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The development and approval of tecoviromat (TPOXX®), the first antiviral against smallpox

Michael Merchlinsky[a,*], **Andrew Albright**[a], **Victoria Olson**[b], **Helen Schiltz**[c], **Tyler Merkeley**[a], **Claiborne Hughes**[a], **Brett Petersen**[b], **Mark Challberg**[c]

[a]Biomedical Advanced Research and Development Authority, 300 C Street SW, Washington DC, 20201, USA

[b]National Center for Emerging and Zoonotic Infectious Disease, Centers for Disease Control and Prevention, Mail Stop G-06, 1600 Clifton Road, NE, Atlanta, 30333, Georgia

[c]National Institute of Allergy and Infectious Diseases, National Institutes of Health, MSC 9825, 5601 Fishers Lane, Rockville, MD, 20851, USA

Abstract

The classification of smallpox by the U.S. Centers for Disease Control and Prevention (CDC) as a Category A Bioterrorism threat agent has resulted in the U.S. Government investing significant funds to develop and stockpile a suite of medical countermeasures to ameliorate the consequences of a smallpox epidemic. This stockpile includes both vaccines for prophylaxis and antivirals to treat symptomatic patients. In this manuscript, we describe the path to approval for the first therapeutic against smallpox, identified during its development as ST-246, now known as tecovirimat and TPOXX®, a small-molecule antiviral compound sponsored by SIGA Technologies to treat symptomatic smallpox. Because the disease is no longer endemic, the development and approval of TPOXX® was only possible under the U.S. Food and Drug and Administration Animal Rule (FDA 2002). In this article, we describe the combination of animal model studies and clinical trials that were used to satisfy the FDA requirements for the approval of TPOXX® under the Animal Rule.

Keywords

Tecovirimat; Smallpox; Variola virus; Antiviral therapy; FDA animal rule

1. The threat of smallpox: past, present, future

Smallpox is one of the most consequential infectious diseases in human history; estimates place the death toll in the 20th century alone at nearly 300 million. Mortality in smallpox epidemics has been historically around 33%. Attempts to control smallpox began with the practice of variolation, where infectious variola virus was inoculated in the skin or inhaled through the nose. Later, the discovery of vaccination by Edward Jenner as an effective prophylaxis against smallpox led, with its implementation, to reduced mortality in much of

*Corresponding author.: Michael.merchlinsky@hhs.gov (M. Merchlinsky.

the world. Advances in vaccine manufacture and stability led the World Health Organization (WHO) in the 1950s to initiate a campaign designed to eradicate smallpox through vaccination. This campaign, which was accelerated greatly in the late 1960s and 1970s, resulted in the declaration by WHO that smallpox was eradicated in May 1980 (Fenner et al., 1988). In the latter stages of the campaign, eradication of smallpox was accomplished by intense surveillance to identify and isolate cases combined with a focused vaccination campaign relying on ring vaccination of contacts and suspected contacts of symptomatic cases of smallpox. Although research on antiviral drugs during this timeframe identified compounds that showed efficacy against poxviruses in tissue culture or mice, none of the compounds demonstrated efficacy when used in patients with smallpox.

While variola virus no longer exists in nature, the possibility of preserved, unrecognized samples or clandestine stocks (Arita, 2014), the potential re-emergence from natural sources, and recent advances in synthetic biology describing the construction of horsepox virus *de novo*, demonstrating that smallpox virus could be constructed from chemical constituents (Noyce et al., 2018) mean the threat of the reemergence of smallpox is not zero. The only legal sources of variola are maintained by the governments of the United States and Russian Federation at WHO-sanctioned repositories, which contain stocks of *Variola virus major* in each country. The eradication of smallpox as a public health threat, the cost and effort associated with mass vaccination, and the serious and sometimes fatal complications associated with the replication-competent vaccines used during the eradication campaign, have led to a diminished public health justification for vaccination of the general population. However, should a smallpox release occur today, it would take place in an unprotected population in the absence of residual or “herd” immunity, meaning that much of the public will be susceptible to infection. The potential consequences of a smallpox epidemic in the present population has led the government of the United States to consider smallpox a high-priority threat, tasking civilian and defense agencies to develop strategies and products to counter that threat.

2. The U.S. Government response to public health threats

2.1. Creation of novel infrastructure: the PHEMCE

To address the varied public health threats that could impact the U.S. population in the 21st century, the government has created a unique infrastructure primarily within the Department of Health and Human Services (HHS) for the development of drugs, biologics, vaccines and devices to serve as medical countermeasures (MCM) against these threats. The agencies responsible for supporting the development of these products work in tandem through a cooperative enterprise known as the Public Health Emergency Medical Countermeasures Enterprise (PHEMCE). Comprised of the Biomedical Advanced Research and Development Authority (BARDA), the National Institutes of Health (and primarily the National Institute of Allergy and Infectious Diseases (NIAID), the Department of Defense (DoD), CDC, FDA, and the Department of Homeland Security (DHS), the PHEMCE works to promote a streamlined approach to drug and device development aimed at addressing a wide range of national and health security threats.

The PHEMCE is designed so that each step of medical countermeasure development is supported by the appropriate arm of the government. The early stages of product identification, proof of concept studies, and early clinical safety trials are supported by NIAID and DoD with FDA oversight. The support and development of products at the later stages of regulatory evaluation proved most difficult as the resources and acumen for manufacturing and late stage clinical trials were not available outside of the major pharmaceutical companies. Under the Pandemic and All Hazards Preparedness Act (PAHPA), since reauthorized as the Pandemic and All Hazards Preparedness Reauthorization Act (CR, 2013), BARDA was created to seek promising MCM candidates under development by NIAID or DoD and provide funding and support to carry out the late-stage development and regulatory approval of MCMs so they could be stored by the US government for deployment in an emergency. Through funding provided under Project BioShield, BARDA, in coordination with sister agencies in the PHEMCE, has achieved the approval of over 40 products and has added a number of MCMs to the Strategic National Stockpile (SNS).

