain statistically significant efficacy data with non-human primates. The use of infection models with high mortality rates may enable demonstration of significance for an effective drug or drug combination. While it is has been proposed to use non-human primate smallpox models with mortality rates similar to the overall average reported in natural variola major infections, relevance of a single reported rate may be limited given the historical variability among outbreaks as well as the many unknowns about exposure and disease course if there were a bioterrorist release.

Cynomolgus macaques can be infected intravenously with variola virus using a very high inoculum ($10^8$ to $10^9$ pfu, Jahrling *et al.*, 2004)[113], producing infections with some characteristics similar to smallpox. A major concern about this model is inconsistent mortality. High viral inocula have been used to overcome the natural block to infection, the mechanism of which is unknown. The need for these high viral inocula may complicate interpretation of studies for reasons mentioned above with respect to host immune functions. Furthermore, it may prove difficult to determine by PCR assay

whether virus has been cleared. The absence of detectable viral genomes with a sensitive PCR assay would be an indication of viral clearance. However, the presence of low levels of viral nucleic acid would not necessarily indicate that replicating virus (viruses, by definition are detected by infectivity) is present. For example, an inoculum of $10^9$ pfu could represent $\geq 10^{10}$ genomes some of which could be trapped in tissues and slowly leached out ($10^{10}$ genomes in a macaque would represent ~$10^6$ genomes/mL of animal volume). Some of the macaque variola and monkeypox virus treatment studies have had detectable nucleic acid in surviving animals at the end of the study.

Similarly, cynomolgus macaques have been infected with very large inocula of monkeypox virus ($\geq 10^7$ pfu) by intravenous, intranasal, intratracheal, and aerosol routes producing disease with some similar characteristics to smallpox. However, many animals infected by intratracheal, intranasal and aerosol routes probably die as a result of bronchopneumonia, sometimes with a disease course that is much shorter than typical smallpox; and the extent of skin and other organ system involvement is not constant across exposure routes. These non-human primate models using monkeypox virus may be more clinically relevant to human monkeypox than human smallpox.

Studies with cynomolgus macaques have shown that these primates are not readily infected with orthopoxviruses and in the case of the current variola model, there are problems with reproducibility (i.e. mortality rates in placebo animals have not been reproducible). Recently, other possible hosts of interest have been identified. Marmosets, another non-human primate, can be lethally infected with relatively low inocula with some orthopoxviruses. Intravenous inoculation with as little as 500 pfu with calpox virus (a cowpox virus isolated from a non-human primate) results in a lethal infection (Kramski *et al.*, 2010[114]; Matz-Rensing *et al.*, 2011[115]). Similarly, lethal infections with monkeypox virus have been observed with an intravenous inoculum of 48 pfu (Mucker et al., personal communication) with incubation times similar to those observed in human smallpox. Additionally, the smaller size of marmosets relative to macaques permits studies with 2-3 times the number of animal subjects. Marmosets may also be safer in that they do not carry herpesvirus B, which is lethal to humans, and are less likely to injure lab workers. Additionally, a recent screen of 38 inbred mouse strains identified 3 strains that were susceptible to monkeypox virus at relatively low inocula via the intranasal ($LD_{50}$ value of 680 pfu) and intraperitoneal ($LD_{50}$ value of 14 pfu) routes (Americo *et al.*, 2010[116]). The susceptibility of marmosets and these mouse strains to variola virus is unknown but may be worth exploring.

### 3.7 Route of Virus Inoculation

The impact of routes of inoculation is not fully established for variola virus but data with other orthopoxviruses indicate that this may be important. The route of infection with ectromelia virus dramatically affects the pathophysiology of the disease, host response, and outcome (Mark Buller, personal communication). Intravenous inoculation of orthopoxviruses may affect the pathophysiology of disease in a number of ways (Mark Buller, personal communication), by shortening the incubation and prodromal phases, by circumventing the "stealth" activity of the large number of virus-encoded host evasion

molecules, by inactivating intracellular mature virus due to complement activation (Vanderplasschen *et al.*, 1998[117]; Moulton *et al.*, 2008[118]), by coating of virions with complement C3b leading to cellular uptake via the complement receptor type 2 expressed on antigen presenting cells and thereby accelerating the humoral response (Moulton *et al.*, 2008[119]; Nielsen *et al.*, 2000[120]), and by infecting a different spectrum of cell types. As mentioned above, monkeypox virus infection of cynomolgus macaques has been evaluated by the intravenous, intratracheal, intranasal and aerosol routes (Mark Challberg, personal communication). The virus is widely disseminated with some tissue specific differences based on route of infection seen in gross pathology, histopathology, and immunostaining.

## 4.0 Regulatory Considerations

Factors such as the species specificity of orthopoxviruses and the limited understanding of the pathophysiologic mechanisms of human smallpox disease make it challenging to develop drugs for treatment of smallpox based on the Animal Rule. However, recognizing the interest in use of the Animal Rule as a possible path to a labeled indication for treatment of smallpox, we have discussed issues related to this pathway below. Thus far, it is unclear whether any single animal model or models can reasonably predict a clinical response to therapy in humans in order to support an indication for treatment of smallpox.

The Division is very interested in facilitating paths forward to the extent that is feasible and scientifically justifiable. We are also mindful of the objections that have been raised to existing variola virus-based models of smallpox and the lack of compelling evidence that non-variola viruses in other models would reliably predict human smallpox outcomes. Therefore, there is value in having a comprehensive development plan for discussion and feedback as data acumulate, clearly delineating how the data accrued in such a program might support such an indication. There may also be additional developmental work that could be performed to further explore the biology of available animal models of infection for poxvirus disease, such as exploring an aerosolized or other route of inoculation (e.g. intra-tracheal or intranasal inoculation), different orthopoxviruses, different viral strains, or different animal species.

### 4.1 Issues with Orthopoxvirus Models of Infection

#### 4.1.1 Variola Virus Infection Models

Although the Animal Rule implies that an infectious pathogen can be considered a "toxic…substance" for purposes of developing products to defend against it (see Federal Register preamble, FR 2002(May 31);67:37988-98), the regulation does not address a situation in which development is proposed to be based entirely on "toxic substances" different from the pathogen for which an indication is sought. As discussed in Section 3.6, a number of problems with non-human primate (NHP)/variola models have been identified, including requirement for a large viral inoculum to cause serious disease, other

experiments showing mild disease with low mortality rates, and variable reproducibility of the NHP/variola models. The Division acknowledges the information gaps that exist based on data from currently available animal models. There are many uncertainties that still need to be addressed regarding any proposed animal model or combination of models, to support further discussion of whether a model or combination of models can be expected to predict efficacy of a product intended for treatment of smallpox. Some examples of these issues include the following.

Existing NHP/variola models typically require an extremely high inoculum by the intravenous (IV) route to cause discernable serious illness in NHPs. This likely reflects the much lower vulnerability of the non-human host to variola virus compared to humans, raising concerns about whether humans with smallpox might react more like immunocompromised animals than like otherwise healthy (and relatively disease-resistant) animals, whether humans would have higher risk of viral recrudescence after therapy and/or emergence of viral resistance, and whether modest inhibition of virus might improve animal survival because of host resilience which may not be translatable to humans. In addition, IV inoculation skips the initial phases of host-pathogen interaction with a virus thought to be highly immunomodulatory, raising further questions about how the pathophysiology of disease in this animal model might differ from the actual human disease state. Although these questions do not necessarily indicate that a different model (e.g. immunocompromised NHP model) would be more predictive, they serve to illustrate the uncertainties regarding attempts to understand complex host-pathogen interactions with a virus believed to have substantial capacity for immune modulation and immune mimicry.

### 4.1.2 Monkeypox Virus Infection Models

Whether and in what ways a surrogate orthopoxvirus in an animal model of infection could be used instead of, or in addition to, a variola virus model to fulfill Animal Rule criteria is an issue for discussion by the Advisory Committee.

With respect to monkeypox virus (MPXV) models, the existence of skin lesions similar to that observed with smallpox does not necessarily reflect similarity of the underlying disease process or mechanisms of mortality, which are not well defined for either disease. The prominence of lymphadenopathy in human monkeypox (compared to smallpox) and some differences in NHP disease reported with monkeypox and variola viruses suggest that there could be important differences in host-pathogen interactions. However, better markers of pathophysiology have not been defined to date

Among the many uncertainties regarding the range of possible NHP models are the lack of clear evidence regarding relevance of different routes of virus exposure, size of viral inoculum, extent of clinically apparent disease manifestations at the time of treatment initiation, duration of treatment relative to duration and course of clinical manifestations, and length of follow-up for disease recrudescence in survivors after treatment cessation. An additional area of uncertainty is the difficulty in extrapolation of results from a different orthopoxvirus in NHPs to treatment of smallpox infection in humans.

### 4.1.3 Rabbitpox Virus Infection Models

The rabbitpox/rabbit model has been proposed as a possible surrogate for pivotal animal studies because it appears to recapitulate some aspects of human smallpox, including viral dissemination and development of secondary skin lesions, as well as some degree of respiratory involvement, depending on the route of transmission. Rabbitpox is an orthopoxvirus closely related to vaccinia virus, which has been associated with outbreaks in rabbit colonies, and which can be transmitted by direct contact, by aerosol exposure, or by intradermal administration of the virus in rabbits. Relatively low inoculum sizes are required to establish lethal rabbitpox infection (Adams and Moyer, 2007)[121]. Whether this or other small animal models can be expected to predict efficacy of a product intended for treatment of smallpox is an issue for discussion by the Advisory Committee.

### 4.1.4 Ectromelia Virus Infection Models

The ectromelia virus/mouse model has been proposed as a possible surrogate for pivotal animal efficacy studies for reasons including the ability to have relatively large sample sizes, and to evaluate a mortality endpoint. The ectromelia virus is an orthopoxvirus associated with outbreaks in mouse colonies in laboratory settings, and is thought to be transmitted naturally among mice in the wild. Similarities and differences between mousepox infection in mice and smallpox in humans have been described by Parker, et al., 2010[122].

### 4.1.5 Dose Rationale

Under the Animal Rule, pharmacokinetic (PK) and pharmacodynamic (PD) information obtained in animals must be sufficient to allow selection of an effective dose regimen in humans. Sponsors should use their proposed animal models to provide detailed supportive data, including PK data from unaffected animals and PK and PD data from affected animals to support selection of an efficacious dose regimen for humans.

### 4.1.6. Efficacy Determinations

The development and evaluation of smallpox antiviral(s) is further complicated by study design issues, including but not limited to, selection of an appropriate primary endpoint. The Animal Rule guidance states that enhancement of survival or prevention of significant morbidity are acceptable primary endpoints. DAVP considers that mortality is likely to be the most interpretable primary endpoint in animal studies of variola virus and other orthopoxvirus infection models.

The use of surrogate endpoints has not generally been considered applicable to smallpox drug development because these animal studies are generally designed to measure clinical benefit over a short time period and because no surrogate marker has been

identified as reasonably likely to predict important clinical outcomes, due in part to the challenges with the consistency and reproducibility of the existing animal models, as well as the limitations in our understanding of the pathophysiology of human smallpox disease. For example, viral load is not well-characterized in relation to clinical outcomes in the available animal models, and insufficient virologic information is available from published literature with human smallpox. Although skin lesions have been discussed and used in some animal studies as a trigger for therapeutic intervention, a reliable and generalizable predictive relationship between number of skin lesions and mortality has not been established.

Currently, FDA prefers a primary clinical endpoint and is considering a mortality endpoint based on clinical criteria for euthanasia, but welcomes additional discussion from the committee.

### 4.2 Summary of Animal Rule Considerations

The Animal Rule was codified in 2002. Assessing applicability of the Animal Rule to antiviral drugs for treatment of smallpox has turned out to be a complex and challenging process. The complexities are illustrated by a brief comparison of available information supporting antiviral development for treatment of smallpox to the expectations of the Animal Rule.

--*Feasibility of ethical human studies*
Treatment of smallpox clearly qualifies for the restriction of Animal Rule development to conditions for which human challenge studies would not be ethical and field studies in naturally occurring disease would not be feasible to perform. No naturally occurring cases have been detected for over thirty years and it would not be ethical to deliberately expose humans to stored variola virus.

--*Reasonably well-understood pathophysiologic mechanisms of the toxic substance*
Given that smallpox last occurred before modern day virologic and other scientific advances, important aspects of smallpox pathophysiology, including determinants and proximate causes of severe and fatal outcomes are not well understood. However, there is little chance for increased understanding in the absence of clinical cases. Reasons for the extremely narrow host range, and determinants of the different pathogenicity of variola virus for different host species, also are not well understood.

--*Effect demonstrated in at least two animal species (or one sufficiently well-characterized for treatment outcome prediction) expected to predict human treatment responses*
Several distinctive features of smallpox research complicate attempts to determine the potential predictive capacity of any animal model or combination of models. No animal species is as susceptible or develops variola disease closely comparable to human smallpox. Animal models using other viral species do not represent the same "toxic substance" and typically also lack close comparability in many other respects. No known effective product is available for use as a positive control – either to demonstrate that an

animal model resembles human disease in sensitivity to treatment effect, or to allow comparison of a new drug to a presumed effective treatment within the model.

--*Endpoint relevant to intended human use*
Mortality was the foremost concern related to smallpox throughout its history. Percent mortality has been an expected endpoint in Animal Rule studies, although even defining mortality in an animal study depends on the appropriateness and transparency of euthanasia criteria within the study. Data from the smallpox era do not clearly support any measurement that might have been proposed as a surrogate marker for outcomes in human disease (and in general, for acute infections that culminate in death or recovery, surrogate markers have been of limited utility[123]). Sequelae such as scarring are, if anything, more difficult to model in animals than mortality; and because scarring was not directly related to disease severity in the pre-eradication era, treatments to reduce sequelae in survivors would have needed to take into account any potential effect on mortality in order to demonstrate the balance of benefit and risk.

--*Pharmacokinetic and pharmacodynamic information from animals and humans sufficient to establish effective dose*
If a smallpox outbreak were to occur, patients undergoing therapy would likely include extremely ill and unstable patients with various stages and intensities of disease progression, for whom dose calculation based on pharmacokinetics in healthy volunteers might not be a good predictor of bioavailability and tissue distribution. Relevant pharmacodynamic or exposure-response parameters are not well established. This issue is not specific to a smallpox treatment indication, but is inherent to all products developed under the Animal Rule, i.e. pharmacokinetic parameters must be determined in both affected and unaffected animals and unaffected humans to extrapolate a dose to affected humans.

