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RESEARCH ARTICLES

Efficacy of three key antiviral drugs used to treat orthopoxvirus infections: a systematic review

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Abstract

Background: In a global political climate increasingly concerned about terrorism, bioterrorism agents such as smallpox would undoubtedly be catastrophic. Since WHO announced the eradication of smallpox in 1980, consequently discontinuing the worldwide vaccination campaign, today's population is either immunologically naïve or has waning levels of protection. Further, up to 25% of today's population are contraindicated for smallpox vaccination due to various immunodeficiency conditions. The aim of this study was to evaluate the efficacy of the anti-DNA antivirals cidofovir (CDV), brincidofovir (BCV) and tecovirimat against smallpox and other orthopoxviruses. As of July 2018, FDA approved tecovirimat as the first treatment for smallpox.

Methods: A systematic review was conducted to identify relevant literature describing the efficacy and safety of CDV, BCV and tecovirimat including *in vitro* and *in vivo* animal studies, human safety trials and human case reports of orthopoxvirus infection.

Results: 158 studies met the inclusion criteria. *In vitro* and *in vivo* animal studies have found that CDV, BCV and tecovirimat are highly efficacious when used therapeutically and prophylactically. They are partially protective in moderate, but not severe, immunodeficiency models. Clinical trials consistently report BCV and tecovirimat to be safe and well tolerated in humans. In human case reports, CDV, BCV and tecovirimat contributed to recovery from orthopoxvirus infection. BCV and tecovirimat demonstrate strong synergistic effect and may reduce risk of antiviral-resistant strains.

Conclusion: BCV and tecovirimat are particularly promising as anti-smallpox agents. Gaps in the literature indicate that further research should focus on developing more robust immunodeficiency and antiviral-resistance models.

Introduction

Though smallpox (causative agent variola virus (VARV)) was eradicated in a global triumph in 1980, it remains a threat as a category A bioterrorism agent. Two known caches of VARV still exist in the United States (US) and Russia, however more stockpiles of the virus could exist elsewhere (1). Advances in synthetic biology has led to increasing concern of smallpox (possibly antiviral-resistant) being synthesised from scratch (2). Given that smallpox vaccination ceased in the 1970s, most of the world's population is immunologically naïve or has waning levels of protection (3). While the first line response to an outbreak would be vaccination, up to 25% of immunocompromised individuals are contraindicated for vaccination. Another available countermeasure is vaccinia immune globulin (VIG). However, it can only be synthesised through the purified blood products of vaccinees and is hence in short supply (4). Therefore, it is imperative to develop other counter-measures that can be used to manage outbreak of smallpox or other orthopoxvirus (OPXV) and smallpox vaccination adverse events (AEs).

The most viable antivirals available for treatment of OPXV are cidofovir (CDV), brincidofovir (BCV) and tecovirimat. CDV (HPMPC; Vistide) is a nucleoside analogue with antiviral activity against dsDNA viruses and is currently approved for treating cytomegalovirus (CMV) in AIDS patients (5). In a smallpox emergency, CDV could be made available by the US Food and Drug Administration (FDA) as an Investigational New Drug (IND) or Emergency Use Authorization (EUA) (6). The mechanism of action is to block viral DNA polymerase, preventing viral replication. BCV (HDP-CDV; CMX001) is an alkoxyalkyl derivative of CDV that has high oral bioavailability – it structurally resembles natural lipids so the compound can be more readily absorbed through the small intestine (7, 8). As BCV is metabolised intracellularly, concentrations are reduced in the kidney, which is the site of dose-limiting toxicity (9-11). BCV has not currently been approved for clinical treatment, except in the instance of compassionate use, which allows unapproved drugs to be used for a seriously ill patient when no other treatment options are available; BCV has been used in patients with CMV who have undergone allogenic haematopoietic cell transplantation (HCT) (12). However, BCV has received Fast Track status and Orphan Drug Designation from the FDA in June 2018 for treatment of smallpox (13). Tecovirimat (ST-246;

TPOXX) is a low-molecular weight compound that is a potent and specific inhibitor of orthopoxvirus replication (14). As of July 2018, tecovirimat was approved as the first treatment for smallpox under FDA's Animal Rule (15).