2.2. The Animal Rule

Products for many of the threats the PHEMCE addresses are difficult to evaluate because the disease or syndrome is very rare or does not exist in nature. Traditional routes of regulatory evaluation are impossible because it is neither feasible to recruit sufficient numbers of patients into a clinical trial nor ethical to expose patients to these specific pathogens to test the efficacy of a drug, biologic or vaccine. In 2009, in an attempt to address this conundrum, FDA released guidance on a novel regulatory pathway established in 2002 called the FDA Animal Rule 21 CFR 314 Subpart I (Approval of New Drug When Human Efficacy Studies Are Not Ethical or Feasible) which was updated and further refined in 2015 (FDA, 2015). The Animal Rule allows sponsors to demonstrate a product's efficacy in animal models in lieu of a human efficacy trial. When using the Animal Rule to evaluate products, all aspects of the regulatory evaluation of a product except for efficacy, such as safety and demonstration of good manufacturing practices, follow traditional product development pathways. Safety is demonstrated by a phase 3 clinical trial in healthy adults designed to detect adverse events at a rate appropriate for the product indication. The requirement for efficacy evaluation using animal models has led FDA to describe the characteristics needed for an animal model to provide support for the approval of a product. A relevant animal model must satisfy four requirements:

1. the disease and the mechanism by which the countermeasure reduces or prevents disease are both well-understood
2. countermeasure efficacy is demonstrated in one or multiple animal models that are considered to be well-characterized and adequate for demonstration of efficacy
3. efficacy endpoints in the animal model are clearly related to the desired outcome in humans such as improved survival or reductions in major morbidity, and
4. the human dose may be selected using data from animals treated at efficacious dose levels.

2.3. Defending against a return of smallpox

The primary weapon in the armamentarium to respond to a smallpox emergency will be vaccines designed to protect the general population from infection if exposed to smallpox. Prophylactic vaccination will prevent the spread and therefore mitigate the magnitude the epidemic. The rare, but significant, adverse events associated with the replication competent vaccines against smallpox, combined with the cost of universal vaccination means that prophylactic universal vaccination is unlikely to be US policy, and in fact routine vaccination was discontinued in the US after 1972 (CDC, 1971). After one is exposed to variola, the ability of the vaccine to protect against smallpox drops off rapidly after a few days (Keckler et al., 2013) and since the response to a smallpox emergency among the civilian population will most likely occur after the detection of sentinel cases, there will be a cohort that can only be protected with a therapeutic countermeasure. Although there were no obvious candidates from the campaign to eradicate smallpox, advances in our understanding of poxvirus molecular biology, the development of high throughput screening, and the successful development of antiviral compounds to treat both RNA and DNA viruses led us to believe a similar approach would work on a virus that expressed over two hundred genes, and thus has numerous potential targets for direct antiviral intervention.

3. The discovery and early evaluation of TPOXX®

In early 2002, NIAID launched an initiative to identify smallpox drugs by evaluating libraries of compounds for the ability to interfere with the replication of vaccinia or cowpox in tissue culture. These viruses are similar to variola but can be studied at BSL-2 facilities. This “prescreen” was performed using libraries of compounds derived from already licensed products and libraries composed of novel chemical entities. Over 300,000 compounds were screened, with potential activity detected for nine chemical scaffolds (Jordan et al., 2010a). The best activity was observed for the tricyclononene carboxamides, and after testing analogs, the lead candidate, a 4-trifluoromethyl phenol derivative, was initially named ST-246 (Jordan et al., 2010a). One of the encouraging observations in the high throughput screening was the lack of cytotoxicity at the concentrations where the compound was active.

3.1. Antiviral activity and mechanism of action

The mechanism of action for TPOXX® was derived by incubating the drug and vaccinia virus in tissue culture at concentrations of the drug that allowed resistant virus to develop. Every resistant virus had a mutation that mapped to the gene corresponding to F13L in vaccinia Copenhagen. Surprisingly, the F13L protein is not required for replication of the virus but is observed during morphogenesis between the outer membrane of the intracellular infectious form of the virus and the new membrane derived from the trans-Golgi. The protein interacts with components of the trans-Golgi that wrap the infectious intracellular viral particles to form the triple-wrapped virus prior to transport to the cell surface and release. Poxviruses replicate by establishing cytoplasmic “factories” where abundant double-membraned infectious virus is produced. Some of the infectious virus is converted into a triplewrapped form that fuses with the cellular membrane before release into the extracellular space. These extracellular viruses accelerate the spread of the infection both *in vitro* and *in vivo*. This mechanism for viral spread appears to be common to all

orthopoxviruses, as the F13L gene is highly conserved throughout all species. The antiviral activity observed in the tissue culture screening did not arise from inhibition of viral replication, but from inhibition of viral spread from cell to cell (Yuang et al, 2005; Grosenbach et al., 2011).

3.2. Initial studies in mouse models of orthopoxvirus infection

The ability of TPOXX® to treat poxvirus infections was tested in several lethal challenge mouse models in both immunocompetent and immunodeficient mice. Treatment of orthopoxvirus-infected immune compromised mice resulted in prolonged survival as long as the drug was present, but when treatment was stopped the disease reappeared and progressed to mortality. The drug was able to cure immunocompetent mice in that it slowed the spread of disease long enough for the host to mount an immune response and clear the virus. These results are consistent with its inhibitory effect of the virus through the interruption of the spread of disease, rather than through the inhibition of replication or destruction of the virus (Berhanu et al., 2009; Grosenbach et al., 2010; Zaitseva et al., 2013).

