Antiviral Research 143 (2017) 278–286

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Efficacy of delayed brincidofovir treatment against a lethal rabbitpox virus challenge in New Zealand White rabbits

Irma M. Grossi [a, *], Scott A. Foster [a], Melicia R. Gainey [b], Robert T. Krile [b], John A. Dunn [a], Thomas Brundage [a], Jody M. Khouri [a]

[a] Chimerix, Durham, NC, USA
[b] Battelle, West Jefferson, OH, USA

ARTICLE INFO

Article history:
Received 22 November 2016
Accepted 4 April 2017
Available online 7 April 2017

Keywords:
Antiviral
Brincidofovir
CMX001
Orthopoxvirus
Smallpox

ABSTRACT

In the event of a bioterror attack with variola virus (smallpox), exposure may only be identified following onset of fever. To determine if antiviral therapy with brincidofovir (BCV; CMX001) initiated at, or following, onset of fever could prevent severe illness and death, a lethal rabbitpox model was used. BCV is in advanced development as an antiviral for the treatment of smallpox under the US Food and Drug Administration's 'Animal Rule'. This pivotal study assessed the efficacy of immediate versus delayed treatment with BCV following onset of symptomatic disease in New Zealand White rabbits intradermally inoculated with a lethal rabbitpox virus (RPXV), strain Utrecht. Infected rabbits with confirmed fever were randomized to blinded treatment with placebo, BCV, or BCV delayed by 24, 48, or 72 h. The primary objective evaluated the survival benefit with BCV treatment. The assessment of reduction in the severity and progression of clinical events associated with RPXV were secondary objectives. Clinically and statistically significant reductions in mortality were observed when BCV was initiated up to 48 h following the onset of fever; survival rates were 100%, 93%, and 93% in the immediate treatment, 24-h, and 48-h delayed treatment groups, respectively, versus 48% in the placebo group ($p < 0.05$ for each vs. placebo). Significant improvements in clinical and virologic parameters were also observed. These findings provide a scientific rationale for therapeutic intervention with BCV in the event of a smallpox outbreak when vaccination is contraindicated or when diagnosis follows the appearance of clinical signs and symptoms.

1. Introduction

Variola virus, an orthopoxvirus and the etiologic agent of smallpox, is responsible for one of the most severe infectious diseases throughout recorded history. The mortality rate from smallpox was ~30% in endemic populations, with death typically occurring ~24–28 days following infection (Fenner et. al., 1988), and survivors are often afflicted by complications including blindness, limb deformities, and various neurologic sequelae (Fenner et al., 1988; Peterson and Damon, 2014). Following a worldwide vaccination campaign, the World Health Organization (WHO) declared smallpox eradicated in 1980 (Fenner et al., 1988).

Consequently, however, 'herd immunity' has been lost, leaving the world's population highly vulnerable to smallpox morbidity and mortality.

Prior to its eradication, smallpox was globally ubiquitous, and owing to the likely existence of undeclared stocks of variola virus retained outside of WHO-designated repository laboratories in the US and Russia (Hansen, 2012), as well as the potential for modern synthetic recreation of the virus from its genomic sequence (Henderson et al., 1999; Strikas et al., 2008), smallpox remains a significant threat due to its potential for use as a biologic weapon. Today, a single case of smallpox would be considered a national public health emergency; accordingly, the US government has advanced strategies to prepare for a possible outbreak.

A vaccine for smallpox (e.g., ACAM2000® [Sanofi Pasteur Biologics LLC], or Dryvax® [Wyeth Laboratories]) is available and considered the first line of defense in an outbreak.

However, there is only a short window (~3 days) following exposure where administration of the vaccine may be beneficial (Centers for Disease Control and Prevention, 2016; Hanna and Baxby, 2002). Although both ACAM2000 and Dryvax are

* Corresponding author. Chimerix, 2505 Meridian Parkway, Suite 100, Durham, NC 27713, USA.
E-mail address: ggrossi@chimerix.com (I.M. Grossi).

http://dx.doi.org/10.1016/j.antiviral.2017.04.002
0166-3542/

considered safe vaccines, serious post-vaccination adverse events can occur (Sanofi Pasteur, 2016). Moreover, the vaccine is contraindicated in people identified as having a higher risk for developing post-vaccination complications and in pregnant women (Fulginiti et al., 2003; Kemper et al., 2002). Accordingly, administering the vaccine to the general population in the absence of an endemic threat is neither practical nor recommended. Therefore, therapeutic agents that can be used for the treatment of smallpox in populations where vaccination is contraindicated, as well as following the onset of symptoms in the event of a smallpox release, are needed.

Brincidofovir (BCV; CMX001) is an orally bioavailable lipid conjugate of cidofovir (CDV) that is converted intracellularly into the active antiviral, CDV-diphosphate (CDV-PP; Hostetler, 2010). The efficacy of BCV as a treatment for smallpox is being evaluated under the US Food and Drug Administration (FDA)'s 'Animal Rule'.