To date, no systematic review has been completed on the potential efficacy of CDV, BCV and tecovirimat against smallpox. To address this gap in knowledge, this systematic review aimed to evaluate the existing research on antiviral efficacy against smallpox and other OPXV and provide a holistic understanding of their effect *in vitro* and *in vivo* animal studies, in human safety trials and reported human cases of OPXV infection.

Objectives

Four specific objectives were designed for each arm of the systematic review and are detailed below (Table 1).

Methods

Search strategy

A systematic review was conducted according to the Preferred Reporting Items for Systematic Review (PRISMA). Studies were identified by searching the electronic literature databases MEDLINE (1946-July 31 2018) and EMBASE (1974-August 1 2018) and hand-searching the reference lists of articles and reviews. The last search was run on 24 September 2018.

Initially, two reviewers (JY and SMR) conducted independent searches to reduce search bias of a single person conducting a search. The two reviewers then had a discussion to finalise the agreed upon search strategy, which allowed the keywords missed by one reviewer to be included. Two searches were conducted using a combination of Medical Subject Headings (MeSH) and text words (Appendix A). The first search (MEDLINE + EMBASE) aimed to identify studies involving *in vitro, in vivo* animal studies and human safety trials, using the following key words and their synonyms: orthopoxvirus, cowpox, ectromelia, monkeypox, smallpox, vaccinia, cidofovir, brincidofovir, tecovirimat. Only MEDLINE was used to conduct the second search, as EMBASE does not have a case report filter. This search aimed to identify cases of human OPXV infections where antivirals were used – results were limited to case reports, and only studies up to 1980 (year of eradication) were included.

Study selection and data extraction

We sought studies that presented quantitative data on the efficacy of antivirals against OPXV. After reviewing results from exploratory searches, it was decided that studies should be divided into four arms with separate eligibility criteria, summarised in Table 2. The types of studies included were: (1) *in vitro* studies, (2) *in vivo* animal model studies, (3) human clinical trials and (4) human case reports. Each search strategy is shown as an individual flow diagram, according to PRISMA (Figure 1).

Studies were screened for relevance by title and abstract. Two reviewers (JY and SMR) independently applied inclusion criteria to all identified and retrieved articles. One reviewer (JY) extracted data from included studies. Case reports, conference reports, reviews and letters to the editor were excluded. Selected papers were limited to the English language and articles in non-English language with English abstracts were excluded. Disagreements were resolved by consensus.

For *in vitro* studies, the chosen outcome measure was the effective concentration of the antiviral capable of inhibiting 50% of cytopathic effect (EC50) or 50% inhibitory concentration (IC50). These measures are used by studies to evaluate and compare different antivirals and identify their potential clinical effectiveness in humans. The EC50 and IC50 measures were also universal to identified studies and allowed for comparison between studies. Studies indicating antiviral efficacy as the main objective were identified as key papers. Studies that had original data, but used CDV, BCV or tecovirimat as reference values, were included but noted as supplementary. For in vivo studies, the outcome measured was impact of antivirals on mortality. Only lethal OPXV challenges were included, and results were grouped by animal model and inoculation route. For human safety or efficacy trials, any drug-related AEs were recorded. Finally, for human case studies, the antivirals were broadened to include VIG. All cases reporting use of these antivirals in any OPXV infection was recorded and the impact on disease progression noted.

Table 1. Systematic review objectives

Study arm	Objective
In vitro	To assess the efficacy of antivirals (CDV, BCV, ST-246) on orthopox viral activity using 50% effective concentration (EC_{50}) as an outcome measure.
In vivo	To assess the efficacy of antivirals in preventing mortality in animal studies compared to placebo.
Clinical safety trials	To assess the safety of antivirals in Phase I, II and III trials compared to placebo**.**
Human case reports	To summarise the use of antivirals in human cases of orthopoxvirus and their effect on disease progression.