3.3. In vitro inhibitory activity against authentic variola virus

The first step in demonstrating the efficacy of TPOXX® against smallpox was the demonstration of the ability of TPOXX® to inhibit the spread of infection in tissue culture. The use of live variola virus is highly regulated, with only two sanctioned laboratories permitted to work with the virus, the CDC BSL-4 facility in Atlanta and a Russian Federation lab in Novosibirsk. Experiments can be performed only after gaining approval of research plans by the WHO Advisory Committee on Variola Virus Research (ACVVR). In order to demonstrate directly the antiviral activity of TPOXX® on variola virus, CDC petitioned WHO ACVVR and received permission to test the antiviral activity *in vitro*. TPOXX® was also tested *in vitro* against a virus stock derived from the central African isolate of monkeypox virus associated with substantial mortality in humans, to determine if the drug would be effective in limiting this human pathogenic poxvirus infection. The data from CDC evaluations confirmed that both viruses were susceptible to inhibition by TPOXX® (Smith et al., 2009).

3.4. Further evaluation under the Animal Rule

The demonstration of potent anti-poxvirus activity and the low level of cytotoxicity suggested that TPOXX® could be an excellent candidate therapeutic against smallpox. The regulatory evaluation of TPOXX® as a smallpox therapeutic required application of the FDA Animal Rule. Evaluation under the Animal Rule would require, in addition to the traditional manufacturing and clinical development steps necessary for drug development, the design and utilization of animal models for efficacy evaluation which meet the qualities spelled out in the Animal Rule guidance document. Meeting these conditions require an understanding of the disease and how it causes morbidity and mortality in both the animal model and humans, an ability to measure the drug exposures associated with efficacy, and a demonstration of efficacy in more than one animal model. Meeting these requirements increases the confidence that the animal model will be predictive of the ability of the countermeasure to affect the outcome of the disease in humans.

3.4.1. Development of an animal model for smallpox—Although the last publicly acknowledged case of smallpox occurred in 1979, the observation of clinical cases during the latter stages of the eradication campaign demonstrated that smallpox was a systemic disease with an incubation period of 7–17 days followed by a prodromal fever preceding a synchronous centrifugal rash (Fenner et al., 1988). The rash pustules were active sites of viral replication and indicative of the severity of the systemic infection as patients with large numbers of lesions were less likely to survive. In the simplest sense, the outcome of the disease was the race between the ability of the systemic disease to kill the host and the ability of the host to generate an immune response that brings the viral infection under control. This is supported by the observation that the highest mortality occurred in populations with the least robust immune systems, such as pregnant women, the very young, and the elderly. The molecular basis for these observations was found with subsequent research on poxviruses which indicates their host range appears to be a function of the immunomodulatory gene products that each virus expresses and the interaction of these gene products with the host immune system. Variola virus encodes many proteins which appear to interfere with the normal immune response to infection in humans (Bratke et al., 2013).

In nature, variola virus has only been observed in humans, posing a challenge in establishing an animal model for the evaluation of MCMs to treat or prevent smallpox. The most obvious animal model to facilitate evaluation of TPOXX® against smallpox would be a nonhuman primate (NHP) infected with variola virus. Research done in the Soviet Union prior to the eradication of smallpox implied some higher apes were susceptible to smallpox and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) team led by Drs. Peter Jahrling and John Huggins carried out a series of experiments at CDC exploring the feasibility of a NHP variola model. After a comprehensive set of experiments exploring different strains of variola virus, doses, and routes of administration, the difficulty in using this species for evaluation of smallpox countermeasures under the Animal Rule were apparent (Jahrling et al., 2004; Wahl-Jensen et al., 2011). Infection of cynomolgus macaques with variola virus at high doses, by aerosol to mimic natural exposure or by intravenous infusion, rarely resulted in illness or death. Fewer than half of the monkeys died at exposures of 1×10^8 pfu delivered intravenously. When the virus dose was increased to 1×10^9 pfu delivered intravenously all animals became sick and died but the resulting disease was emblematic of the rare, but universally fatal, hemorrhagic form of smallpox, or the rare form classified as flat smallpox, where in contrast to normal rash observed in most smallpox cases, the lesions expand to form a continuous unbroken pustule. Therefore, this model did not satisfy the criteria for relevance under the FDA Animal Rule since it would be difficult to demonstrate statistically relevant reduction in mortality without extremely large study groups and the disease, when fatal, did not resemble the typical clinical rash disease observed in most humans, calling into question the predictive value of this model. Experiments using TPOXX® in limited numbers of cynomologus macaques infected with variola were attempted (Huggins et al., 2009; Mucker et al., 2013). In these studies it was impossible to show a statistically meaningful improvement in mortality against smallpox since the group sizes were too small and some of the untreated animals survived. However, a reduction in lesion count and oral viral shedding was observed in treated animals, an

indirect, but encouraging, sign of efficacy as higher lesion count and viremia level were seen to be predictive of mortality in humans with smallpox (Fenner et al., 1988).

3.4.2. Use of nonhuman primate models of orthopoxvirus disease under the Animal Rule—During the early stages of product development for smallpox vaccines, the regulatory path for their evaluation was not established and efforts to develop models for evaluation of the vaccines under the FDA Animal Rule were explored. Using the intravenous challenge model used to test the efficacy of next generation vaccines (Earl et al., 2004) as a starting point, an interagency group was established and supported by NIAID to suggest lines of research and to evaluate potential animal models for regulatory use. In the end, this effort was not applied to the evaluation of smallpox vaccines since their evaluation was predicated on non-inferiority trials comparing their elicited immune response to vaccines with demonstrated efficacy against smallpox. However, the group pioneered many of the techniques subsequently used by product developers and BARDA to establish appropriate animal models for regulatory use including a central repository of a well-characterized challenge reagent, proficiency testing at multiple locations, and use of statistics to identify symptoms and triggers for medical intervention.