New Zealand White rabbits (NZW) intradermally inoculated with rabbitpox virus (RPXV), strain Utrecht, is a well-characterized animal model of lethal orthopoxvirus infection (Chapman et al., 2010; Adams et al., 2007; Rice et al., 2011b). RPXV causes a disease course in rabbits that closely resembles that of smallpox in humans. This includes an asymptomatic incubation period followed by disseminated infection characterized by fever, severe respiratory complications, secondary skin lesions, and a high mortality rate (Chapman et al., 2010; Adams et al., 2007; Rice et al., 2011b). The ~2-week course of disease in RPXV infection mirrors the 4-week course of human smallpox infection, with disease progression in rabbits approximately three times the rate of the human disease course (Fig. 1) (Chapman et al., 2010; Rice et al., 2011a,b).

Treatment with BCV upon development of secondary lesions using this model has been evaluated in proof-of-concept efficacy studies (Rice et al., 2011a,b), where it showed statistical significance in reducing mortality. BCV also demonstrated significant reductions in mortality when treatment was initiated following the detection of secondary lesions in a blinded, randomized Phase II study. The lowest effective dose regimen from that study, 20 mg/kg followed by 5 mg/kg doses at 48 and 96 h, was selected for use in the current study (Trost et al., 2015). Unlike secondary lesions, fever has been shown to be a more reliable and objective clinical indicator of RPXV infection following challenge for establishing the onset of the disease (Rice et al., 2011b; Nalca and Nichols, 2011), and was therefore designated as the randomization trigger for treatment in the current study.

The primary goal of this study was to assess the survival benefit, compared to placebo, of an efficacious dose regimen of BCV in rabbits (20/5/5 mg/kg at 48-h intervals) that produces rabbit exposures less than or equal to human exposures associated with the intended human dose regimen for smallpox. Additionally, the study was designed to identify the window of effective therapeutic intervention by means of immediate and delayed treatment following confirmation of disease onset in a lethal RPXV animal model of orthopoxvirus infection. The design of this study doubled as a Phase 3 pivotal study under the FDA Animal Rule, intended to both provide evidence of efficacy for regulatory approval and to provide additional information to guide BCV use in the event of a real world release setting. Secondary objectives included evaluation of the incidence, severity, and progression of clinical events associated with RPXV infection when immediate or delayed BCV treatment was administered compared with placebo. RPXV DNA viral load and infectious virus, as measures of potential infectivity, were also evaluated.

2. Materials and methods

The study was conducted at Battelle's Biomedical Research Center (West Jefferson, OH, USA) in compliance with the US FDA's Good Laboratory Practice guidance.

2.1. Test systems

NZW rabbits were received from Covance Research Products (Denver, PA, USA) and maintained according to Battelle's standard operating procedures. One week prior to infection with RPXV, rabbits were implanted with temperature transponder chips for monitoring body temperature (Transponder Type: IPTT-300; BioMedic Data Systems, Inc, Seaford, DE).

2.2. Study challenge

Plaque-purified RPXV, strain Utrecht, master stock was obtained from Richard W Moyer (University of Florida College of Medicine, Gainesville, FL, USA) and prepared as described by Trost et al. (2015). A target lethal inoculum of 300 plaque-forming units [PFU]) was diluted in Dulbecco's phosphate-buffered saline. RPXV inoculations were staggered over 2 days, with the actual virus concentration administered confirmed by plaque assay as 350 PFU and 316 PFU by day of inoculation. A total of 146 NZW aged 17 weeks (+ 4 and + 5 days) and weighing 2.0–2.7 kg were inoculated with the intention to randomize at least 24 animals to each treatment group. Ketamine (22–50 mg/kg) and xylazine (3–10 mg/kg) were administered intramuscularly to the epaxial muscles of the lower back to anesthetize animals prior to challenge.

Anesthesia complications resulted in the deaths of two animals during challenge. RPXV was administered intradermally as bilateral injections of equal volume (i.e., target of 150 PFU in 100 μL per injection) to the thighs of each rabbit (Trost et al., 2015).

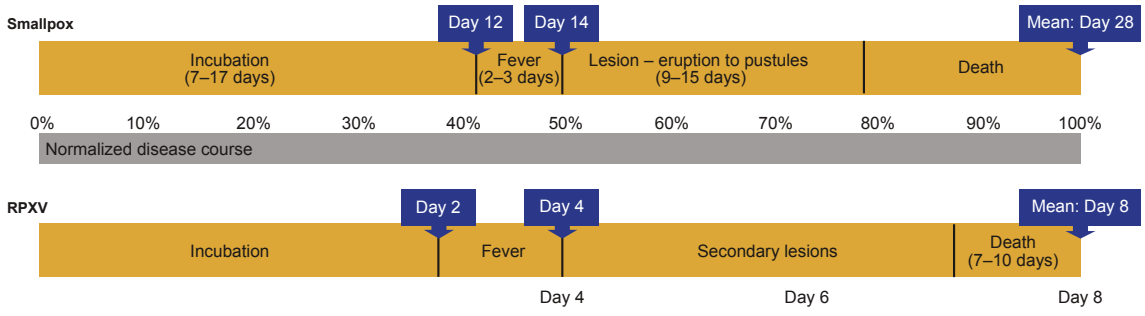

Fig. 1. Comparison of the disease course for rabbitpox and smallpox. RPXV, rabbitpox virus. Adapted from information in (Chapman et al., 2010; Rice et al., 2011a,b).