Table 2. Eligibility criteria for each study arm

Inclusion criteria	Exclusion criteria
In vitro studies	
▪ Must assess CDV, BCV or tecovirimat in lethal OPXV challenges ▪ Any cell line, culture or assay was included ▪ EC_{50}/IC_{50} recorded ▪ Studies where the above antivirals were not the main subject (i.e. used as comparison) but displayed original results were also included ▪ There was no date restriction ▪ English language	▪ Did not assess EC50/IC50 ▪ Did not assess activity against OPXV ▪ Incomplete or unreported results ▪ Results not from original experiments
In vivo studies	
▪ Assessed CDV, BCV or tecovirimat against an OPXV in any animal ▪ All regimes of CDV, BCV or tecovirimat alone or in combination with another antiviral or vaccination ▪ Studies where the above antivirals were not the main subject (i.e. used as comparison) but displayed original results ▪ Animals of any immune status ▪ Mortality/survival rate recorded ▪ There was no date restriction ▪ English language	▪ Studies without trial design such as case reports, conference reports, reviews, letters to the editor ▪ Studies not including CDV, BCV or tecovirimat ▪ Not investigating OPXV ▪ No control arm
Human trials	
▪ Phase I, II or III trials assessing CDV, BCV or tecovirimat efficacy and/or safety ▪ Studies could assess antiviral efficacy against other viruses ▪ Reviews that included data about trials were included ▪ There was no date restriction ▪ Studies had to be in English language	▪ Studies not including CDV, BCV or tecovirimat in human subjects
Human case reports	
▪ Any case report involving use of CDV, BCV, tecovirimat or VIG against OPXV infection ▪ Studies had to be in English language	▪ Not including CDV, BCV or tecovirimat ▪ Not involving OPXV ▪ Case not human