The animal model best characterized by this group, the intravenous challenge of cynomolgus macaques with monkeypox virus, became the central model used for TPOXX® development. The intravenous challenge model recapitulates the latter stages of a smallpox infection, essentially skipping the incubation period, by infecting with an intravenous bolus of monkeypox that disseminates systemically. The infected monkeys display a synchronous centrifugal rash approximately 4 days post infection, become feverish and progressively more ill, reaching euthanasia criteria around 10 days post-infection (Huggins et al., 2009). Since the intended indication for TPOXX® is as a therapeutic against smallpox, a model which establishes a systemic disease in monkeys similar to the latter stages of a smallpox infection is appropriate for evaluation of therapeutic efficacy. The high mortality and reproducibility of this model made it applicable to evaluation of TPOXX® under the Animal Rule.

Research with monkeypox requires BSL-3 containment. There is much greater capacity for animal research at facilities with BSL-3 containment rooms compared to variola virus research that can only be carried out at the CDC BSL-4 lab. The requirements for personal protection for BSL-4 research make studies meeting FDA requirements for good laboratory practices more difficult to carry out. In order to provide coverage long enough to ensure the host could mount an immune response, a 14-day treatment regimen was used beginning three or four days post-challenge. Initiation of treatment four days post-challenge was considered a therapeutic model of efficacy since all monkeys exhibited the rash diagnostic of smallpox infection in humans. A series of challenge studies using this model were supported by NIAID and DoD and carried out at the USAMRIID facilities at Fort Detrick. In these studies the effective dose of TPOXX® was determined and studies showing dose response, the effect of delaying treatment and the effect of shortening treatment duration were performed (Jordan et al., 2009; Huggins et al., 2009).

4. The creation of BARDA and the further development of TPOXX

BARDA was created by Congressional legislation in 2007 to shepherd the late-stage development of MCMs and provide them in sufficient quantities to the SNS to address potential emergencies. BARDA achieves this through the establishment of public-private partnerships where BARDA provides funds and subject matter expertise to expedite the regulatory pathway for the MCM under development by a sponsor. One of the responsibilities assigned to BARDA was the development of therapeutic antivirals against smallpox. After releasing a request for proposals for smallpox antivirals, a contract was awarded to SIGA Technologies, Incorporated (New York, New York) in 2009 for the development and acquisition of a smallpox therapeutic. At the time of contract award, there were two main problems slowing the development of TPOXX®. The first problem was the optimization of the manufacture of TPOXX® and the second was the demonstration of efficacy under the existing animal models.

4.1. Optimization of manufacture

SIGA made significant advances while under NIAID support in establishing a reliable manufacturing process with defined starting materials. SIGA collaborated with the manufacturing subject matter experts at BARDA to optimize the process to ensure production of a single isomer at sufficient scale to meet the USG requirements for the SNS. In order to facilitate the response to large medical emergencies, Congress established a series of storage facilities called the SNS located across the United States to hold and distribute MCMs, devices, and equipment in an emergency. A MCM can be delivered to the SNS when the MCM has demonstrated sufficient safety and efficacy and good manufacturing practices (GMP) to allow distribution under an Emergency Use Authorization (EUA). The EUA is a regulatory mechanism allowing the distribution of MCMs after the declaration of a medical emergency by the Secretary of Health and Human Services. The pre-EUA status is not considered a regulatory endpoint, but recognition of amassing sufficient data during pursuit of approval to allow widespread distribution without the level of informed consent and oversight expected in a clinical trial when a product is under Investigational New Drug (IND) status prior to approval. The FDA has regulatory mechanisms in place, such as the Emergency IND or Emergency Expanded Access Protocols to provide countermeasures under IND to a limited number of patients in an emergency (FDA,2018a). The effective response to some smallpox emergency scenarios would require the widespread distribution of TPOXX®. A package containing the safety data from the phase 1 safety trial, the efficacy data from the NHP monkeypox (MPOX) model, and the data demonstrating GMP from the engineering runs supported by BARDA was submitted to FDA by CDC since it is the USG agency expected to distribute the drug in an emergency. The first doses of TPOXX® were delivered to the SNS under pre-EUA in early 2013.

4.2. Demonstration of efficacy under the Animal Rule

The second major hurdle for the development of TPOXX® was determining the path to demonstrate efficacy using animal models to satisfy the Animal Rule. The obvious problems with a variola virus challenge model meant that the only data supporting efficacy was from

the indirect evidence from the intravenous monkeypox virus challenge model. In order to solicit input on possible pathways for demonstrating efficacy for smallpox countermeasures under the Animal Rule, the FDA's Center for Drug Evaluation and Research (CDER) organized an Antiviral Drugs Advisory Committee in December 2011, consisting of a panel of scientists and clinicians. The committee was asked to address the following agenda: "The committee will discuss pathways for the development of drugs intended to treat variola virus infection (smallpox) in the event of an outbreak, including the use of animal models of other orthopoxviruses (the group of viruses that includes smallpox) as potential evidence of efficacy". Presentations were made to the panel describing the present status of research supporting the efficacy of TPOXX® and potential orthopoxvirus animal models by representatives from FDA, CDC, BARDA, NIAID, sponsors of smallpox countermeasures including SIGA, and members of the scientific community conducting orthopoxvirus research. The consensus of the committee was that models based on orthopoxvirus infections in susceptible hosts could be used to support the efficacy of countermeasures against smallpox. The committee also felt it was important to test the countermeasure in multiple models to increase confidence in its efficacy, particularly due to the difficulty in executing animal studies at BSL-4 facilities and the limitations of the variola virus challenge model.