--*Other supporting data Agency may take into account if available*
The Animal Rule specifies that "In assessing the sufficiency of animal data, the agency may take into account other data, including human data, available to the agency." Many other products with animal model data as major components of approval discussions have also had some degree of human information that could be considered together with the experimental animal data. For smallpox treatment, in general, such information has been much more limited and/or less encouraging than for other development plans related to threat agents.

## 5.0 Proposed Drug Development Plans for Treatment of Smallpox

This section summarizes some of the issues identified in the sponsors' proposed development plan to date, outlines areas for discussion, and provides alternative proposals for comment. Our overall goal is to work with sponsors and stakeholders to develop a plan for obtaining adequate evidence to determine whether the product is reasonably likely to confer a clinically meaningful benefit.

## 5.1 CMX001

CMX001 is an orally administered lipid conjugate of cidofovir that is currently under development for treatment of human smallpox infection. Animal studies have been conducted in various small animals (mice and rabbits) and in non-human primates (NHPs). One of the main questions under discussion is whether it will be possible to fulfill the criteria of the animal rule for a therapeutic agent for smallpox on the basis of studies conducted in animal models other than non-human primates (NHPs), and without animal studies using variola virus (VARV).

Because cynomolgus macaques metabolize CMX001 very rapidly to an inactive form, relative to humans and other animals, it is not possible to evaluate CMX001 activity in this NHP model of orthopoxvirus infection. Therefore, to demonstrate activity in NHP studies of MPXV or VARV might require exploring whether an appropriate case could be made for pharmacokinetic extrapolation if cidofovir, the active metabolite of CMX001, were administered intravenously to NHPs as a surrogate drug for CMX001. Published studies have reported antiviral activity of IV cidofovir in settings such as an NHP-MPXV model (Huggins et al, 2002), and in a mouse model of cowpox (Bray, et al., 2000)[124]. CMX001 was evaluated in the NHP-MPXV model in two studies that led to the finding of low CMX001 exposures in monkeys. Presumably because of low CMX001 exposures in monkeys, no mortality benefit was observed between CMX001 treated and placebo animals. This finding is particularly important, since mortality has been assumed to be the principal outcome of interest for human smallpox.

Instead of any additional NHP and/or variola studies, the sponsor proposes to use the mouse-ectromelia model (with inoculation via the respiratory route) and rabbit-rabbitpoxvirus model (with inoculation via the intradermal route) for its efficacy studies to fulfill Animal Rule criteria for human smallpox.

Available data with CMX001 in the rabbit-rabbitpoxvirus (RPV) studies are considered preliminary. Intradermal viral inoculation was used in the majority of these studies; while animal-to-animal spread was used in Studies UF-008 and UF-009. Results of selected rabbit-RPV studies submitted by the sponsor are summarized in Appendix I.

Study UF-008 failed to show a survival benefit for CMX001 treatment. All rabbits who received CMX001 or placebo survived RPV infection when CMX001 was initiated at the onset of skin lesions.

Study UF-009 used a 10-fold higher viral inoculum (1000 pfu per animal) than that used in Study UF-008 (100 pfu per animal). In UF-009, CMX001 was initiated at the time of lesion onset and 3/3 placebo animals died, compared to none of the placebo animals that received the lower viral inoculum (100 pfu per animal) in studies UF-007 and UF-008. In UF-009, of the 9 animals who received 1, 2, or 3 doses of CMX001 at the time of lesion onset, 3 animals died.

Studies UF-010, UF-011, and UF-012 were randomized, controlled, double-blind trials where CMX001 was initiated at the onset of skin lesions. In each of these studies, 100 pfu RPV per animal was inoculated by the intradermal route. In Studies UF-010, UF-011, and UF-012, all animals began treatment (CMX001 vs. placebo) at either Day 3 post-inoculation (PI) or Day 4 post inoculation at the time of skin lesion onset. In UF-010, mortality in animals that received placebo was 83% (10/12); compared to 8% (1/12) in animals who received 3 doses of CMX001 at days 3, 4, or 5 PI. In UF-011, mortality in animals that received placebo was 10/12 (83%) compared to 5/12 (42%) in animals who received a single dose of CMX001 3 to 4 days PI. In UF-012, mortality in animals that received placebo was 11/12 (92%) compared to 4/12 (33%) in animals that received 2 doses of CMX001 at 3, 4, or 5 days PI. In these 3 studies, mortality in the placebo group was fairly consistent, ranging from 83-92%, and was lower in CMX001 treated animals, ranging from 8% to 42%, with the lowest mortality observed in animals who received 3 doses CMX001.

The results observed in Studies UF-010, UF-011, and UF-012 differ considerably from those observed in Studies UF-008 and UF-009 in which RPV inoculum was 100 pfu and 1000 pfu per animal respectively, administered by the aerosolized route, and treatment was initiated in approximately half of the animals by Day 7 PI, and in almost all animals by Day 9 PI at the time of skin lesion onset. These differences could potentially be attributed to differences in RPV pathogenesis due to different routes of inoculation, and differences in timing of treatment initiation.

Results with CMX001 in Studies UF-010, UF-011, and UF-012 with the current rabbit-rabbitpoxvirus model appear promising; but should be confirmed with additional studies (e.g. randomized, double-blinded, controlled trials with larger numbers of animals, conducted under Good Laboratory Practices [GLP]) to demonstrate consistency of dose and duration, route of viral inoculation, and timing of treatment initiation relative to onset of clinical manifestations that occur in the majority of animals.

At present, DAVP's comments on the analyses of the data from the mouse model of infection with ectromelia are based on the summary data submitted for review. In several of these studies, study drug was initiated either before viral inoculation or post-viral inoculation but before the onset of clinically evident disease, consistent with pre- or post-exposure prophylaxis. For treatment/therapeutic studies, we anticipate that skin lesions and other constitutional signs would provide the basis for determining when to initiate study agent for a given animal in any proposed study. In general, initiation of the drug at the onset of skin lesions or at later time-points (e.g. 24 hours or more after the onset of skin lesions and/or other clinical manifestations that are consistently observed in the majority of animals studied). Other study design issues that may affect the interpretability of the data include differences in dose, timing of initial dose, duration of dosing, viral inoculum, study endpoints (including mortality based on euthanasia criteria), and duration of follow-up post-inoculation. Additional potential limitations include lack of blinding and non-compliance with Good Laboratory Practices (GLP).

In addition to orthopoxviruses, CMX001 also has *in vitro* activity against herpesviruses and adenoviruses. The overall drug development program for CMX001 includes ongoing early phase human clinical trials to evaluate the safety and efficacy of pre-emptive therapy with CMX001 to prevent adenovirus (ADV) and cytomegalovirus (CMV) infections following hematopoietic stem cell transplantation (HSCT). These trials may provide supportive data on safety of CMX001 in immunocompromised patients, and efficacy of CMX001 against other double-stranded DNA viral infections in this population. However, extrapolation of efficacy for pre-emptive treatment of adenovirus or CMV infection to smallpox infection would be difficult because of the differences in the virus, disease pathogenesis, and course of infection.

### 5.2 ST-246

ST-246 is an oral drug that is currently under development for treatment of human smallpox infection under the Animal Rule. One of the main questions under discussion is whether it will be possible to fulfill the Animal Rule for a therapeutic agent for smallpox on the basis of studies conducted in non-human primates (cynomolgus macaques) with monkeypox virus (MPXV) rather than variola virus. See Appendix II for a summary of monkeypox and variola studies in non-human primates (NHPs) submitted by the sponsor.

Antiviral activity of ST-246 is limited only to orthopoxviruses. Human clinical trials are either not possible (e.g. for treatment of variola virus infection, i.e. smallpox, which has been eradicated), or may be challenging (e.g. conducting studies for treatment of human monkeypox infection, which occurs mainly in the Democratic Republic of Congo may be difficult or not feasible depending on the evolving epidemiology of the disease and on local conditions). Available human data with ST-246 are limited to small healthy adult volunteer studies completed for evaluation of safety and pharmacokinetics and several single-patient Emergency INDs for vaccinia infection.

Animal studies have been conducted in various small animals (mice, ground squirrels, rabbits, prairie dogs) and in non-human primates (NHPs). The NHP model has been further evaluated in preliminary efficacy studies with monkeypox virus (MPXV) as well as with variola virus (VARV). In these NHP studies, mortality (based on euthanasia criteria) has been evaluated as the primary endpoint since mortality has been assumed to be the principal outcome of interest for human smallpox. Evaluation of the specific euthanasia criteria used in each study is crucial to assess the clinical significance of a mortality-based primary endpoint.

In earlier studies, considerable variability in study results was observed, due, in part, to study design issues such as the use of composite clinical and/or virologic criteria to determine when animals underwent euthanasia. Initial NHP-MPXV studies (i.e., AP06-21, AP-06-021G) showed 100% mortality (in 3/3 and 4/4 animals who received placebo, respectively). In Study AP06-21, mortality was 0% among animals treated with 3, 10, 30 and 300 mg/kg ST-246 for 14 days starting on day 3 post MPXV inoculation prior to development of skin lesions (most animals had skin lesions at day 4 or later) or other manifestations of illness. In Study AP-06-021G, no deaths were observed in animals

treated with 10 or 20 mg/kg/day for 14 days starting at days 3 or 4 post MPXV inoculation prior to development of skin lesions or other manifestations of illness. The timing of treatment initiation in these studies would most likely be considered to represent post-exposure prophylaxis rather than treatment. As noted above, the mortality findings in these studies are somewhat difficult to interpret because of the use of virologic criteria as a component of the euthanasia criteria, resulting in some non-moribund NHPs undergoing euthanasia because of high viral loads. In Study AP-06-21E6, none of the animals (0/5) treated with 10 mg/kg/day ST-246 for 14 days starting on day 5 post MPVX inoculation died through day 28. All of these animals developed skin lesions on day 4 post inoculation. However, the single animal in this study who received placebo died due to a bacterial infection rather than monkeypox infection. No efficacy conclusions could be made from Study AP-06-21E6.

More recently completed NHP-MPXV studies (AP-09-026G, SR10-037F, SR10-038F, and FY10-087) have based euthanasia solely on clinical findings. These studies show generally consistent results, such as near-uniform mortality in placebo NHPs, and apparent mortality benefit in ST-246 treated NHPs if treatment was initiated within 1-2 days of skin lesion onset.

Results with ST-246 in Studies AP-09-026G, SR10-037F, SR10-038F, and FY10-087 in the current NHP-MPXV model appear promising; but should be confirmed with additional studies (e.g. randomized, double-blinded, controlled trials with larger numbers of animals, conducted under GLP) to demonstrate consistency of dose and duration, route of viral inoculation, and timing of treatment initiation relative to onset of clinical manifestations that occur in the majority of animals.

Available NHP-VARV data have identified some issues that might benefit from additional research to help improve this animal model. In Study 1470, in which NHPs were inoculated with $1x10^8$ pfu/mL variola virus (VARV), 2/2 animals who received placebo died, compared to 0/6 overall for animals treated with high dose ST-246 (300 mg/kg/day). ST-246 was started on the day of variola virus inoculation or the day after inoculation. Thus, in this study, timing of treatment initiation was more consistent with post-exposure prophylaxis than with treatment based on clinical manifestations. In addition, virologic parameters were included in the euthanasia criteria, making the results of this study difficult to interpret. Mortality has also been inconsistent in the NHP-VARV model. In Study 1745, in which NHPs were inoculated with $1x10^8$ pfu/mL variola virus, none of the 7 animals that received placebo died; while 1 of 7 (14%) animals died that were treated with 10 mg/kg/day ST-246 for 14 days started at day 3 or 4 post variola virus inoculation. The majority of animals developed skin lesions on day 4 post-inoculation. In this study, euthanasia criteria were based solely on clinical findings. However, because none of the placebo animals died in this study, the efficacy findings with regard to mortality are difficult to interpret. In this study, there appeared to be a somewhat greater initial viral load decline during ST-246 treatment in comparison to that observed in placebo animals. However, after a few days, viral load curves were quite similar in treated and control animals. As has been noted with other diseases, it may be very difficult to assess whether viral load curves with modest or temporary differences

between treatment groups are clinically meaningful indicators of differences in disease outcome.

There are considerable challenges with conducting NHP-VARV studies; and significant refinement of the current model will be necessary in order to conduct meaningful drug treatment studies in this model. Mortality rates in the current NHP-VARV model have been inconsistent for placebo-treated VARV-infected animals as discussed above. The biology of the NHP-VARV model needs additional exploration including, but not limited to other routes of inoculation, other viral strains, other animal host species, (e.g. another NHP such as the marmoset, that might require lower viral inocula to produce lethal disease), because available data suggest VARV infection (via IV inoculation) in NHPs does not closely resemble human smallpox. Additional challenges with study of VARV include the availability of only one study site (CDC), species specificity of VARV, difficulty obtaining sufficient numbers of NHPs for clinically meaningful animal efficacy studies, and possible future eradication of all variola stocks.

### 5.3 Experience with Poxvirus Infections in Critically Ill Human Patients

Combinations of antiviral therapies (vaccinia immune globulin, cidofovir or CMX001, and ST-246) have been used together with complex and extensive supportive care in a few critically ill patients with serious vaccinia virus infection following vaccine exposure in the setting of comorbid conditions predisposing to such complications[125]. These uncontrolled anecdotal experiences do not allow conclusions about the contribution of various components of specific therapy and supportive care to patient outcomes. They do serve to illustrate some of the questions that could arise about mechanisms of severe disease and complications in susceptible hosts (whether or not the more severe aspects of vaccinia illness in immunocompromised patients could be considered comparable to severe aspects of variola illness if it were to occur in the general population, given the apparently unique predisposition of humans to variola illness), about prediction of pharmacokinetics and dosing in the seriously ill humans who would have the greatest unmet medical need in the event of a smallpox occurrence, and about evaluation of disease progression during therapy.

## 6.0 Highlights of Key Issues and Discussion Points

The following brief summaries bring together examples of the central issues that have arisen, and have been introduced in earlier sections of this document, in considering potential Animal Rule pathways for development of drugs that might be used to treat patients ill with smallpox if an outbreak were to occur. Each issue summary is followed by some pertinent discussion points.

### 6.1 Issues Related to Pathophysiology

The first tenet of the animal rule calls for a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product.