Animal models for the study of orthopoxviruses

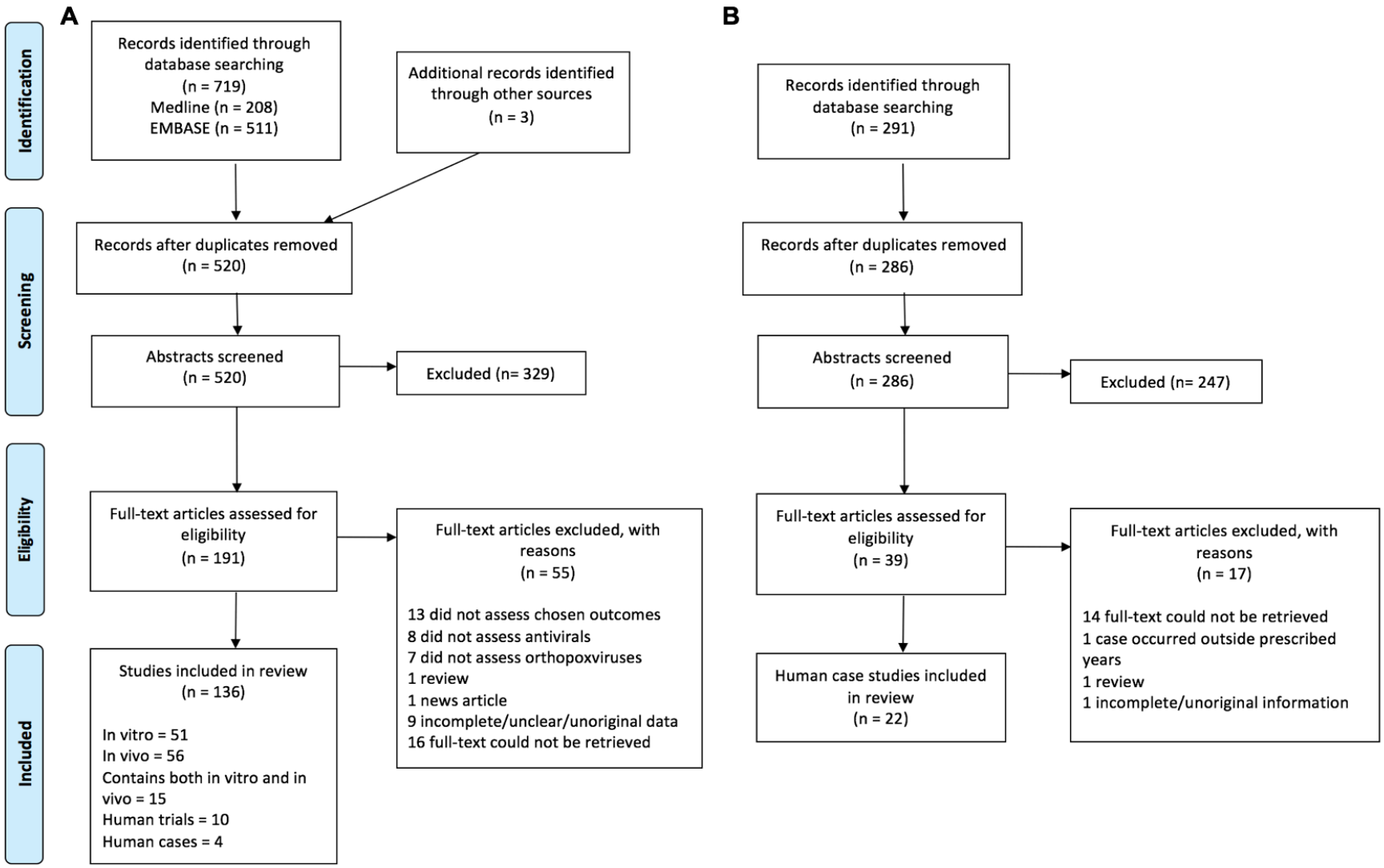
As VARV is specifically human pathogenic, no one animal model can reproduce all the disease characteristics of VARV. Consequently, many models have been developed to mimic certain disease characteristics, with varying inoculation, OPXV and drug routes (16, 17). Though large animal studies in non-human primates (NHP) are advantageous due to similarities with humans, they are limited by small sample size and cost. Therefore, many small animal models have been developed with focus on respiratory models (intranasal, aerosol and intratracheal inoculation) as the likely route of infection in a bioterrorism event (18).

VARV models using NHP are commonly used, but only produce mild generalised infection and rash (18). Aerosol inoculation requires very high viral doses (measured as plaque forming units (PFU)), and intravenous models manifest differently depending on PFU; 10^9 PFU (high) causes haemorrhagic VARV-like disease (almost 100% fatal and rare in humans), while 10^8 PFU (low) results in a 'lesional' model (though mortality is inconsistent) (18, 19). As such, other animal models are often used. Only 2 studies used VARV model (16, 19).

In cowpox (CPXV), aerosol and intranasal routes induce systemic smallpox-like disease. Aerosol produces more severe pulmonary disease, targeting the lower respiratory tract, while intranasal targets the upper respiratory tract due to the larger particles (18). In this review, 12 and 2 studies, respectively, were found on intranasal and aerosol CPXV models, and 1 study on both.

Vaccinia virus (VV) models have used mice or rabbits. Intranasal or aerosol inoculation in mice requires very high PFU to achieve lethality (10^4-10^5 PFU) (18). Intranasal route produces haemorrhagic VARV-like lesions, and lethal infection in BALB/c mice requires higher PFU of Western Reserve (WR) vaccinia strain compared to C57BL/6 mice (18, 20). SKH-1 hairless mice are used for dermal infections (18). Intradermal rabbit models more closely resemble disease compared to aerosol. However, antiviral efficacy has only been tested in mice via intranasal and intravenous routes, yielding 13 and 1 studies respectively.