The committee recommended further studies in three animal models: the monkeypox virus intravenous challenge model in NHPs developed by NIAID; the rabbitpox virus intradermal challenge model in rabbits; and the intranasal challenge model of ectromelia virus (mousepox) in Balb/C mice (FDA, 2011). At the time of the advisory committee meeting, the rabbit and mouse models had been described in reports in academic scientific journals, but had not been evaluated to see if they met the stringent standards required for evaluation of countermeasures under the Animal Rule. The application of multiple animal models to the evaluation of smallpox antivirals was only feasible if the mechanism of action of the countermeasure is the same in each model, the antiviral target gene product is conserved among the challenge viruses, and the activity of the countermeasure is not host-specific, all of which are true for TPOXX®.

Recognizing the critical role that animal models occupied in regulatory development, BARDA established a mechanism to develop animal models and carry out nonclinical studies in a product independent manner. A number of contract research organizations (CROs) were evaluated by BARDA for their ability to execute animal studies at a level commensurate for submission to FDA for product development. The eligible CROs could respond to task orders released by BARDA for solving specific nonclinical challenges. After the discussion at the Advisory Committee, contracts were awarded to members of this network to develop both the intradermal rabbitpox virus challenge model in rabbits and the intranasal ectromelia virus challenge model in mice. In both cases, the CRO used the published data describing the publication history of the models in the scientific literature (Adams et al., 2007; Parker et al., 2010) as a starting point and performed additional studies to determine if the models could serve to evaluate smallpox countermeasures under the Animal Rule. Characterization of the model included the use of uninfected animals during natural history studies to ensure potential symptoms of disease and triggers for medical intervention were reproducible, unambiguous and objective, the use of statistical evaluation

of these triggers, and the use of serial timed euthanasia and necropsy to follow the disease progression.

A pre-IND was established with CDER for protocol submission and feedback prior to study execution and a dialogue with CDER was maintained throughout the model characterization process including face-to-face meetings post-study to explain the results and solicit advice, resulting in both models achieving a level of stringency acceptable for product evaluation under the Animal Rule (Garver et al., 2016; Perry et al., 2018). The intent of the studies was to provide a framework of an animal model for any product developer to adapt to their needs. This approach saves time and money as it obviates the need for each product sponsor to develop an independent animal model. In addition, it provides the most humane approach since fewer animals will be used if the model is developed once and shared among product developers. BARDA did not use the data from the studies to solicit CDER for specific study designs, but instead provided the data to all developers so they could approach CDER and design an efficacy study with the appropriate triggers for medical intervention.

After discussions with CDER, SIGA Technologies decided to test the efficacy of TPOXX® using the rabbitpox virus intradermal challenge rabbit model as a second model to evaluate efficacy under the FDA Animal Rule in addition to the efficacy data already submitted using the MPOX model in NHPs. Rabbits were inoculated with a lethal dose of rabbitpox virus and four days post-challenge given placebo or 14 days of TPOXX®. Every animal treated with placebo reached pre-determined euthanasia criteria between days 5 and 10 post infection while nearly every rabbit that received TPOXX® survived at each of the four drug concentrations tested. At four days post challenge, all rabbits had fever and contained measurable levels of rabbitpox virus DNA in the blood. The two rabbitpox virus challenge studies and four TPOXX® efficacy studies in NHPs were performed under good laboratory practices (GLP), a level of documentation, data oversight, and monitoring equivalent to that expected for a clinical trial. A study run under GLP also utilizes validated assays and is subject to FDA inspection. This level of oversight is expected by FDA if an Animal Rule study is to be used as the basis of the efficacy data for the regulatory approval of any drug, including TPOXX®.

5. Establishing human dosage for the treatment of smallpox

The most difficult part of evaluating a drug for approval under the Animal Rule is arriving at a dose of the drug that is reasonably assumed to provide clinical benefit when used to treat the disease in humans. Regulatory evaluation using the Animal Rule still requires the demonstration of drug safety through clinical trials in healthy adults. The results from these trials, in conjunction with those from toxicology studies in animal models, help establish the limits of safety, and in doing so, define the upper limit of the human dose. Efficacy is demonstrated in animal models that satisfy the requirements of the FDA Animal Rule. The process used to establish an efficacious human dose through bridging efficacy and safety data is best summarized in the most recent version of the Animal Rule Guidance published by FDA in 2015 (FDA, 2015). This document describes in detail the process by which FDA was able to use the results from efficacy and safety studies to arrive at a dose for the approval of TPOXX®.

5.1. Obtaining pharmacodynamic and pharmacokinetic data

The dose of a drug approved under the Animal Rule relies on good pharmacodynamics (pD) and pharmacokinetics (pK) data. The pK and pD of the drug can be easily measured in uninfected humans in clinical trials and in both uninfected and infected animals treated at multiple drug doses including that used to demonstrate efficacy. In the case of the evaluation of TPOXX® under the Animal Rule the arguably most important piece of data, the pK and pD of the drug in people infected with smallpox, are the only data that cannot be collected. The sponsor can triangulate between the pK data in uninfected and infected animals and uninfected humans to establish a human dose if the pK and pD parameters in infected animals are easily predicted from the observed exposures in the uninfected animals. If the pK data is the same in both infected and uninfected animals, it is reasonable to assume that the observed pK parameters in uninfected humans are a good estimate of drug exposures during infection. If the pK parameters are different in uninfected and infected animals, it may be impossible to predict the drug exposure in a human infected with the disease. For TPOXX®, the pK parameters were conserved between infected and uninfected animals.