In the situation under discussion today, the toxic substance is variola virus and the toxicity of concern is the illness likely to develop if humans were exposed to variola virus (either lacking timely vaccination or with illness developing despite vaccination). Human smallpox has been eradicated; but threat of using variola virus as a bioterrorism agent remains and the extent of the threat is difficult to estimate. Therefore, development of medical countermeasures is necessary; and animal studies appear to be the best option for drug development for this indication. The pathophysiology of variola virus infection (smallpox) is not well understood, making it difficult to know which elements of variola infection and pathogenesis in humans are most important to recapitulate in an animal model of variola infection; however, the limited information available suggests there could be important differences as well as similarities in the disease process with different host-pathogen combinations. Currently, an animal model for variola infection which reproduces all aspects of variola virus infection in humans has not been developed; and whether the current NHP model(s) of variola virus infection are able to fully predict human response to therapy remains in question. These issues may need to be considered in assessing the level of certainty in extrapolating from animal models of orthopoxvirus infection to human smallpox.

### 6.2 Issues Related to Model Selection

The second tenet of the animal rules calls for the effect to be 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 response in humans.

Variola virus infection in established NHP models thus far does not closely resemble human smallpox except for development of a (usually mild) rash illness. Typically, a large viral inoculum via the intravenous route has been required to establish serious infection, potentially bypassing the initial steps in smallpox pathogenesis. Thus, a different animal model of variola virus, a combination of variola and non-variola models, or at least two other animal models of orthopoxvirus infection may be necessary. For example, a model of a naturally occurring orthopoxvirus infection initiated at low viral

inoculum by the natural route of exposure in an animal host (e.g. ectromelia in mouse or rabbitpox in rabbits) may more closely resemble some aspects of human smallpox and/or may contribute some types of information that might be considered more suitable components of an approach for establishing drug efficacy and extrapolating to humans. Models permissive of serious disease including high levels of viral replication after a low viral inoculum might address some aspects of poxvirus disease in susceptible hosts, while models using a higher inoculum might allow exploration of additional aspects of the challenges of therapy after intensive exposure (including issues such as emergence of resistance, and whether this is affected by initiation of treatment after high-level viral challenge and/or by initiation of treatment after a natural incubation period culminating in high levels of endogenous viral replication).

In previous discussions of related issues (see the draft guidance, "Smallpox (variola) infection: developing drugs for treatment or prevention" on the FDA guidance page at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071179.pdf), sponsors were advised to consider studying a range of models in preparation for discussing the appropriateness of proceeding to more difficult and restricted variola studies. In the absence of a model clearly comparable to or predictive of smallpox in humans, the possibility was raised of exploring both models using viral species to which the selected host species was known to be highly susceptible at low inoculum levels, and models in which aggressive viral challenges were used to ensure reproducible serious disease manifestations. Characterization and use of small animal models was also suggested as a means of exploring variables such as drug dose and timing of treatment initiation relative to illness. In general, based on the possibilities raised at that time, preliminary supporting studies in a suitable range of models might contribute to focused design and discussion of proposals for a smaller number of replicable, robust studies using scarcer resources (animal or viral species) at a later stage of development. As the availability of scientific information in this field advances, additional discussion may be warranted of whether use of several different models can adequately reproduce enough aspects of human smallpox to support a conclusion of likely benefit in humans with smallpox illness; and how such a combination of models might be selected, if there is no single compelling model.

### 6.3 Issues Related to Study Endpoints

The third tenet of the animal rule states that the animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.

Mortality has been assumed to be the principal outcome of interest for human smallpox. Other severe morbidities associated with smallpox could include blindness and significant scarring, as well as complications such as secondary bacterial infections. However, these conditions may be even more difficult to assess in an animal model of infection. To date, no compelling evidence has been presented for use of non-mortality

endpoints as sole predictors of human mortality benefit in this setting. Additionally, DAVP favors mortality endpoints given the lack of a well-established capacity for specific measurements of viral load or skin lesions (which have been discussed as potential surrogate endpoints) to fully predict or determine disease severity or mortality.

Consideration of non-mortality endpoints also raises important questions with regard to timing and magnitude of antiviral effect that might be associated with beneficial effects on overall outcome, and how to assess whether meaningful benefit would likely be achieved with the treatment scenarios likely to occur in the average case if an outbreak were to occur. Similar issues have arisen in relation to other acute viral illnesses where, for example, certain clinical or virologic measurements at the time of presentation might be related to outcome but the magnitude and rapidity of drug effect needed to improve clinical outcomes might be very difficult to predict (see for example discussions in the guidance document "Influenza: Developing Drugs for Treatment and/or Prophylaxis," on the FDA guidance page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064980.htm). These scenarios are unlike chronic infections with HIV or HCV in which sustained viral replication can be detected over a prolonged period and can be expected to continue in the absence of treatment, and for which substantial evidence exists directly relating treatment-induced changes in specific viral measurements in specific timeframes, as measured in controlled trials, to longer-term outcomes.

### 6.4 Examples of Other Key Study Design Issues

**Timing of treatment initiation:**

For a smallpox development program, animal studies should be designed with clinical criteria that are clinically relevant. In an outbreak setting, treatment of smallpox would likely begin for patients after the onset of clinical signs and symptoms (e.g. rash with fever). For treatment/therapeutic studies in animals, we would anticipate that skin lesions and/or other constitutional signs would provide the basis for determining when to initiate study agent for a given animal. Additionally, initiation of the drug at other later time-points (e.g. 24 hours or longer after the onset of skin lesions and/or other clinical signs or symptoms that are consistently observed in the majority of animals studied) would provide important information about how long therapy can be delayed and still provide a therapeutic benefit. Treatment prior to viral inoculation or before onset of symptoms would be considered pre-exposure or post-exposure prophylaxis, respectively.

Partly because of the importance of vaccine in prevention planning and the need for treatment of persons who nevertheless might develop disease if an outbreak were to occur, the focus of today's discussion is on how to assess whether a drug is likely to work for treatment of established illness. In an actual outbreak situation, some persons known to have smallpox contact might be recognized and treated preemptively in very early stages of smallpox illness if vaccination was missed or failed (and others might be treated but not actually have smallpox or even exposure, further complicating potential risk-benefit issues), but others might not be recognized as contacts or might not have

symptoms recognized until skin manifestations were fully characteristic or until systemic disease was well advanced. Assessment of whether benefit is likely for such patients has been considered an important component of evaluating the potential place of a candidate antiviral in smallpox preparedness, although exploration of effects over a range of treatment times and exposure/disease scenarios in animal models has also been considered potentially informative.

**Duration of treatment and post-treatment follow-up:**

For a given animal model, studying different durations of therapy, and extending the study duration beyond 28 days (which has been used in many NHP studies to date as the timepoint for scheduled euthanasia) in order to study the development of the immune response and to assess for potential viral reemergence after clearance of residual drug from tissue compartments, may be important for predicting efficacy in humans.

If viral replication continues in the presence of drug, particularly at sub-optimal concentrations, viral resistance may develop. Determining whether drug resistance occurs in animal models will be important for assessing the potential for drug resistance in humans. If drug resistance were to emerge during or after treatment, then combinations of antiviral agents for treatment of smallpox might be important to explore. In addition, if prolonged treatment is necessary to avoid viral recrudescence, the difficulty of extrapolating optimal treatment duration from animals to humans could be enhanced.

**Route of viral inoculation:**

The impact of routes of inoculation is not fully established for variola virus but data from other orthopoxviruses suggest that this study design issue may be important. Intravenous inoculation of orthopoxviruses in animals may affect the pathophysiology of disease differently compared to the respiratory route of variola virus transmission in humans. Sponsors are encouraged to provide detailed rationale supporting their proposed route of viral inoculation and are also encouraged to explore aerosolized routes of viral inoculation where feasible in an effort to improve the current animal models.

**Viral inoculum size:**

It is thought that variola virus only required a few infectious units for human infection, although viral replication could reach high levels during the course of infection. This species-specific host susceptibility to serious disease after presumed low-level exposure has posed challenges in evaluating the comparability of animal models using a variety of host-pathogen combinations, the potential alternative or complementary uses of models that can produce serious disease with a low inoculum and those that require a very high viral inoculum, and the potential relevance of drug effects observed in either type of model to prediction of effects in humans.

Ectromelia (mousepox virus; Moscow strain) and rabbitpox virus (Utrecht strain, related to vaccinia) are able to produce lethal infection with low viral inoculum in their respective mouse and rabbit hosts. However, it is unclear how to correlate these surrogate

viruses and surrogate animal models to human smallpox in the absence of any variola data.

Non-human primate models of smallpox using variola virus have needed high inocula to produce disease, and available data have shown inconsistent mortality results. Other surrogate orthopoxviruses used in non-human primate studies (e.g. monkeypox virus) have also needed high inocula to produce lethal disease. Preliminary data with other animal models (e.g. marmoset) suggest that lower doses of monkeypox virus could be used to produce lethal disease. Additional work could be considered to determine whether the marmoset or other animal hosts might allow lower doses of variola virus to be used.

### 6.5 Issues Related to Pharmacokinetics and Dosing of Candidate Products

The fourth tenet of the animal rule calls for data or information on the pharmacokinetics (PK) and pharmacodynamics (PD) of the product or other relevant data or information in animals and humans that would allow selection of an effective dose in humans. Human PK data of the investigational agent, combined with PK and PD data in an acceptable animal model, is essential for identification of an efficacious dose in humans under the animal rule. The human dose must provide similar or higher exposure relative to that observed at an efficacious dose in an animal model. Sponsors should obtain PK data for the investigational agent in healthy subjects as well as in healthy and affected animals such that exposure in animals can be appropriately bridged to select a dose for humans. It should also be noted that drug exposures in otherwise healthy and seriously ill patients may differ significantly for the same dose, and that if tissue distribution of the virus and/or the drug differs between host species, the ability to predict outcomes may be affected.

### 6.6 Issues Related to Potential for Additional Supporting Data

The Animal Rule notes that other data including human data may be taken into account if available when the agency assesses sufficiency of animal data.

Additional supportive data in other populations are important for an overall development program. For any development proposal, the sponsor's willingness and ability to propose, design, and carry out clinical trials in any appropriate naturally occurring human poxvirus diseases, as well as to design investigational protocols that could be adapted both to expand access and to collect meaningful data in an emergency setting, will be important for drug development and may make a major contribution to assessment of appropriate emergency use mechanisms.

For example, consideration could be given to studying efficacy and safety of smallpox drug(s) in naturally occurring human poxvirus infections such as vaccinia virus infection (e.g. naturally circulating variants as well as complications of smallpox vaccine) and monkeypox virus infection. Such clinical data, along with data from animal studies conducted with these orthopoxviruses, could provide supportive data as part of an overall registrational program.

FDA also encourages sponsors to prepare human study protocols that could be implemented quickly, if warranted, in an emergency situation (e.g. possible outbreak or biothreat situations). Such protocols should be designed to collect as much interpretable safety and efficacy data as possible. Preferably, such protocols should be planned and reviewed well in advance of such a situation. Sponsors are encouraged to submit such protocols for early review and feedback in addition to animal study protocols and results.

If applicable, clinical trials in humans to evaluate drug efficacy in non-orthopoxvirus disease, may provide supportive data with regard to drug safety and could confirm the potential for efficacy and exposure-response relationships (against other viruses for which activity has been demonstrated) in humans.

**Appendix I: Summary of selected rabbit/rabbitpox studies**
**Sponsor: Chimerix**
**Investigational agent: CMX001**

**Summary of selected rabbit-rabbitpoxvirus studies with CMX001 initiated at onset of skin lesions**

| Study | Description | Viral Inoculum | CMX001 regimen | Timing of CMX001 relative to viral inoculation | Results | Comments |
|---|---|---|---|---|---|---|
| UF-008 | Randomized, placebo-controlled efficacy of CMX001 in treatment of NZW rabbits infected with rabbitpox virus (Utrecht strain) by animal-to-animal spread **via aerosolized route of viral inoculation**: treatment at lesion onset (Part I); **Not blinded.** | 100 pfu per rabbit | 20 mg/kg x 1 dose | Day 8 PI in 3/3 rabbits. | 0/3 mortality at Day 21 PI | Did not show a mortality difference between CMX001 vs. placebo. |
| | | | 20 mg/kg x 2 doses* | Day 8 PI in 1/3 rabbits<br>Day 9 PI in 1/3 rabbits<br>Day 10 PI in 1/3 rabbits. | 0/3 mortality at Day 21 PI | |
| | | | 20 mg/kg x 3 doses* | Day 7 PI in 1/3 rabbits<br>Day 8 PI in 1/3 rabbits<br>Day 9 PI in 1/3 rabbits | 0/3 mortality at Day 21 PI | |
| | | | Placebo | N/A | 0/3 mortality at Day 21 PI | |
| | | | | | | |
| UF-009 | Randomized, placebo-controlled efficacy of CMX001 in treatment of NZW rabbits infected with rabbitpox virus (Utrecht strain) by animal-to-animal spread **via aerosolized route of viral inoculation**: treatment at lesion onset (Part II); **Not blinded.** | **1000** pfu per rabbit | 20 mg/kg x 1 dose | Day 7 PI in 2/3 rabbits<br>Day 8 PI in 1/3 rabbits | 1/3 mortality at Day 21 PI | Trend toward mortality difference between CMX001 vs. placebo.<br><br>Used higher viral inoculum than UF-008. |
| | | | 20 mg/kg x 2 doses* | Day 7 PI in 1/3 rabbits<br>Day 8 PI in 1/3 rabbits<br>Day 9 PI in 1/3 rabbits | 1/3 mortality at Day 21 PI | |
| | | | 20 mg/kg x 3 doses* | Day 7 PI in 2/3 rabbits<br>Day 9 PI in 1/3 rabbits | 1/3 mortality at Day 21 PI | |
| | | | Placebo | N/A | 3/3 mortality at Day 21 PI | |
| | | | | | | |
| UF-010 | Blinded, randomized, placebo-controlled efficacy of CMX001 in treatment of NZW rabbits infected with rabbitpox virus (Utrecht strain) by direct intradermal challenge of each animal: | 100 pfu/rabbit | 20 mg/kg x 3 doses* | Day 3 PI in 5/12 rabbits<br>Day 4 PI in 6/12 rabbits<br>Day 5 PI in 1/12 rabbits | 1/12 mortality at Day 14 PI | Lesions appeared earlier in UF-010 (direct viral inoculation) compared to UF-008 and UF-009 (animal-to-animal |
| | | | Placebo | N/A | 10/12 mortality at Day 14 PI | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | treatment initiated at appearance of secondary lesions. | | | | | spread). |
| | | | | | | |
| UF-011 | Blinded, randomized, placebo-controlled efficacy of CMX001 in treatment of NZW rabbits infected with rabbitpox virus (Utrecht strain) by direct intradermal challenge of each animal: treatment initiated at appearance of secondary lesions. | 100 pfu/rabbit | 20 mg/kg x 1 dose | Day 3 PI in 3/12 rabbits<br>Day 4 PI in 9/12 rabbits | 5/12 mortality at Day 14 PI | Lesions appeared earlier in UF-011 (direct viral inoculation) compared to UF-008 and UF-009 (animal-to-animal spread). |
| | | | Placebo | N/A | 10/12 mortality at Day 14 PI | |
| | | | | | | |
| UF-012 | Blinded, randomized, placebo-controlled efficacy of CMX001 in treatment of NZW rabbits infected with rabbitpox virus (Utrecht strain) by direct intradermal challenge of each animal: treatment initiated at appearance of secondary lesions. | 100 pfu/rabbit | 20 mg/kg x 2 doses* | Day 3 PI in 3/12 rabbits<br>Day 4 PI in 6/12 rabbits<br>Day 5 PI in 3/12 rabbits | 4/12 mortality at Day 14 PI | Lesions appeared earlier in UF-012 (direct viral inoculation) compared to UF-008 and UF-009 (animal-to-animal spread). |
| | | | Placebo | N/A | 11/12 mortality at Day 14 PI | |
| | | | | | | |

*Doses were given every other day; pfu, plaque forming units; PI, post-inoculation
In these studies, primary endpoint was mortality (by euthanasia based on several clinical criteria). Study duration was Day 21 PI for Studies UF-008 and Study UF-009. Study duration was Day 14 PI for Studies UF-010, UF-011, and UF-012.

CMX001 was initiated after appearance of skin lesions. Lesion onset ranged from 3-5 days PI; the majority of animals developed lesions at Days 4-5 PI. Additional details are provided below:

<u>Study UF-008</u>
CMX001: lesion onset occurred on Day 7 PI in 1/9 rabbits, on Day 8 PI in 5/9 rabbits, on Day 9 PI in 2/9 rabbits, and on Day 10 PI in 1/9 rabbits.
Placebo: lesion onset occurred on Day 7 PI in 2/3 rabbits, and on Day 8 PI in 1/3 rabbits.

Study UF-009
CMX001: lesion onset occurred on Day 4 PI in 2/9 rabbits, on Day 7 PI in 3/9 rabbits, on Day 8 PI in 2/9 rabbits, and on Day 9 PI in 2/9 rabbits.
Placebo: lesion onset occurred on Day 4 PI in 2/3 rabbits, and on Day 8 PI in 1/3 rabbits.

Study UF-010
CMX001: lesion onset occurred on Day 3 PI in 5/12 rabbits, on Day 4 PI in 6/12 rabbits, and on Day 5 PI in 1/12 rabbits.
Placebo: lesion onset occurred on Day 2 PI in 1/12 rabbits, Day 3 PI in 5/12 rabbits, and on Day 4 PI in 6/12 rabbits.

Study UF-011
CMX001: lesion onset occurred on Day 3 PI in 3/12 rabbits, and on Day 4 PI in 9/12 rabbits.
Placebo: lesion onset occurred on Day 3 PI in 6/12 rabbits, Day 4 PI in 2/12 rabbits, and on Day 5 PI in 4/12 rabbits.

Study UF-012
CMX001: lesion onset occurred on Day 3 PI in 3/12 rabbits, on Day 4 PI in 6/12 rabbits, and on Day 5 PI in 3/12 rabbits.
Placebo: lesion onset occurred on Day 3 PI in 2/12 rabbits, Day 4 PI in 7/12 rabbits, and on Day 5 PI in 3/12 rabbits.

**Appendix II: Summary of selected non-human primate (NHP)/monkeypox and variola studies**
**Sponsor: SIGA**
**Investigational agent: ST-246**

**Summary of selected non-human primate (NHP) studies with intravenous route of viral inoculation**

| Study | Description | Viral Inoculum | ST-246 regimen | Timing of ST-246 relative to viral inoculation | Results | Comments |
|---|---|---|---|---|---|---|
| MONKEYPOX VIRUS (MPXV) | | | | | | |
| AP06-21 | Double-blind, randomized, placebo-controlled study to evaluate oral ST-246 in the lesional MPXV cynomolgus monkey model (conducted at USAMRIID, MD) | MPXV strain ZAI 1979-005 ($5x10^7$ pfu/mL) | Placebo | N/A | 3/3 mortality at Day 33 PI | Euthanasia based on virologic criteria as well as clinical criteria. |
| | | | 3 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 33 PI | |
| | | | 10 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 33 PI | |
| | | | 30 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 33 PI | |
| | | | 300 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 33 PI | |
| AP-06-021G (GLP) | Double-blind, randomized, placebo-controlled repeat dose efficacy study of the therapeutic window of the proposed primate equivalent of the human dose (400 mg/day) of oral ST-246 Polyform I in cynomolgus monkeys infected with MPXV (conducted at USAMRIID, MD) | MPXV strain ZAI 1979-005 ($5x10^7$ pfu/mL) | Placebo | N/A | 4/4 mortality at Day 42 PI | Euthanasia based on virologic criteria as well as clinical criteria. |
| | | | 10 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 42 PI | |
| | | | 20 mg/kg/day x 14 days | Day 3 PI in 3/3 NHPs[a] | 0/3 mortality at Day 42 PI | |
| | | | 10 mg/kg/day x 14 days | Day 4 PI in 3/3 NHPs[a] | 0/3 mortality at Day 42 PI | |
| | | | 20 mg/kg/day x 14 days | Day 4 PI in 3/3 NHPs[a] | 0/3 mortality at Day 42 PI | |

| | | | | | | |
|---|---|---|---|---|---|---|
| AP-06-21E6 (Non-GLP) | Double-blind, randomized, placebo-controlled study to evaluate oral ST-246 in the lesional MPXV cynomolgus monkey model Study (conducted at USAMRIID, MD) | MPXV strain ZAI 1979-005 ($5x10^7$ pfu/mL) | Placebo | N/A | 1/1 mortality at Day 28 PI (death was due to $2^o$ bacterial infection, not due to MPXV infection) | No efficacy conclusions can be made since the single placebo NHP appeared to die to an illness other than MPX infection. |
| | | | 10 mg/kg/day x 14 days | Day 5 PI in 5/5 NHPs[a] | 0/5 mortality at Day 28 PI | |
| AP-09-026G (Non-GLP) | Double-blind, randomized, placebo-controlled study to evaluate the minimum effective therapeutic dose of oral ST-246 Polyform I in cynomolgus monkeys infected with MPXV (conducted at USAMRIID, MD) | MPXV strain ZAI 1979-005 ($5.5x10^7$ pfu/mL) | Placebo | N/A | 7/7 mortality at Day 42 PI | All NHPs had lesion onset at Day 4 PI.<br><br>Euthanasia based on clinical criteria. |
| | | | 0.3 mg/kg/day x 14 days | Lesion onset in 5/5 NHPs | 4/5 mortality at Day 42 PI | |
| | | | 1 mg/kg/day x 14 days | Lesion onset in 5/5 NHPs | 5/5 mortality at Day 42 PI | |
| | | | 3 mg/kg/day x 14 days | Lesion onset in 5/5 NHPs | 1/5 mortality at Day 42 PI | |
| | | | 10 mg/kg/day x 14 days | Lesion onset in 5/5 NHPs | 1/5 mortality at Day 42 PI | |
| | | | | | | |
| SR10-037F (Non-GLP) | Double-blind, randomized, placebo-controlled study to evaluate the impact of delayed ST-246 treatment on efficacy following intravenous (IV) challenge with lethal MPVX challenge (conducted at Southern Research Institute, Frederick, MD) | MPXV strain ZAI 1979-005 ($5x10^7$ pfu/mL) | Placebo | N/A | 3/3 mortality at Day 56 PI | Euthanasia based on clinical criteria. |
| | | | 10 mg/kg/day x 14 days | Day 4 PI in 6/6 NHPs | 1/6 mortality at Day 56 PI | |
| | | | 10 mg/kg/day x 14 days | Day 5 PI in 6/6 NHPs | 1/6 mortality at Day 56 PI | |
| | | | 10 mg/kg/day x 14 days | Day 6 PI in 6/6 NHPs | 3/6 mortality at Day 56 PI | |
| | | | | | | |
| SR10- | Double-blind, randomized, | MPXV | Placebo | N/A | 3/4 mortality at Day | Euthanasia based |

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|---|---|---|---|---|---|---|
| 038F (Non-GLP) | placebo-controlled study to evaluate the impact of duration of ST-246 treatment on efficacy following intravenous (IV) challenge with lethal MPXV challenge in cynomolgus monkeys (conducted at Southern Research Institute, Frederick, MD) | strain ZAI 1979-005 ($5x10^7$ pfu/mL) | | | 28 PI | on clinical criteria. |
| | | | 10 mg/kg/day x 3 days | Day 4 PI in 4/4 NHPs | 2/4 mortality at Day 28 PI | |
| | | | 10 mg/kg/day x 5 days | Day 4 PI in 6/6 NHPs | 0/6 mortality at Day 28 PI | |
| | | | 10 mg/kg/day x 7 days | Day 4 PI in 6/6 NHPs | 0/6 mortality at Day 28 PI | |
| | | | 10 mg/kg/day x 10 days | Day 4 PI in 5/5 NHPs | 1/5 mortality at Day 28 PI | |
| | | | | | | |
| FY10-087 (Non-GLP) | Randomized, placebo-controlled study to evaluate ST-246 pharmacokinetics in cynomolgus monkeys infected intravenously with MPXV (conducted at Southern Research Institute, Frederick, MD)<br><br>**Not blinded.** | MPXV strain ZAI 1979-005 ($5x10^7$ pfu/mL) | Placebo | N/A | 6/6 mortality at Day 28 PI | Euthanasia based on clinical criteria.<br><br>Not specified if NHPs had MPX disease at time of death. No necropsies were performed. |
| | | | 3 mg/kg/day x 14 days | Day 4 PI in 6/6 NHPs | 0/6 mortality at Day 28 PI | |
| | | | 10 mg/kg/day x 14 days | Day 4 PI in 6/6 NHPs | 0/6 mortality at Day 28 PI | |
| | | | 20 mg/kg/day x 14 days | Day 4 PI in 6/6 NHPs | 0/6 mortality at Day 28 PI | |
| | | | | | | |
| VARIOLA VIRUS (VARV) | | | | | | |
| Study 1470 | Randomized, placebo-controlled study to evaluate Oral ST-246 in the lesional VARV cynomolgus monkey model (conducted at CDC)<br><br>**Not blinded.** | Harper variola strain ($1x10^8$ pfu/mL) | Placebo | N/A | 2/2[b] at Day 22 PI | High ST-246 dose used. Study design more similar to post-exposure prophylaxis due to timing of ST-246 relative to viral inoculation. |
| | | | 300 mg/kg/day x 14 days | Day 0 PI in 3 NHPs | 0/3 at Day 22 PI | |
| | | | 300 mg/kg/day x 14 days | Day 1 PI in 3 NHPs | 0/3 at Day 22 PI | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Euthanasia based on virologic criteria as well as clinical criteria. |
| Study 1745 (GLP-like) | Double-blind, randomized, placebo-controlled, repeat-dose efficacy study of the therapeutic window of the proposed primate equivalence of the human dose (400 mg/day) of oral ST-246 Polyform I in cynomolgus monkeys infected with VARV (conducted at CDC) | Harper variola strain ($1x10^8$ pfu/mL) | Placebo | N/A | 0/7 mortality at Day 23 PI | Euthanasia based on clinical criteria.<br><br>No variola virus-related mortality was noted. ST-246 could not be evaluated for efficacy or prevention of mortality. |
| | | | 10 mg/kg/day x 14 days | Day 3 PI in 1/7 NHPs<br>Day 4 PI in 6/7 NHPs | 1/7 mortality at Day 23 PI | |

Mortality is assessed as unscheduled euthanasia prior to the end-of-study.
PFU, plaque forming units; EOT, end-of-treatment; PI, post-inoculation; MPXV, monkeypox virus; VARV, variola virus
[a]In NHP-MPXV model, the majority of NHPs develop skin lesions at Day 4 PI. Many NHPs (placebo and ST-246 groups) had detectable viral loads at EOT.
[b]In Study 1470, one placebo NHP was assessed by investigators as moribund when euthanized. The other placebo NHP was non-moribund when euthanized. It appears that the non-moribund NHP in the placebo group might have been euthanized due to elevated viral load (8 log10 copies/ml), not due to clinical criteria. All NHPs (placebo and ST-246 groups) had detectable viral loads at EOT.

## APPENDIX III

### Examples of evidence elements

After the 1999 IOM report on variola research[126] and especially after the events of 2001, interest in treatments for possible bioterrorism-related smallpox increased. FDA has issued draft guidance describing the challenges of drug development for smallpox treatment, and has participated in a wide variety of discussions in multiple venues including an FDA-sponsored two-day workshop in 2009. Numerous issues of potential relevance to evaluation of an antiviral for smallpox treatment have been identified or hypothesized: some of these are illustrated in the following Appendix Table, which however is not definitive and does not encompass all possible evidence elements. A fully perfect evidence base for such a drug is recognized as unlikely to be achievable; attempts have been made to identify what types of evidence would be most persuasive, but even these might be infeasible in many respects. Given the strongly shared hope that there will never be another case of smallpox, coupled with concern about the major public health implications if an outbreak were to occur and the need for best feasible decisions on use of any intervention that might appear promising in such a situation, more discussion of the best uses of imperfect evidence – and ways of improving that evidence even though still imperfect – is needed. This Table is not intended as a scoring system but as an aid to thinking through some of the complexities of drug development for smallpox treatment by listing features of extreme hypothetical "stronger case" (A) and "weaker case" (Z) elements extracted from discussions of proposals/inquiries, guidance, workshop and briefing activities, as context for possible options that might be considered.