5.2. Choosing an animal model for modeling the human dose

The second consideration for establishing the human dose is picking the appropriate animal model for dose selection. Since efficacy had been demonstrated in more than one animal model, FDA chose to model the human dose from the least favorable animal model, i.e. the animal model requiring the highest exposures as measured by pK and pD parameters to demonstrate efficacy. Analysis of the efficacy data by SIGA indicated that the mean steady state concentration, or maintaining a sufficiently high C_{min}, was the most important correlate of efficacy. This association makes logical sense considering the mechanism of action for TPOXX® is blocking a viral-cellular protein interaction associated with the transition of infectious intracellular virus to an extracellular form of the virus that can exit the cell. Even a transitory drop in TPOXX® concentration could result in the production of the extracellular virus which could exit the cell and establish new independent infections since TPOXX® has no effect on virus entry or replication. The drug exposures required for efficacy in the NHP model, as determined by C_{min} values were higher than those required for efficacy in rabbits. This is consistent with the biology of the challenge models, since the MPOX model, because of its intravenous route of challenge, establishes an immediate systemic infection which is treated four days later as lesions form, whereas the intradermal challenge in the rabbit model establishes a local infection, and symptoms for intervention arise coincident with the viral spread associated with the establishment of the systemic disease, slightly sooner than intervention in the MPOX model. Therefore, pK data in the NHPs was used to model the human dose (Jordan et al., 2009; Leeds et al., 2013).

5.3. Determining the human dose

The last consideration in assigning the appropriate human dose is the selection of a dose which can reasonably be expected to be effective since the pK and pD parameters exceed those measured at the effective dose in animal studies and is safe because the pK and pD parameters are lower than observed at the doses associated with adverse events, taking into account the data from clinical safety trials and toxicology studies. For TPOXX®, the only

disturbing safety signal was the observation of seizures in dogs at very high C_{max} values. In a series of phase 1 clinical safety studies, TPOXX® was safe, with only mild adverse events detected at the tested doses and regimens. Another consideration in establishing the TPOXX® dose was food-related bioavailability, because the uptake of TPOXX® was approximately twice as effective when the patient took the drug while ingesting a meal with fat compared to the fasted state (Chinsangaram et al., 2012; Jordan et al., 2010b). The mortality data from dose-ranging efficacy studies in the MPOX model demonstrated that a statistically significant increase in survival was observed at doses at or above 3 mg/kg. When secondary endpoints, such as lesion count and viral load were also considered there was a dose dependent benefit that resulted in a reduction in disease severity at or above doses of 10 mg/kg. The human dose was modeled after the more conservative 10 mg/kg treatment regimen associated with maximum clinical benefit. When SIGA modeled the human dose using a standard two compartment analysis, the data supported a human dose of 400 mg/day dose to provide everyone coverage equivalent to or higher than the 3 mg/kg dose in NHPs with a 600 mg/day dose providing similar exposures equivalent to the 10 mg/kg dose in NHPs (Amantana et al., 2013). The FDA modeling was consistent with these observations but in order to ensure adequate exposures, even in the fasted state, CDER suggested that SIGA run their pivotal safety trial at 600 mg given twice daily (bis in die, BID).

5.4. Performance of a pivotal safety trial

SIGA conducted a placebo-controlled safety trial in 449 adult volunteers, with 359 receiving TPOXX® at 600 mg BID. The pK data showed that, in a fed or fasted state, exposure levels exceeded the exposures observed in the 10 mg/kg dose in NHPs. Importantly, the pK parameter associated with efficacy, the C_{min}, was 4-fold-8-fold higher in humans than in the MPOX model at a dose of 10 mg/kg. In addition to this study in healthy adults, SIGA executed a series of clinical trials in specific patient groups such as those with renal or liver deficiencies, performed drug-drug- interaction studies, and other studies that are part of a normal drug development pathway to inform FDA as to the proper use of the drug. The pK and pD results from the clinical trial were consistent with the expected values from the dose modeling performed by FDA ensuring that everyone will receive a drug exposure high enough to be effective and low enough to be safe.

6. Final evaluation and approval of TPOXX for the treatment of smallpox

CDER convened an Antimicrobial Drugs Advisory Committee Meeting (AMDAC) on May 1, 2018 to evaluate the package of data associated with the development of TPOXX® (tecovirimat) and to address the question: “Based on the available data, does the risk-benefit profile of tecovirimat support its use for the treatment of human smallpox?” The committee was unanimous in its approval that TPOXX® was appropriate for treatment of smallpox. The majority of the session consisted of discussions focusing on the role of TPOXX® in a smallpox response such as post-exposure prophylactic use, its potential use with vaccination, and urging the medical community to acquire more information as to its utility in special populations such as pediatrics and pregnant women (FDA, 2018b). On July 13, 2018, FDA approved the license for TPOXX® as NDA 208,627 under the provisions of 21 CFR 314

Subpart I (Approval of New Drug When Human Efficacy Studies Are Not Ethical or Feasible).

7. Plans for the future

The approval of TPOXX® provides the public health community with a countermeasure to treat patients in a smallpox emergency that previously had no recourse. The studies performed during its development showed the wide applicability of the compound as it has demonstrable efficacy when used against monkeypox virus as a post-exposure prophylactic, and as a compassionate treatment for those experiencing adverse events from vaccination with vaccinia virus. BARDA and the USG are still supporting the development of smallpox antivirals since the approval of another smallpox therapeutic with an alternative mechanism of action would provide the opportunity for combination treatment to greatly reduce the chances that antiviral resistance will arise to reduce the effectiveness of treatment At present, SIGA has manufactured and delivered two million treatment courses of TPOXX® to the SNS with the last delivery occurring in September 2017. Presently, SIGA is developing alternative formulations of TPOXX® to treat dysphagic and pediatric populations and will continue to manufacture and deliver TPOXX® as required to the SNS to maintain preparedness.