Table: Matrix illustrations for smallpox treatment discussion

| Attribute[iii] | Case A (Stronger Case – includes *all* attributes listed for #1 through 5 below)[iv] | Potential cases B-Y (any variation between A and Z: many gradations possible for each | Case Z (Weaker Case)[vi] |
|---|---|---|---|

[iii] Focus is on package proposals for antiviral products to treat established smallpox illness, as that has been the principal area of interest for these products, while vaccines have had the predominant pharmaceutical role in prevention and control discussions. If antivirals were to be considered primarily for prophylactic use or for treatment of naturally occurring nonvariola poxvirus infections, some elements of the discussion might become simpler and others more complex: potentially a topic for another day and another meeting.
[iv] Not all attributes of "stronger case" can necessarily be achieved at any one time, and even the best achievable combination might not fully address all aspects of the "Animal Rule" (see additional discussion in introductory material). However, potential contribution of each attribute to strength of evidence might be useful to consider when discussing the level of conviction that might be based on any one proposed development design, and the potential impact of adding or removing certain types of evidence.
[v] Listed attributes can have both qualitative and quantitative variability such that detailed discussion of a comprehensive list is not feasible in a limited time. Illustrative examples may be selected for discussion.
[vi] Just as a program containing most or all "stronger case" attributes might not reach the level of evidence expected in many other types of development programs and might leave open issues for further discussion before determination of whether an approval decision could be supported, a program containing most or all "weaker case" attributes would be unlikely to support late-stage regulatory action on an indication but would not necessarily rule out experimental use of a product if an emergency setting were to arise in which

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| | | element)[v] | |
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| 1. Principal model | | | |
| a. Virus selection | Variola isolate with known association with historical virulent human illness | | Non-variola, uncertain association with human disease occurrence or severity |
| b. Host selection | Optimal, well-defined resemblance to human host-pathogen interactions | | Little or unknown resemblance to human host-pathogen interactions |
| c. Route of viral challenge | Respiratory with evidence that respiratory exposure type is relevant to historic human disease understanding | | Route with no clear human corollary (e.g. intraperitoneal) |
| d. Inoculum size | Appropriate range (e.g. low inoculum of virulent virus in susceptible host, plus exploration of more aggressive challenges)[vii] | | Requirement for very high inoculum (low virulence and/ or low host susceptibility)[viii] |
| e. Disease characteristics | Substantial incubation before disease appearance, skin lesions prominent but not sole manifestation, multiorgan involvement with pathophysiology/pathology and mode of mortality comparable to best understanding of historical human disease,[ix] virus tissue distribution comparable to best understanding of historical human disease, high mortality, recovery with no latency or recrudescence in survivors | | Disease poorly documented or highly divergent from historical smallpox descriptions |
| f. Consistency | High | | Study-to-study variability |
| g. Information on existing treatments | Exposure-response similar to known effective and known ineffective human treatments[x] | | Animal results inconsistent with available human results, or no information |
| | | | |
| 2. Administrative characteristics of animal models and studies | | | |
| a. GLP | Compliant; prospective discussion of any deviations and agreement on adequate | | Non-compliant; data not signed by study director and/or Quality Assurance |

it appeared likely to be relevant. The principal proposed and/or practicable development plans fall somewhere in between, and the intention is to focus discussion in that territory.

[vii] No host with fully comparable susceptibility is anticipated; host specificity was essential to eradication.

[viii] Very high inoculum might also introduce artificial immune stimulus further limiting extrapolation.

[ix] Human disease understanding is limited; it is hoped there will never be more cases; any new information that might enhance understanding of historical disease could also enhance discussion of models. Historical descriptions of disease and expert opinions on pathophysiology were both heterogeneous. If bioattack might cause disease different from historical pattern, extrapolation would be even more difficult.

[x] No known effective treatment for established human smallpox illness exists for comparison.

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| | alternative safeguards of data quality and integrity; data signed by study director and/or Quality Assurance Officer; inspections performed by DSI and no major issues noted that would affect study quality | | Officer; facility found out of compliance or DSI cannot complete inspection; no adequate documentation of measures to assure data integrity and quality |
| b. Animal welfare | Compliant; meets or exceeds all animal care and use guidelines; protocols approved by IACUC | | Compliant[xi] |
| c. Reproducibility | Study can be reproduced in more than one GLP-compliant, inspected facility; data reproduced with minimal variability between studies | | Study performed at single site, non-GLP or unknown compliance |
| | | | |
| 3. Principal study characteristics and design | | | |
| a. Protocol submission | Advance submission to FDA with adequate lead time for review/feedback; comments taken into consideration, agreement reached on incorporation into protocol | | Submitted when study is imminent or initiated, and/or feedback not considered (or not implemented following discussion) |
| b. Monitoring | Intensive (frequency and documentation comparable to veterinary intensive care settings and/or to clinical trials in severe human diseases; use of telemetry and/or 24/7 remote observation where appropriate) | | Infrequent and cursory |
| c. Product proposed for study | Same drug substance, product, formulation, and route of administration as proposed for humans; well-documented rationale for comparing animal and human dosing regimens | | Different substance or product without convincing basis for extrapolation; or markedly different tissue distribution of drug relative to virus in animals and humans |
| d. Logistics and duration of therapy | Relevant and feasible in circumstances considered likely if a human outbreak were to occur | | Impractical or illogical relative to postulated human situations |
| e. Follow-up duration | Adequate post-recovery, post-treatment follow-up of survivors to assess for risks of disease recrudescence or major sequelae of disease or treatment, and for level of protection in re-exposure | | Too short for reliable outcome assessment |
| f. Intervention trigger | Well-defined, prospectively agreed evidence of clinical | | Arbitrary timepoint after viral inoculation, before or not |

[xi] Studies not meeting animal welfare criteria should not be conducted or considered at any evidence level.

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| | illness comparable to likely human signs and symptoms associated with disease recognition and treatment | | related to clear-cut disease manifestations |
| g. Range of dose and timing studied | Either preliminary or pivotal studies designed to show effect across wide range of doses and treatment initiation times, including and bracketing those plausibly associated with human treatment situation | | Single arbitrary dose and timing studied; relevance to human setting implausible or unclear |
| h. Principal endpoint | Percent mortality; defined documented animal euthanasia criteria agreed upon as yielding interpretable information for prediction of likely mortality in human disease; euthanasia decisions made and individual case descriptions documented by personnel without knowledge of treatment assignments or stake in study outcome | | Laboratory or clinical measurement without clearly established documentation of meaningful predictive or surrogate function[xii]; or euthanasia with decisions not adequately documented or adequately separated from interest in study outcome |
| i. Supportive care | Consistent with best veterinary practice; relevant to likely attempts at care if human cases were to occur; following clear, consistent, prospectively agreed triggering criteria; designed to enhance reliability and relevance of analysis of study drug effects | | None or arbitrary |
| j. Statistical approach | Robust, well-powered sample size calculations and statistical analysis plan, prospectively reviewed and discussed before study | | Under-powered sample size, post hoc outcome analyses |
| k. Data completeness | Summaries, tabulations, raw data, case narratives, and submission quality and accessibility meeting standards similar to expectation for pivotal clinical trials as well as toxicology studies in usual development programs | | Summaries without supporting information |
| l. Results | Large dramatic effect size across multiple doses and treatment initiation times | | Effect size modest, lacking in statistical robustness, and/or highly dependent on precise dose or treatment initiation time |
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[xii] Some early manifestations historically correlated with mortality, not necessarily predictive or causal in humans, or historically important non-mortality outcomes, raise additional complexities in extrapolation.

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| 3. Additional animal models and study designs | At least two prospectively discussed additional models illustrating different levels of host susceptibility to pathogen, pathogen relatedness to smallpox, and intensity of challenge; meeting most criteria listed under #1; studied at multiple sites for reproducibility, and designed to fill in gaps based on discussion of principal model; illustrating range of doses and treatment initiation times associated with benefit, especially if not fully evident from primary model | | Absent or with many inadequacies similar to above listings in this column |
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| 4. In vitro and cell culture data | | | |
| a. Mechanism of action | Well-defined and conserved across related viral species | | Unknown or not conserved |
| b. Resistance emergence | High genetic barrier to resistance documented in related viruses that can be safely studied; clear rationale for similar expectations with variola; post-treatment viral isolates from animal studies and any related human cases analyzed and not resistant | | Rapid or single-mutation emergence of resistance in cell culture passage of related viruses and/or resistant virus isolated after treatment of animals or humans |
| c. Breadth of activity | Highly active against multiple relevant viral strains/species | | Variable activity across viral strains and species |
| | | | |
| 5. Human data | | | |
| a. Efficacy/activity | Some controlled trial data suggestive of benefit in related naturally occurring human disease | | Anecdotal (or controlled) data suggesting disease progression and/or resistance emergence during treatment of other diseases |
| b. Exposure-response | Data available to support exposure-response assessment and selection of relevant PK parameters from similar products and diseases | | Data inconsistent or absent |
| c. Safety/PK | Robust database supporting safety, PK, and dosing in populations with varying demographics and illness severity relevant to intended target population; adequate information to assess and manage interactions with other pharmaceuticals likely to be used concomitantly | | Anecdotal (or controlled) data suggesting serious toxicity and/or lack of adequate dosing predictions; interaction data lacking, or raising difficult-to-manage concerns |

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| d. Protocol development | Well-designed protocols to optimize feasibility and interpretability, and balance data collection and access needs while providing appropriate information for patient and provider decision-making, across a range of putative circumstances: if an outbreak emergency were to occur; if development is feasible and appropriate for closely or distantly related human diseases in which well-controlled clinical trials might be conducted; or if individual cases of related human disease occur that cannot be studied in controlled trials but might be treated under expanded access | | Protocols that offer misleading information to patients and providers, cannot yield interpretable data, and/or include analyses likely to produce erroneous or misleading conclusions |

## APPENDIX IV.

## Notes and Citations

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[1] General descriptions can be found in a number of reviews and summaries including but not limited to Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and Its Eradication. Geneva, WHO, 1988; Dixon CW. Smallpox. London, J&A Churchill, 1962; Rao AR. Smallpox. Bombay, Kothari Book Depot, 1972; Breman JG and Henderson DA, Diagnosis and management of smallpox, N Engl J Med 2002;346:1300-1308. Descriptions in the current overview draw heavily on these sources, plus additional more detailed or primary sources cited below.

[2] Range was often suggested to be 7-17 days and average around 12 days. A retrospective analysis of historical data suggested "The upper confidence limit of the 99th percentile of the incubation period is 22.2 days…" (Nishiura H, Brockmann SO, Eichner M. Extracting key information from historical data to quantify the transmission dynamics of smallpox. Theoret Bio Med Model 2008;5:20).

[3] In addition to citations at beginning of this section, also see Bancroft IR, Clinical observations on variola. J Med Res 1904;11:322-44 (describing "about twelve hundred cases" from an epidemic of about 1600 cases with 11.1% mortality). Even the nature of the most characteristic lesions was debated, e.g. Bras 1952, "are smallpox lesions of a primary inflammatory nature (Bärensprung, 1854), or are they primarily degenerative, as Weighert (1874) accepted? The divergent opinions may perhaps be explained by the dissimilar techniques…."

[4] Occasional sequelae of long-ago smallpox are still reported: see for example Arora A, Agarwal A, Kumar S. Osteomyelitis variolosa: a report of two cases. J Orthopaed Surg 2008;16:355-8.

[5] Initially distinguished by differences in phenotype on chorioallantoic membranes of embryonated eggs (see discussion in Downie AW, Dumbell KR, Ayroza Galvao PA, Zatz I. Alastrim in Brazil. Trop Geogr Med 1963;15:25-8). For more recent phylogenetic comparisons, see for example Li Y et al. On the origin of smallpox: correlating variola phylogenics with historical smallpox records. PNAS 2007;104:15787-92.

[6] Dixon (1962 and 1948) suggested a confident diagnosis of smallpox could usually be made by the second day of rash and fifth day of illness, but that clinical classification was best determined a few days later.

[7] This tabulation is not exhaustive, but includes a few examples of the more extensively documented schemata. Other category lists used or mentioned in various sources included variola hemorrhagica (purpura variolosa or "black smallpox") and variola vera (variola confluens, variola discreta, and varioloid) (Brayton ND, Pathology and treatment of smallpox: an analysis of over two thousand cases and of fifty autopsies. JAMA 1903;41:233-8; quoting Osler); variola vera (uncomplicated, with hemorrhage, abortive, or with complications), variola pustulosa hemorrhagica, and purpura variolosa (Councilman WT, Magrath GB, Brinckerhoff WR, The pathological anatomy and histology of variola. J Med Res 1904;11:12-135); hemorrhagic, confluent, and discrete (Mukherjee et al, Bull WHO 1974;51:219-55); division of hemorrhagic into type I and type II (Downie AW et al., Haemorrhagic smallpox, J Hyg 1969;67:619-29); hemorrhagic, malignant, and characteristic (Henderson DA et al. for the Working Group on Civilian Biodefense, Smallpox as a biological weapon. Medical and public health management. JAMA 1999;281:2127-37); mild, moderate, severe, and grave based on lesion counts (mentioned in sources such as Hooper JW et al., Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. J Virol 2004;78:4433-43); lesion density classes 1,2,3, and 4 based on forearm counts and confluence (Thomas DB et al., Endemic smallpox in rural East Pakistan. II: Intravillage transmission and infectiousness. Am J Epidemiol 1971;93:373-83); classical (discrete and confluent), purpura variolosa, and variola hemorrhagica pustulosa (Haviland JW. Purpura variolosa. Its manifestations in skin and blood. Yale J Biol Med 1952;24:518-24) etc. Translations between classification schemata are not always well-defined or straightforward. Some investigators pointed out lack of clear demarcation between classifications, including presence of hemorrhage in cases not classed as hemorrhagic and presence of pox lesions on close examination in early purpuric cases [e.g. Dixon 1962 notes potential even in "discrete" cases for "small haemorrhages to occur in the skin or mucous membranes" and "Some clinicians have, however, applied the term "haemorrhagic" to this type of case and have assumed that some particular treatment has affected the course of the disease which is essentially benign. It is for this reason that I firmly believe the word "haemorrhagic" should be omitted in the description of any type of smallpox." Bras

1952 series of autopsies (176 human and 1 orangutan in Indonesia) "We could not conclude that purpura variolosa was so very different in nature from variola vera, as many authors state."]

[8] Ricketts TF, Byles JB. The Diagnosis of Smallpox. London, Cassell and Company, 1908.

[9] Dixon CW, 1962. p. 6.

[10] Rao AR, 1972. p. 8.

[11] Fenner F, et al., 1988. P. 4.

[12] See for example Fenner et al., p. 97.

[13] Dixon CW. Smallpox in Tripolitania, 1946: an epidemiological and clinical study of 500 cases, including trials of penicillin treatment. J Hyg 1948;46:351-77.

[14] Dixon (1962) p. 104, "Every writer on smallpox over the last 150 years has pointed out that in his experience the amount of blindness due to smallpox was much less than that quoted by previous authors." Councilman 1904, "…it appears that this must have been much more frequent formerly than it is now."

[15] Dixon (1962, p. 57) notes some cases of variola minor were reported as "discrete with more than 500 pocks on the face" although most had fewer.

[16] Fenner et al. (1988), pages 96-7 and 101.

[17] This uncertainty persists with recent virologic investigations of stored strains: see for example Olson VA et al., Smallpox virus plaque phenotypes: genetic, geographical and case fatality relationships, J Gen Virol 2009;90:792-8. Questions have also been raised about whether variola strains might be altered in ways that would affect virulence in a bioweapons setting (see for example Tucker JB, Zilinskas R. The 1971 smallpox outbreak in the Soviet city of Aralsk: implications for variola virus as a bioterrorist threat. Introduction. Crit Rev Microbiol 2003;29:81-95; plus accompanying articles Zelicoff AP, An epidemiological analysis of the 1971 smallpox outbreak in Aralsk, Kazakhstan, Crit Rev Microbiol 2003;29:97-108; Sarynov E, Kulmakhanov B, Makatox Z, Report on measures taken to contain and eradicate the smallpox outbreak locale in the city of Aralsk (September/October, 1971), Crit Rev Microbiol 2003;29:109-44; and Sarynov E, Kulmakhanov B, Report on measures taken to contain and eradicate the smallpox outbreak locale in the city of Aralsk, Part II, Crit Rev Microbiol 2003;29:145-158; Atlas RM, Clover R, Commentary on implications of the 1971 outbreak in Aralsk, Kazakhstan, for U.S. smallpox vaccination policy (No. 1), Crit Rev Microbiol 2003;29:159-61; also discussions such as Finkel E, Engineered mouse virus spurs bioweapon fears. Science 2001;291:585.)

[18] Fenner et al. (1988) p. 246. "The reasons for the difference in severity [with cutaneous variolation] are not known….In any event the virus was not attenuated and one of the major disadvantages of variolation was that it could spread to susceptible contacts to produce severe natural smallpox." Dixon 1948 (p. 364) describes 13 mother-infant pairs: "The type of infection in mother and child was the same in six, in four the severity in the mother was very much greater than that in the infant, and in three the infant's disease was more severe than that of the mother."

[19] For example Bhatnagar V et al., Transmission patterns of smallpox: systematic review of natural outbreaks in Europe and North America since World War II. BMC Publ Health 2006;6:126; Tovey D. The Bradford smallpox outbreak in 1962: a personal account. J Royal Soc Med 2004;97:244-7 gives an example in which several cases were unrecognized until multiple exposures took place, despite existing "intimation of a possible outbreak" in a non-endemic area (in this instance suspicion was reportedly raised by peripheral blood smears considered suggestive of "overwhelming virus infection: with nucleated red blood cells, myelocytes, fragmenting granulocytes and vacuolation of the protoplasm, condensed nuclear bodies and atypical plasma cells and some Türck cells"); similarly Nelson PD, Smallpox in Saint Paul and Minneapolis, 1924-5. Minnesota Med 2004;87(5):34-9..

[20] Variola minor was reported not to carry similarly elevated risk in pregnancy (Megale P, Angulo JJ, Perderneiras CA. Variola minor in Braganca Paulista County 1956. Bull Soc Pathol Exot 1979;72:11-20) even if "more severe" than other variola minor cases (Downie AW, Dumbell KR, Ayroza Galvao PA, Zatz I. Alastrim in Brazil. Trop Geogr Med 1963;15:25-8) and occasionally associated with fatalities (2 known pregnant women and 3 neonates among 12 deaths reported in a summary of 1492 variola minor cases, De Quadros CCA, Morris L, Azeredo da Costa E, Arnt N, Tigre CH, Epidemiology of variola minor in Brazil based on a study of 33 outbreaks. Bull WHO 1972;46:165-71). Brilliant LB (Occupationally-acquired smallpox in an IgM-deficient health worker. Bull WHO 1981;59:99-106) describe "one of the last cases of variola major" as having two crops of variola lesions after a vaccine take, severely ill but recovering, and state "there are no previous reports of smallpox in persons with an altered immune capacity….").

[21] For example, Sarkar and Mitra (A search for the causes of severity in smallpox. J Ind Med Assoc 1968;51:272-4; also see Sarkar JK, Mitra AC, Chakravarty MS, Relationship of clinical severity, antibody level, and previous vaccination state in smallpox. Trans Royal Soc Trop Med Hyg 1972;66:789-92) reported patients with hemorrhagic smallpox had low or undetectable antibody titers but patients with confluent smallpox had higher titers than those with discrete smallpox. These authors also reported different virulence for chick embryos of viruses from cases of varying severity, but these findings were not widely reproduced and were noted not to accord with observed differences in severity along contact chain (hemorrhagic cases transmitting to contacts who developed milder disease and vice versa). Mack TM, Noble J, and Thomas DB (A prospective study of serum antibody and ptotection against smallpox. Am J Trop Med Hyg 1972;21:214-8) discussed difficulty of relating antibody types exactly to disease risk. Cutpoints and timing of antibody measurement varied across studies, with levels at disease onset possibly of different significance from levels at exposure (see for example Sarkar JK, Mitra AC, Muhkerjee MK, The minimum protective level of antibodies in smallpox, Bull WHO 1975;52:307-11). Also see Koplan JP, Foster SO, Smallpox: clinical types, causes of death, and treatment. JID 1979;140:440-1. ("There are no obvious host differences in the patients seen in each of the clinical types. Nor is there evidence of a difference in quantity of virus infecting the individuals grouped in the different types.")

[22] See for example Baxby D, Rev Med Virol 2002;12:201-9, review of Hanna W, Studies in Small-pox and Vaccination, Bristol, John Wright and Sons, 1913; Mack TM, Thomas DB, Ali A, Khan MM, Epidemiology of smallpox in West Pakistan. I. Acquired immunity and the distribution of disease. Am J Epidemiol 1972;95:157-68; Albert MR et al., Smallpox manifestations and survival during the Boston epidemic of 1901 to 1903. Ann Intern Med 2002;137:993-1000..

[23] Fenner 1988; Sivils M, Dissecting the pamphlet literature of the Boston smallpox inoculation controversy, Literature and Medicine 2011;29:39-57.

[24] For a 20th century example see Tao CS, A study of the smallpox prevalence and inoculation data in some districts of Kiangsu Province. Trans Ninth Congr Far East Assoc Trop Med 1934;2:499-501.

[25] Fenner p. 253, "Fresh scab material was supposed to be stored "on the person" (i.e., at about 37º C) for up to a month, a prescription that ensured that it contained little active virus, but consisted in large part of inactivated virus."

[26] Dixon (1948 and 1962) also "suggested… that the virus extruded through the skin, perhaps modified by its passage, is in some way different from the virus from the respiratory tract" and cited both insufflation variolation and epidemiologic periodicity of disease in support of lesser infectiousness of virus in scabs.

[27] Fenner et al., p. 188, "Man was the natural host of variola virus, and a priori it might be expected that a single viable virus particle, lodged in an appropriate site, could be infectious, although because of non-specific protective mechanisms a larger dose would usually be required. However, assays could obviously not be carried out and this must remain a supposition …."

[28] See for example reports of the Meschede and Monschau hospital outbreaks (Wehrle PF, Posch, Richter KH, Henderson DA. An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. Bull WHO 1970;43:669-79; Gelfand HM, Posch J, The recent outbreak of smallpox in Meschede, West Germany. Am J Epidemiol 1971;93:234-7); and Centers for Disease Control and Prevention, Smallpox – Stockholm, Sweden, 1963; MMWR 1996;45:538-545. However, the vast majority of cases appeared attributable to close exposure to recognizably ill persons (e.g. discussion in Mack T, A different view of smallpox and vaccination. N Engl J Med 2003;348:460-3; and Mack T, Smallpox in Europe, 1950-71. JID 1972;125:161-9.).. Attempts to detect small-particle-borne and large-particle-borne virus using impingers and settling plates (Downie AW et al., The recovery of smallpox virus from patients and their environment in a smallpox hospital; Bull WHO 1965;33:615-22) suggested that large-droplet was more likely than airborne spread, in keeping with most epidemiologic data. Swabs from the circumoral area and the pillow were more likely to yield virus than either air sampling or swabs from the skin of the back (including undisrupted lesions); the authors suggest saliva contamination. Opinions varied over time as to whether reports of fomite-associated infection were related to contact with scabs or were better explained by residua from oral/respiratory secretions.

[29] Downie AW et al. Studies on the virus content of mouth washings in the acute phase of smallpox. Bull WHO 1961;25:49-53. In assays of mouth washings from patients with early rash, "…in terms of infective doses of virus for the chick embryo, …some of the specimens had 300 or more pock-forming units of virus in 0.1 ml of the specimen." Assays using chorioallantoic membrane and cell culture were both reported highly sensitive with the egg method "less fastidious to the inoculum quality" (Shchelkunov SN,

Marennikova SS, Moyer RW; *Orthopoxviruses Pathogenic for Humans*, New York, Springer, 2005; Page 309). Using other poxvirus species, attempts were made to estimate the ratio of virus particles visible by electron microscopy to chorioallantoic membrane pock counts: one group reported ratios ranging from 170 to 12.3 and cited other investigators with results varying both higher and lower.

[30] See for example Mukherjee MK et al., Pattern of intrafamilial transmission of smallpox in Calcutta, India," Bull WHO 1974;51:219-25: "The attack rate in vaccinated contacts was significantly less than in unvaccinated contacts, but there was not such difference in the case rates caused by severe and mild index cases. Rao 1972 p. 15, "there has not been even a single instance in which a second haemorrhagic case has occurred amongst the contacts of a series of 385 cases of Haemorrhagic smallpox studied by the author. Conversely, there have been many instances in which non-haemorrhagic types occurred amongst the contacts." Rao 1972 p. 16, "the haemorrhagic type of cases, as a source, do not transmit the disease more readily to the familial contacts…." Bancroft 1904 "the relation of ["purpura variolosa"] to other forms of the disease was wholly indeterminate. In two instances it followed exposure to mild forms of variola vera. In no instance did one case of the purpuric form give rise to another. Cases of variola traceable to purpura variolosa as a source of infection showed no uniformity in type."

[31] See for example Bozzette SA et al., A model for a smallpox-vaccination policy, N Engl J Med 2003;348:416-25, outlining a number of hypothetical scenarios to defend against, ranging from a smallpox threat turning out to be a hoax, to accidental, hostile, or voluntary contamination of a few individuals followed by person-to-person spread, to aerosolization of varying extent and intensity in densely occupied spaces. For any hypothetical scenario, both the initial number of cases and the capacity for person-to-person spread contribute to the potential impact of an event and the risk-benefit balance of interventions. For a sample of discussions using a variety of modeling approaches, see Meltzer MI et al, Modeling potential responses to smallpox as a bioterrorist weapon, Emerg Infect Dis 2001;7:959-69; Halloran ME et al., Containing bioterrorist smallpox, Science 2002;298:1428-32; Legrand J et al., Modeling responses to a smallpox epidemic taking into account uncertainty, Epidemiol Infect 2003;132:19-25; McKenzie FE, Smallpox models as policy tools, Emerg Infect Dis 2004;10:2044-7; Riley S, Ferguson NM, Smallpox transmission and control: spatial dynamics in Great Britain, PNAS 2006;103:12637-42; Burke DS et al., Individual-based computational modeling of smallpox epidemic control strategies, Acad Emerg Med 2006;13:1142-9; Glasser JW et al., Evaluating public health responses to reintroduced smallpox via dynamic, socially structured, and spatially distributed metapopulation models, Clin Infect Dis 2008;46(Suppl 3):S182-94.

[32] See for example Bancroft 1904, "no relation exists between the length of the initial stage and the severity of the disease."

[33] See for example Rao (1972) p. 22, "ordinary" smallpox: "The lesions appear in such quick succession, that it is difficult to follow the timing of occurrence of lesions on the different parts of the body….. Within a matter of 24 hours the whole rash appears and after that, normally no fresh lesions develop."

[34] See for example Bancroft (1904) description of "successive crops" of lesions in some "abortive" or "varioloid" cases; also Dixon 1962.

[35] Marsden J. On the diagnosis of smallpox. Br J Clin Practice 1958;12:333-41. On the other hand, variola minor patients might have both delayed appearance of focal rash with prolonged prodrome, and rapid evolution of the rash once it appeared (Marsden J, Variola minor. Month Bull Min Health 1952;11:74-7). Councilman et al. (1904) noted "premature formation of crusts, due to the early, and in many instances the traumatic, rupture of vesicles" in autopsies of children dying in the first week of disease.

[36] Rao 1972 p21-2, "flat semiconfluent" "fatality rates were about 95 percent in the unvaccinated and 60 percent in the vaccinated"; "flat discrete" "case fatality rate is equally bad among the unvaccinated…." P. 25, "ordinary confluent" "The fatality rate in our series was 62 percent in the unvaccinated and about 26 percent in the vaccinated."

[37] Dixon 1962 p. 25-6, "Whereas the latter ["malignant case"] has signs of a severe general infection but a relatively poor rash, the benign case has a spectacular eruption and relatively little evidence of toxaemia. In recognizing these two distinct syndromes in smallpox it can be seen that it is not true to say that severity is directly correlated with the extent of the rash." P. 28, suggests even "benign semi-confluent" cases could have "well over a thousand lesions." Rao 1972 p. 6-7, "…the density of rash in the non-haemorrhagic types is not in itself of great prognostic significance….Classification based upon the count of the actual number of lesions is impracticable and further it is most unreliable in assessing the prognosis….both the nature and evolution of the lesions and the density of rash have prognostic significance. Differences,

however, are most marked according to the first variable e.g., the fatality rates are higher amongst the Flat discrete cases than amongst the Ordinary confluent cases and, similarly Ordinary discrete cases are more frequently fatal than Modified confluent cases."

[38] See Rao citations above, and summary of same data in Fenner et al., p. 4.

[39] Downie 1954, "no knowledge of where the virus is multiplying" during incubation

[40] See citations under "diseases virologically related to smallpox" below.

[41] The seminal article on ectromelia as a model for viral diseases including "smallpox, chickenpox, measles, and rubella" utilized serial virologic and pathologic examination after infection through the skin (Fenner F, The pathogenesis of the acute exanthems. Lancet 1948;2:915-20). Fenner reports results suggesting initial multiplication at the site of inoculation and in regional lymph nodes with virus detected within hours of inoculation, transient viremia after several days seeding the reticuloendothelial system with subsequent viral replication in liver and spleen, release of virus by necrotic cells at those sites in a secondary viremia seeding the skin, and replication of virus in the skin for a few days before lesions became apparent. Both at the inoculation site and in distantly seeded skin, Fenner reported "macroscopic changes were first detected when the virus titre had almost reached its maximum"; and concluded that "invasion" by virus occurs during the incubation period and "symptoms and signs develop only when multiplication of the virus has almost reached a maximum" across a variety of acute viral illnesses.