8. Lessons learned from the development and approval of TPOXX

TPOXX® development was only possible through a collaborative effort including all members of the PHEMCE. The initial screening and identification of TPOXX® was spearheaded by CDC and NIAID; the early stages of product manufacture, the proof of concept animal studies, the development of a NHP animal model, and phase 1 studies were supported by NIAID through multiple grants and contract funds; the critical nonclinical studies to demonstrate efficacy were performed by DoD at USAMRIID facilities; and the support of GMP manufacturing, support of the clinical trials necessary to demonstrate safety, the development of the second animal model required to demonstrate efficacy were provided by BARDA. Even so, without the support of FDA through frequent interactions, including an Advisory Committee meeting canvasing the scientific community for appropriate animal models for efficacy evaluation, and discussions to design studies to inform the appropriate safe human dose, the approval of TPOXX® would not have been possible. It was only through the collaborative effort of the PHEMCE that SIGA was able to leverage the technical and financial expertise to negotiate the regulatory path and provide the American public with a new and vital addition to the armamentarium against smallpox (Grosenbach et al., 2018).

The approval of TPOXX® represents the first approval of a small molecule from discovery to licensure using the FDA Animal Rule. The development and addition of this therapeutic small molecule provides a vital tool in the USG response to a public health threat from smallpox as the cost of goods, stability, and operational advantages for distribution makes TPOXX® an ideal MCM in a smallpox emergency. The approval of TPOXX® provides a path, as circuitous as it may appear, for the approval of additional small molecules for public health response.

References

Adams MM, Rice AD, Moyer RW, 2007 Rabbitpox virus and vaccinia virus infection of rabbits as a model for human smallpox. J. Virol 81, 11084–11095. [PubMed: 17686856]

Amantana A, Chen Y, Tyavanagimatt, Jones KF, Jordan R, Chinsangaram J, Bolken TC, Leeds JM, Hruby DE, 2013 Pharmacokinetics and interspecies al-lometric scaling of ST-246, an oral antiviral therapeutic for treatment of orthopoxvirus infection. PLoS One 8 (4), e0061514.

Arita I, 2014 Discovery of forgotten variola specimens at the National Institutes of Health in the USA. Expert Rev. Anti. Infect. Ther 12, 1419–1421. [PubMed: 25373507]

Berhanu A, King DS, Mosier S, Jordan R, Jones KF, Hruby DE, Grosenbach DW, 2009 Infection ST-246 inhibits in vivo poxvirus dissemination, virus shedding, and systemic disease manifestation Antimicrob. Agents Chemother 53, 4999–5009. [PubMed: 19752270]

Bratke KA, McLysaght A, Rothenburg S, 2013 A survey of host range genes in poxvirus genomes. Infect. Genet. Evol 14, 406–425. [PubMed: 23268114]

CDC, 1971 Public Health Service recommendations on smallpox vaccination. MMWR (Morb. Mortal. Wkly. Rep.) 20, 339.

Chinsangaram J, Honeychurch KM, Tyavanagimatt S, Leeds JM, Bolken TC, Jones KF, Jordan R, Marbury T, Ruckle J, Mee-Lee D, Ross E, Lichtenstein I, Pickens M, Corrado M, Clarke JM, Frimm AM, Hruby DE, 2012 Safety and pharmacokinetics of the anti-orthopoxvirus compound ST-246 following a single daily oral dose for 14 days in human volunteers. Antimicrob. Agents Chemother. 56, 4900–4905. [PubMed: 22777041]

CR, 2013 Pandemic and All-Hazards Preparedness Reauthorization Act 2013 Title IV S. 401402 (USA).

Earl PL, Americo JL, Wyatt LS, Eller LA, Whitbeck JC, Cohen GH, Eisenberg RJ, Hartmann CJ, Jackson DL, Kulesh DA, Martinez MJ, Miller DM, Mucker EM, Shamblin JD, Zwiers SH, Huggins JW, Jahrling PJ, Moss B, 2004 Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. Nature 428, 182–185. [PubMed: 15014500]

FDA, 2002 New drug and biological drug products: evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not feasible or ethical Fed. Register 2002 67 (105), 37988–37998.

FDA, 2011 In: U.S. Food and Drug Administration. Summary Minutes of the Antiviral Drugs Advisory Committee Meeting Held December 14–15 2011, . http://wayback.archive-it.org/7993/20170113193108/http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AntiviralDrugsAdvisoryCommittee/UCM294882.pdf.

FDA, 2015 Product Development under the Animal Rule Guidance for Industry. https://www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm399200.htm.

FDA, 2018a Home Page for EUA. www.fda.gov/EmergencyPreparedness/default.htm.

FDA, 2018b In: Summary Minutes for the Anti-infective Drugs Advisory Committee Meeting, May 1, 2018, . https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/UCM605890.pdf.

Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID, 1988 Smallpox and its Eradication. World Health Organization, Geneva, Switzerland.

Garver J, Weber L, Vela EM, Anderson M, Warren R, Merchlinsky M, Houchens C, Rogers JV, 2016 Ectromelia virus disease characterization in the BALB,/c mouse: a surrogate model for assessment of smallpox medical countermeasures. Viruses 8, 203–221.