[42] See for example Sarkar JK, Mitra AC, Mukherjee MK, De SK. Virus excretion in smallpox. 2. Excretion in the throats of household contacts. Bull WHO 1973;48:523-7. "Throat swabs of 34 of 328 family contacts of 52 smallpox cases, examined 4-8 days after the onset of the disease in the family, were positive for variola virus. The log titre of virus per swab ranged from 2 to 3.95….Only 4…developed clinical smallpox…." The authors noted their findings were difficult to reconcile with other reports of failure to isolate virus from pre-eruption oral sample from smallpox patients, and with "field experience" that transmission occurred during "manifest disease."

[43] Downie et al., 1961, "Generally speaking, the patients with severe disease…tended to show more virus in the mouth washings….However, this correlation was not very close as some patients with relatively mild disease occasionally showed a large amount of virus …." Sarkar JK et al. (Virus excretion in smallpox. 1. Excretion in the throat, urine, and conjunctiva of patients. Bull WHO 1973;48:517-22) reported titers up to 6 logs in "hemorrhagic" and "confluent" cases and up to 4 logs in "discrete" cases, peaking around day 3 if sampled that early and then gradually diminishing in nonfatal and 11 of 13 fatal cases (2 patients who died by day 6 had constant titers of 5 and 3 logs respectively reported on days 3, 4, and 5); found no relationship to vaccine status; and obtained inconsistent results with urine assays.

[44] Fenner et al., p. 44, "by analogy with other poxvirus infections viraemia would have been expected to be primarily cell-associated"; Also from a small number of samples, a suggestion of greater amounts of virus in cells than serum was reported (Downie et al., Virus and virus antigen in the blood of smallpox patients. Their significance in early diagnosis and prognosis. Lancet 1953;2:164-6). A number of authors (e.g. Dixon 1948) suggested that the timing of rare cases in neonates with presumed antenatal transmission was consistent with maternal viremia at the end of the incubation period but not earlier.

[45] Downie AW, McCarthy K, Macdonald A. Viraemia in smallpox. Lancet 1950;2:513-4. Downie AW, McCarthy K. Pathogenesis of variola. Chapter 18, p. 194-215, in Hartman FW et al., (ed.), Dynamics of virus and rickettsial infections. New York, Blakiston, 1954. In addition to the very small numbers of reports having serial samples from individual patients, Mitra AC et al. (Viraemia in haemorrhagic and other forms of smallpox. J Indian Med Assoc 1966;47:113-4) obtained virus from blood of all 15 "haemorrhagic type" patients examined and only 1 of 30 other patients examined, while speculating that more of the latter might have been positive except that they tended to be admitted to hospital later; of the 15 hemorrhagic cases, each with one measurement at the time of admission ranging from days 3 to 6 of illness, log titers of 4 or greater were reported in 5 of 8 sampled on day 3 and none sampled later, while log titers less than 3 were reported from 6 of 7 sampled later than day 3 and 1 of 8 day 3 samples. Downie and Dumbell (The isolation and cultivation of variola virus on the chorio-allantois of chick embryos. J Pathol Bacteriol 1947;189-98) reported isolation of virus from blood of a hemorrhagic case on day 6 of disease.

[46] Downie et al. 1969 citation above. These and other reports also suggested that soluble antigen in blood carried a worse prognosis than the ability to isolate virus from the blood.

[47] Alivisatos GP, Violaki-Paraskeva M. Die Virämiredauer nach Vakzination und Variolaerkrankung. Zeitschrift für immunitätsforschung 1959;117:230-243.

[48] Downie AW, Dumbell KR. Survival of variola virus in dried exudate and crusts from smallpox patients. Lancet 1947;1:550-3. "There appears to be little relation between the virus content of crusts and the stage of the disease at which they were collected; at least our findings showed that the crusts of case 6, collected on the 24th day of illness, apparently contained virus in amount comparable to that in crusts removed from other patients on the 10th-15th day of illness." Mitra AC, Sarkar JK, Mukherjee MK. Virus content of smallpox scabs. Bull WHO 1974;51:106-7. "Log titres of smallpoxvirus (pock-forming units per 0.1 g of scab) were estimated…from 3.72 to 6.54 with no diminution as convalescence progressed, and they were not related to the primary vaccination status of the patient or the clinical severity of the disease." Recent reanalysis of transmission times again recall the doubts raised in the pre-eradication era about transmission potential in late convalescence (Nishiura H, Eichner M, Infectiousness of smallpox relative to disease age: estimates based on transmission network and incubation period. Epidemiol Infect 2007;135:1145-50).
[49] Even in circumstances where much more information is available, assessment of the relative importance of viral measurements at disease onset, timing and rate of changes, and magnitude and timing of any reported treatment-related changes has been difficult and sometimes controversial for acute viral illnesses: see the discussion of virologic measurements and their uses in the guidance document "Influenza: Developing Drugs for Treatment and/or Prophylaxis," available on the FDA website at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064980.htm.
[50] Dixon 1948 p. 362, "As pointed out by Ricketts & Byles (1908) and others, smallpox patients either die on the first or second day in the fulminating type, between the twelfth and fourteenth day from toxaemia, or from sepsis or bed sores in the third week or later."
[51] Henderson et al. (1999) p. 2130.
[52] Councilman et al. (1904) thought skin, soft palate, pharynx, esophagus pustules/pocks "fundamentally specific and due to the presence of a parasite peculiar to the disease"; hematopoietic/lymphoreticular, kidney, and liver abnormalities among "Associated lesions of indeterminate specificity"; and "Associated lesions, bacterial in origin" to be frequent contributors to pathogenesis "through channels created by [variola] or incidental to the lowering of resistance." Numerous authors before, at the time, and since differed in opinion on one or more of these issues but aside from the pathogen's terminologic change from "parasite" to virus, similar conclusions have been reflected in many other sources.
[53] Examples, laryngeotracheal obstruction: Brayton 1903. Heart failure and pulmonary edema: Ricketts 1908. Antigen-antibody complexes: Fenner 1988, Henderson 1999 Toxemia: Dixon. Bacterial superinfection: Councilman et al. Koplan and Foster 1972 cited above: "…the cause of death of this infection has not been clearly established..." (recommends expectant fluid and electrolyte management, treatment of superinfection, and attempts to treat DIC based on clinical picture). Bras G (The morbid anatomy of smallpox. Documenta de Medicina Geographica et Tropica 1952;4:303-51) noted bronchitis or pneumonia in a substantial proportion of his 177 autopsies (including one orangutan), but often characterized as "mild" – therefore perhaps not considered as primary cause of death. Found hemorrhage of varying types and degrees, raising questions about classification – also found distinction between discrete and confluent to be "impracticable." Found vesicles in all purpura cases. Lillie RD(Smallpox and vaccinia: the pathologic histology. Arch Pathol 1930;10:241-91) reviewed literature from late 19th and early 20th century and described bronchopneumonia as a major factor in varying proportions of patients across different autopsy series, also with variable reports of the relative contribution of primary viral infection and bacterial superinfection; also described reports of renal tubular degeneration, cloudy swelling in liver, and lymphadenopathy, lymphoid necrosis, or lymphadenitis. Councilman et al autopsy series (1904) described wide variety of pulmonary, hepatic, renal, and cardiac findings, including "cloudy swelling" of liver and/or myocardium in some cases; referred to days 10-13 as "lethal period"; reported "a general invasion of the tissues by cocci seems to be one of the most characteristic features of the disease" and these "are brought to the lesions by the blood" and "streptococcus septicemia is so generally present in fatal cases that it can almost be regarded as a part of the disease."
[54] See for example Jahrling PB et al., Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. PNAS 2004;101:15196-200.
[55] See Martin DB, The cause of death in smallpox: an examination of the pathology record. Mil Med 2002;167:546-51. Concludes "…symptoms are nonspecific until the papules first appear at the height of the viremia. Available evidence suggests that a patient's fate is determined at this point…."

[56] Coagulation abnormalities: Roberts JF et al. Haemorrhagic smallpox. 1. Preliminary haematological studies. Bull WHO 1965;33:607-13. McKenzie PJ et al. Haemorrhagic smallpox. 2. Specific bleeding and coagulation studies. Bull WHO 1965;33:773-82.
[57] See for example Perkins RG, Pay GO, Streptococcus pyogenes in variola. J Med Res 1903;10:180-195. These authors reported streptococci cultured not only from autopsy material and postmortem blood cultures but also from antemortem blood cultures in 7 of 8 fatal cases and 1 of 5 nonfatal cases, but not from most lesion cultures; they considered the bacteria "subsidiary" but possible contributors to "hasten death" or "delay convalescence" in some cases, but too diverse for antiserum treatment. Even after midcentury, Rao AR, Prahlad I, Swaminathan M (A study of 1000 cases of smallpox, J Ind Med Assoc 1960;35:296-307) reported "Deaths from smallpox were mostly due to septicaemia produced by secondary invading organisms." Ikeda K (The blood in purpuric smallpox. JAMA 1925;84:1807-13) reported some cases of "purpuric" smallpox had cocci visible on peripheral blood smear, while others had negative blood cultures.
[58] Fenner et al. p. 49, "In ordinary-type variola major the rash affected the sebaceous glands severely (Bras, 1952b) and permanent facial pockmarks occurred in 65-80% of survivors (Mack et al., 1970; Jezek et al., 1978d). The sebaceous glands were not affected in flat-type smallpox and the few patients who survived this type of infection were rarely severely pockmarked." Some authors described the scars in survivors of malignant or flat disease categories as superficial, papery, and/or similar to burn scars.
[59] Dixon 1962 p. 105, "It is important to appreciate that while the scarring on the remainder of the body diminishes with the passage of time, scarring on the face is more noticeable at the end of a year than it is when the patient is first discharged from the hospital." P. 100, "many patients who on discharge from hospital have only level pigmented scars, which the clinician hopes will resolve without a blemish, show classical pocks when seen six months to a year later"
[60] Jezek Z, Basu RN, Arya ZS. Problem of persistence of facial pock marks among smallpox patients. Ind J Publ Health 1978;22:95-101. These authors also reported differences in scar persistence between male and female patients, and noted that the age differences they observed (children more likely to escape scarring) were in contradiction to an earlier publication showing an opposite distribution.
[61] Jezek Z, Hardjotanojo W. Residual skin changes in patients who have recovered from variola minor. Bull WHO 1980; 58:139-40.
[62] Jezek Z, Hardtonojo W, Rangaraj AG. Facial scarring after varicella. A comparison with variola major and variola minor. Am J Epidemiol 9181;114:798-803.
[63] Bacteria were variably reported to be present in smallpox skin lesions, and possibly related to systemic sepsis. See for example Hines LE, The bacteriology of the skin lesions in smallpox, JID 1922;31:89-91.
[64] Bras G. Observations on the formation of smallpox scars. Arch Pathol 1952;54:149-56.
[65] Regan TD, Norton SA. The scarring mechanism of smallpox. J Am Acad Dermatol 2004;50:591-4.
[66] See for example Dixon 1962, Boeckh 1946.
[67] See for example Stolte JB, Sas GJ. Chloramphenicol and A.C.T.H in smallpox. Lancet 1951;2:715-7. Possible lower fever, greater "sense of well-being" noted in one of 3 cases but authors "feel…A.C.T.H. in this disease is fraught with danger." Steroids were noted as given to one of the 3 fatal cases in Aralsk (citations above) and have been mentioned in treatment of recent cowpox cases (discussed below).
[68] See for example Dixon 1948; Johnstone D, Fluker J, A suggested scheme for smallpox treatment. Month Bull Min Health 1949;8:188-9. Corkill 1951; Krishnamurthy 1951 (both described in more detail below); Stolte & Sas 1951 cited above; Marsden JP, Coughlan WJ, Antibiotics in smallpox, Lancet 9151;2:711-3; Breen GE, Antibiotics in smallpox, Lancet 1951;2:713-4.
[69] For example, Dixon (1948) compared 51 patients classified as "benign" confluent, semiconfluent, or discrete, receiving penicillin 30,000 units q4h from about days 5-8 of rash, to 105 "similar" cases for whom no penicillin was available, and reported no effect of earlier initiation on papular or vesicular rash but a marked improvement in the subsequent pustule and scabbing stages, with early indications of only "very superficial scarring" but uncertainty about subsequent "weathering" effects; 0 vs. 9 "septic complications"; and "slightly shorter" length of hospitalization but not statistically significant and in any case determined mostly by time to shed all scabs. Corkill NL (Epidemic smallpox in Jiddah. Treatment with antibiotics. BMJ 1951;2:663-7) suggested case fatality was lower in an outbreak where penicillin, streptomycin, and "aureomycin" were used, apparently comparing with historical expectations; believed suppuration was reduced making nursing easier and fever less persistent. Krishnamurthy VN (Treatment of smallpox. Antiseptic 1951;48:656-8) reported among 16 cases (treated with topical spray) "all the 4 controls, one of

the two cases treated with Penicillin and both the cases treated with Streptomycin died, but 7 out of 8 cases treated with combined spraying of Penicillin and Streptomycin survived with very little pitting or scarring."

[70] Teissier P, Marie PL, Essais de sérothérapie variolique. Compt Rendu Acad Sci 1921;155:1536-9: reported recovery after convalescent serum injection (usually subcutaneous) in 1 patient with hemorrhagic smallpox, 3 grave variola with hemorrhagic rash, 3 of 6 confluent variola including a pregnant women, and 1 of 3 young children. Couzi G, Kircher JP, Immunothérapie de la variole, Bull Inst Hyg Maroc 1941;59-68, reported dramatic improvement in outcomes of seriously ill patients, by comparison to their previous experience with sulfa and xylol – referring to the latter as "le traitement classique de la variole." Johnstone and Fluker (cited above) reported trying convalescent serum in a few cases with "…no evidence that it was of therapeutic value…."

[71] Downie AW, Dumbell KR, Pox viruses. Annu Rev Microbiol 1956;10:237-252: "Most of the experimental work on chemotherapy in the pox virus group has used vaccinial infection of tissue cultures, chick embryos, and mice as model systems." Later authors reported limited work in monkey models: e.g. Rao AR et al., Preparation HOE 105 (citenazone) in chemoprophylaxis of variola in monkeys: a preliminary communication. Ind J Med Res 1973;61:818-30, reported one of the oral thiosemicarbazones (apparently not one of those selected for human clinical trials) appeared to lessen disease in variolated monkeys when administered as late as 72 hours but not at 96 hours after viral inoculation.