Grosenbach DW, Berhanu A, King DS, Mosier S, Jones KF, Jordan R, Bolken TC, Hruby DE, 2010 Efficacy of ST-246 versus lethal poxvirus challenge in im-munodeficient mice. Proc. Natl. Acad. Sci. USA 107, 838–843. [PubMed: 20080762]

Grosenbach DW, Jordan R, Hruby D, 2011 Development of the Small-Molecule Antiviral ST-246 as a Smallpox Therapeutic. Future Virol. 6, 653–671. [PubMed: 21837250]

Grosenbach DW, Honeychurch K, Rose EA, Chinsangaram J, Frimm A, Maiti B, Lovejoy C, Meara I, Long P, Hruby DE, 2018 Oral tecovirimat for the treatment of smallpox. N. Engl. J. Med 379, 44–53. [PubMed: 29972742]

Huggins J, Goff A, Hensley L, Mucker E, Shamblin J, Wlazlowski C, Johnson W, Chapman J, Larsen T, Twenhafel N, Karem K, Damon IK, Byrd CM, Bolken TC, Jordan R, Hruby D, 2009 Nonhuman primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246. Antimicrob. Agents Chemother 53, 2620–2625. [PubMed: 19349521]

Jahrling PB, Hensley LE, Martinez MJ, Leduc JW, Rubins KH, Relman DA, Huggins JW, 2004 Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. Proc. Natl. Acad. Sci. U.S.A. 101, 15196–15200. [PubMed: 15477589]

Jordan R, Goff A, Frimm A, Corrado ML, Hensley LE, Byrd CM, Mucker E, Shamblin J, Bolken TC, Wlazlowski C, Johnson W, Chapman J, Twenhafel N, Tyavanagimatt S, Amantana A, Chinsangaram J, Hruby DE, Huggins J, 2009 ST-246 antiviral efficacy in a nonhuman primate monkeypox model: determination of the minimal effective dose and human dose justification. Antimicrob. Agents Chemother. 53, 1817–1822. [PubMed: 19223621]

Jordan RJ, Leeds JM, Tyavanagimatt S, Hruby D, 2010a Development of ST-246 for treatment of poxvirus infections. Viruses 2, 2409–2435. [PubMed: 21994624]

Jordan R, Chinsangaram J, Bolken TC, Tyavanagimatt SR, Tien D, Jones KE, Frimm A, Corrado ML, Pickens M, Landis P, Clarke J, Marbury TC, Hruby DE, 2010b Safety and pharmacokinetics of the antiorthopoxvirus compound ST-246 following repeat oral dosing in healthy adult subjects. Antimicrob. Agents Chemother 54, 2560–2566. [PubMed: 20385870]

Keckler MS, Reynolds MG, Damon IK, Karem KL, 2013 The effects of post-exposure smallpox vaccination on clinical disease presentation: addressing the data gaps between historical epidemiology and modern surrogate model data. Vaccine 31, 5192–5201. [PubMed: 23994378]

Leeds JM, Fenneteau F, Gosselin NH, Mouksassi MS, Kassir N, Marier JF, Chen Y, Grosenbach D, Frimm AE, Honeychurch KM, Chinsangaram J, Tyavanagimatt SR, Hruby DE, Jordan R, 2013 Pharmacokinetic and pharmacodynamics modeling to determine the dose of ST-246 to protect against smallpox in humans. Antimicrob. Agents Chemother 57, 1136–1143. [PubMed: 23254433]

Mucker EM, Goff AJ, Shamblin JD, Grosenbach DW, Damon IK, Mehal JM, Holman RC, Carroll D, Gallardo N, Olson VA, Clemmons CJ, Hudson P, GHruby DE, 2013 Efficacy of tecovirimat (ST-246) in nonhuman primates infected with variola virus (smallpox). Antimicrob. Agents Chemother 57, 6246–6253. [PubMed: 24100494]

Noyce RS, Lederman S, Evans DH, 2018 Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS One 13 (1), e0188453. [PubMed: 29351298]

Parker S, Siddiqui AM, Painter G, Schriewer J, Buller RML, 2010 Ectromelia virus infections of mice as a model to support the licensure of anti-orthopoxvirus therapeutics. Viruses 2, 1918–1931. [PubMed: 21994714]

Perry MR, Warren R, Merchlinsky M, Houchens C, Rogers JV, 2018 Rabbitpox in New Zealand white rabbits: a therapeutic model for evaluation of poxvirus medical countermeasures under the FDA Animal Rule Fron. In Cell. and Inf. Micro 8, 1–13.

Smith SK, Olson VA, Karem KL, Jordan R, Hruby DE, Damon IK, 2009 In vitro efficacy of ST-246 against smallpox and monkeypox. Antimicrob. Agents Chemother. 53, 1007–1012. [PubMed: 19075062]

Wahl-Jensen V, Cann JA, Rubins KH, Huggins JW, Fisher RW, Johnson AJ, de Kok-Mercado F, Larsen T, Raymond JL, Hensley LE, Jahrling PB, 2011 Progression of pathogenic events in cynomolgus macaques infected with variola virus. PLoS One 6, e24832. [PubMed: 21998632]

Yuang G, Pevear DC, Davies MH, Collett MS, Bailey T, Rippen S, Barone L, Burns C, Rhodes G, Tohan S, Huggins JW, Baker RO, Buller RML, Touchette E, Waller K, Schriewer J, Neyts J, DeClerq E, Jones K, Hruby D, Jordan R, 2005 An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge. J. Virol 79, 13139–13149. [PubMed: 16189015]

Zaitseva M, Shotwell E, Scott J, Cruz S, King LR, Manischewitz J, Diaz CG, Jordan R, Grosenbach DW, Golding H, 2013 Effects of postchallenge administration of ST-246 on dissemination of IHD-J-Luc vaccinia virus in normal mice and in immune-deficient mice reconstituted with T cells. J. Virol 87, 5564–5576. [PubMed: 23468500]