[72] Focus of this tabulation is on compounds that were studied in cell culture and/or animal models and then in clinical trials in established smallpox illness.

[73] Marboran is the best known of a number of thiosemicarbazone derivatives reported to have some activity against poxviruses. See for example Bauer DJ et al., The chemotherapy of variola major infection. Bull WHO 1962:26:727-32. Marsden JP. Case of malignant smallpox treated with Compound 33T57. Br Med J 1962;2:524. Ker FL. Smallpox treated with Compound 33T57. Br Med J 1962;2:734. Bauer et al., Prophylactic treatment of smallpox contacts with N-methylisatin β-thiosemicarbazone (compound 33T57, Marboran). Lancet 1963;2:191-6. Ferguson DL, Some observations on the role of methisazone ('Marboran') in the prophylaxis of smallpox in a rural area. S A Med J 1964;38:868-9. Landsman JB, Grist NR. Controlled trial of Marboran on group vaccinated against smallpox. Lancet 1964;1:330. Bauer DJ. Clinical experience with the antiviral drug Marboran® (1-methylisatin 3-thiosemicarbazone). Ann N Y Acad Sci 1965;130:110-7. De Valle et al., Methisazone in prevention of variola minor among contacts. Lancet 1965;2:976-8. Bauer DJ. Chemoprophylaxis of smallpox and treatment of vaccinia gangrenosa with 1-methylisatin 3-thiosemicarbazone. Antimicrob Agents Chemother 1965;5:544-7. Herrmann EC Jr. Sensitivity of herpes simplex virus, vaccinia virus, and adenoviruses to deoxyribonucleic acid inhibitors and thiosemicarbazones in a plaque suppression test. Appl Microbiol 1968;16:1151-5. Rao AR et al. Chemoprophylaxis and chemotherapy in variola major. Part I. An assessment of CG 662 and Marboran in prophylaxis of contacts of variola major. Ind J Med Res 1969;57:477-83. Rao AS et al. Chemoprophylaxis and chemotherapy in variola major. Part II. Therapeutic assessment of CG 662 and Marboran in treatment of variola major in man. Ind J Med Res 1969;57:484-94. Bauer DJ et al. Prophylaxis of smallpox with methisazone. Am J Epidemiol 1969;90:130-45. Cho CT et al. Methisazone and monkey pox virus: studies in cell cultures, chick embryos, mice and monkeys. Am J Epidemiol 1970;92:137-44. Heiner GG et al. Field trials of methisazone as a prophylactic agent against smallpox. Am J Epidemiol 1971;94:435-49.

[74] Sidwell RW et al. In vivo antiviral properties of biologically active compounds. II. Studies with influenza and vaccinia viruses. Appl Microbiol 1968;16:370-92. Dixon GJ et al. Antiviral activity of 9-β-D-arabinofuranosyladenine. V. Activity against intracerebral vaccinia virus infections in mice. Antimicrob Agents Chemother 1968;8:172-9. Allen LB, Sidwell RW. Target-organ treatment of neurotropic virus diseases: efficacy as a chemotherapy tool and comparison of activity of adenine arabinoside, cytosine arabinoside, idoxuridine, and trifluorothymidine. Antimicrob Agents Chemother 1972;2:229-233. Klein RJ, Friedman-Kien AE, Brady E. Treatment of poxvirus infections in rabbits with 9-β-D-arabinofuranosyladenine. Antimicrob Agents Chemother 1974;5:409-12. Allen LB et al. Inhibition of experimental deoxyribonucleic acid virus-induced encephalitis by 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate. Antimicrob Agents Chemother 1975;8:468-73. Nishiyama S et al. Comparative study of the antiviral activity of acyl derivatives of 2,2'-anhydro-1-β-D-arabinofuranosylcytosine and other nucleosides against encephalitis in mice. Antimicrob Agents Chemother 1977;11:198-201. Babiuk LA et al. Comparison of the antiviral effects of 5-methoxymethyl-deoxyuridine with 5-iododeoxyuridine, cytosine arabinoside, and adenine arabinoside. Antimicrob Agents Chemother 1975;8:643-50. Sidwell RQ

et al. Viral keratitis-inhibitory effect of 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate. Antimicrob Agents Chemother 1975;8:463-7. Hyndiuk RA et al. Treatment of vaccinial keratitis with vidarabine. Arch Ophthalmol 1976;94:1363-4. Worthington M, Cunliffe M. Treatment of fatal disseminated vaccinia virus infection in immunosuppressed mice. J Gen Virol 1977;36:329-33. Werner GT, Metzger E, Sauer O. Adenine arabinoside monophosphate in experimental vaccinia virus infections. Adv Ophthalmol 1979;38:72-81. Koplan JP et al. Treatment of variola major with adenine arabinoside.., JID 1979;131:34-9.

[75] Slack R et al., A new antiviral agent: 4-bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone, M and B 7714. Nature 1964;204:587; Rao AR, McFadzean JA, Squires S, The laboratory and clinical assessment of an isothiazole thiosemicarbazone (M & B 7714) against pox viruses, Ann NYAS 1965;130:118-27; Rao AR, McFadzean JA, Kamalakshi K. An isothiazole thiosemicarbazone in the treatment of variola major in man. A controlled trial and laboratory investigations. Lancet 1966;1:1068-72; Rao AR et al., Assessment of an isothiazole thiosemicarbazone in the prophylaxis of contacts of variola major, Lancet 1966;1:1072-4

[76] de Clercq E et al., Effect of cytosine arabinoside, iododeoxyuridine, ethyldeoxyuridine, thiocyanatodeoxyuridine, and ribavirin on tail lesion formation in mice infected with vaccinia virus. Proc Soc Exper Biol Med 1976;151:487-90; Herrmann EC Jr, Sensitivity of herpes simplex virus, vaccinia virus, and adenoviruses to deoxyribonucleic acid inhibitors and thiosemicarbazones in a plaque suppression test. Appl Microbiol 1968;16:1151-5; Tagaya I, Amano H, Incomplete inhibition of variola virus focus formation in HeLa cell cultures by cytosine arabinoside. Microbiol. Immunol 1977;21:335-8. Hossain MS et al., Treatment of smallpox with cytosine arabinoside. Lancet 1972;2:1230-2. Monsur KA et al., Treatment of variola major with cytosine arabinoside. JID 1975;131:40-3 ("Whereas the isolations from throat and skin, which occurred in both treated and untreated patients, reflect the inability of drug to penetrate the pustular lesions, the isolation of virus from blood on day 7 from three of four patients treated with Ara-C (compared with zero of six control patients) suggests that the drug itself may have interfered with the defense mechanisms of the body.") Dennis DT et al., Failure of cytosine arabinoside in treating smallpox. Lancet 1974;2:377-9.

[77] Rada B, Blaškovič D. Some characteristics of the effects of 6-azauridine on vaccinia virus multiplication, in comparison with those of 5-iododeoxyyuridine. Acta Virol 1966;10:1-9. Jaffari SMH, Hussain A, 6 azauridine in smallpox. Ind Jour Med Res 1969;57:809-14. Elis J, Rašková H (New indications for 6-azauridine treatment in man. A review. Eur J Clin Pharmacol 1972;4:77-81) report what appear to be the same patients but do not reference the earlier publication nor mention the "further studies" said to be "in progress" at that time. Koplan et al (above) refer to this study as "inadequately designed" and unconfirmed. A related drug, studied for psoriasis, was removed from the US market in the 1970s because of thrombotic events (Gitel SN et al. The thrombogenicity of 6-azauridine. Haemostasis 1979;8:54-57).

[78] See Rao et al. article on CG 662 and Marboran cited above.

[79] See for example Boeck V, Smallpox among US soldiers in Korea, Bull US Army Med Dept 1946;6:45-58 and Small pox among US Army forces in Korea, J Mil Med Pacific 1946;2:2-16. Respiratory symptoms were occasionally also reported during the pre-eruptive period (Angulo JJ, Perderneiras AA, Megale P, Sakuma ME. Variola minor in Braganca Paulista County, 1956. Bull Soc Pathol Exot 1977;70:282-8).

[80] See for example Haviland 1952; Ikeda 1925 ("It was a frequent experience in the receiving ward of this hospital to find clinically purpuric cases sent in undiagnosed or as suspected scarlet fever or measles during and even subsequent to the height of the epidemic.").

[81] See for example Ricketts 1908, Dixon 1948 ("in mild cases modified by successful vaccination the eruption tends to be as superficial as that of chickenpox"), Marsden 1958 ("the usual mistake is to regard an attack of modified smallpox as chicken-pox because it is mild"); Seward JF et al., Development and experience with an algorithm to evaluate suspected smallpox cases in the United States, 2002-2004, CID 2004;39:1477-83, Moore ZS et al., Chickenpox or smallpox;: the use of the febrile prodrome as a distinguishing characteristic. CID 2004;39:1910-7 ("7%-17% of unvaccinated chickenpox case patients meet the smallpox febrile prodrome criteria"); CDC, Investigation of a smallpox rumor – Mexico, MMWR 1985;34:343-4); ProMEDmail Archive Number 20100325.0953 (March 25, 2010), Undiagnosed illness – Uganda (Bukuda) chickenpox suspected; also note potential for concurrent circulation (Sarkar JK et al., Concurrent smallpox and chickenpox, Bull WHO 1976;54:119-122); and occasional other rarities (Mukherjee MK et al., Coxsackie virus infection simulating smallpox. Indian J Dermatol 1976;22:86-8). Top FH and Peck LE (A small outbreak of smallpox in Detroit. Am J Publ Health 1943;33:490-498) report

an outbreak in which 6 of the eventual 18 cases were initially diagnosed as chickenpox. Madeley CR (Diagnosing smallpox in possible bioterrorist attack, Lancet 2003;361:97-8, "…differentiating smallpox from varicella, is difficult even when those concerned are seeing both diseases regularly.") Kerrod E, Geddes AM, Regan M, Leach S, Surveillance and control measures during smallpox outbreaks, Emerg Infect Dis 2005;11:291-7, mention outbreak control difficulties in Liverpool 1902-3 associated with misdiagnosis as chickenpox. Gregory G (Observations on vaccination and small-pox, more especially with reference to the theory of vaccine influence, and the relation subsisting between the cicatrix and the character of the consecutive variola. Med Chir Trans 1841;24:15-29) referred to mild smallpox cases in vaccinated persons as "varicelloid" and listed "varicella vera" as a diagnosis in several non-smallpox patients admitted to the smallpox hospital.

[82] See for example Downie A, MacDonald A, Smallpox and related virus infections in man. Brit Med Bull 1953;9:191-5. "In our experience cases of generalized vaccinia are not as uncommon as the literature suggests. We have during the last few years examined material from several cases in adults vaccinated for the first time during smallpox outbreaks. In such cases the focal lesions in the skin may be similar to those of variola although the distribution is not so decidedly centrifugal as in that disease…."

[83] Boeck (cited above) did report lymphadenopathy as part of the smallpox prodrome in soldiers in Korea, but authors directly comparing the two diseases tended to remark on prominent and sometimes massive lymphadenopathy as characteristic of monkeypox but not of smallpox. Bras 1952 smallpox autopsy series "Like other investigators, we saw very few changes in the lymph glands." Councilman 1904 smallpox autopsy series reported nodes "may show some swelling" but often not. Lillie reported lymphadenopathy described by some 19th century authors on smallpox.

[84] See for example Alcamí A, Smith GL. Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity. J Virol 1995;69:4633-9. Gubser C. Smith GL. The sequence of camelpox virus shows it is most closely related to variola virus, the cause of smallpox. J Gen Virol 2002;83:855-72. Shchelkunov SN et al. Analysis of the monkeypox genome. Virology 2002;297:172-94. Gileva IP et al. Properties of the recombinant TNF-binding proteins from variola, monkeypox, and cowpox viruses are different. Biochim Biophys Acta 2006;1764:1710-8. Ciulla E et al. Evolutionary history of orthopoxvirus proteins similar to human complement regulators. Gene 2005;355:40-7. Esposito JJ et al. Genome sequence diversity and clues to the evolution of variola (smallpox) virus. Science 2006;313:807-12. Liszewski MK et al. Structure and regulatory profile of the monkeypox inhibitor of complement: comparison to homologs in vaccinia and variola and evidence for dimer formation. J Immunol 2006;176:3725-3734. Weaver JR, Isaacs SN. Monkeypox virus and insights into its immunomodulatory proteins. Immunol Rev 2008;225:96-113. Alzhanova D et al. Cowpox virus inhibits the transporter associated with antigen processing to evade T cell recognition. Cell Host Microbe 2009;6:433-45. Hughes AL, Irausquin S, Friedman R. The evolutionary biology of poxviruses. Infect Genet Evolut 2010;10:50-9. O'Gorman WE et al. Alternate mechanisms of initial pattern recognition drive differential immune responses to related poxviruses. Cell Host Microbe 2010;8:174-85. Hendrickson RC et al. Orthopoxvirus genome evolution: the role of gene loss. Viruses 2010;2:1933-67. Alzhanova D, Früh K. Modulation of the host immune response by cowpox virus. Microbes Infect 2010;12:900-9. Bahar MW et al. How vaccinia virus has evolved to subvert the host immune response. J Struct Biol 2011;175:127-34.

[85] Jezek Z, Kriz B, Rothbauer V. Camelpox and its risk to the human population. J Hyg Epidemiol Microbiol Immunol 1983;27:29-42. Bera BC et al. Zoonotic cases of camelpox infection in India. Vet Microbiol 2011;152:29-38.

[86] See for example Grey SE, Belshe RB. Poxvirus zoonoses – putting pocks into context. N Engl J Med 2004;350:324-7. Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. Vet Microbiol 2010;140:229-36. Reynolds MG et al. The incidence of molluscum contagiosum among American Indians and Alaska natives. PLoS One 2009;4:e5255.

[87] See for example Magrath G, Brinckerhoff WR. On experimental variola in the monkey. J Med Res 1904;11:230-47. Brinckerhoff WR, Tyzzer EE. A critical review of the literature on experimental variola and vaccinia in the monkey. J Med Res 1906;14:223-31. Brinckerhoff WR, Tyzzer EE. On the occurrence of variola vera in monkeys and in the Orang Utan. J Med Res 1906;14:308-20. Hahon N. Smallpox and related poxvirus infections in the simian host. Bacteriol Rev 1961;25:459-76. . Janssen RJ et al. The effects of 6-mercaptopurine on variola infections in rhesus monkeys. I. The influence of the drug on the resistance and immunological response of the infected host. JID 1962;111:155-162. Cheville

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