Figure 1. (A) Search 1 yielding *in vitro* and *in vivo* animal studies, human clinical trials and human case reports (B) Search 2 yielding human case reports



For rabbitpox virus (RPXV) models, aerosol and intradermal inoculation produce similar disease progression to human smallpox (18). This is a good model for airborne VARV transmission as infected rabbits can transmit airborne infection. Two and four studies were found on aerosol and intradermal inoculation, respectively, as well as 1 study on both.
Ectromelia virus (ECTV) model shares many disease features with smallpox, and is conducted in mice, for which the knowledge of genetics and immunology is extensive (18). Disadvantages are that mice are naturally infected via the skin, and the major cause of death is liver disease. A commonly studied model is lethal intranasal ECTV in A/Ncr mice, which causes 100% mortality within 7-10 days. As humans are less susceptible to OPXV, a low dose intranasal infection of C57BL/6 mice (resulting in 60-80% mortality) may be a more suitable model (21). Further, ECTV modified with interleukin-4 (IL-4) gene is particularly useful as the virus is lethal to naturally resistant and vaccinated mice (22). This review found 7 studies for intranasal inoculation, 2 for aerosol and 1 for both.

Monkeypox virus (MPXV) is a zoonotic OPXV which is a public health concern in its own right; it is endemic in regions of Africa and epidemic in the US following importation of infected African animals (23). NHP MPXV models are well established, and 5 relevant studies were identified. Small-animal models are also useful; 5 papers describe African dormice, ground squirrels, prairie dogs, marmots and STAT1-deficient C57BL/6 mice as highly susceptible and capable of producing human-like disease (23-26).

Camelpox (CMLV) is most similar genetically to VARV (27). However, it has only recently been used in animal models as immunocompetent mice are naturally resistant (28). Immunodeficient athymic nude mice were found to be susceptible, establishing the first small animal model for this virus. Only 1 study was found (28).

Results

A total of 1010 studies from search strategies A and B were identified on the efficacy of CDV, BCV and tecovirimat including *in vitro* and *in vivo* animal studies, human clinical trials, and on human cases of OPXV infection. After removal of duplicates, non-English language and studies that did not test the chosen antivirals, 806 abstracts were reviewed. Of these, 230 full-text articles were reviewed and 158 articles met the inclusion criteria (Figure 1).

The included studies were separated into 4 groups corresponding to each arm of the review: there were 51 *in vitro* studies, 56 *in vivo*, 15 containing both, 10 human clinical trials, and 26 human case reports.

In vitro findings

A total 66 studies tested the efficacy of CDV, BCV and/or tecovirimat *in vitro*. Of these, 22 studies assessed the antiviral drugs as the main objective (Table 3); the remaining 44 used the antivirals as reference drugs for testing models or novel drugs (Appendix B). CDV was by far the most studied, appearing in 57 studies. Comparatively, BCV was studied in 9 papers and tecovirimat in 12 papers.

CDV was efficacious *in vitro* against a broad range of dsDNA viruses including adeno-, herpes-, irido-, hepadna-, papilloma-, polyoma- and poxviruses (29). Of OPXV, VARV is highly sensitive to CDV (30-32). However, due to CDV's poor oral availability and nephrotoxicity, BCV has been of interest as a safer alternative. BCV consistently demonstrates higher potency and selectivity *in vitro,* exceeding CDV in cowpox virus (CPXV), vaccinia virus (VV), MPXV, VARV, ectromelia virus (ECTV) and camelpox virus (CMLV) challenges (33-43). BCV appears to be particularly efficacious against VARV, with EC_{50} values approximately 271-fold higher than CDV (43).

Similarly, tecovirimat has a high level of potency that is specific to OPXV (44). The efficacy of tecovirimat exceeds CDV in CPXV, VV, CML, VARV, MPXV, ECTV (39, 41, 44-49). EC_{50} values are consistent even in different cell lines, and tecovirimat can also completely inhibit plaque formation, virus-induced cytopathic effect and formation of extracellular VV (15, 50). Importantly, tecovirimat is specifically active against multiple strains of VARV and MPXV (44, 51). Tecovirimat also inhibited CDV-resistant CPXV *in vitro*.

In vivo findings in healthy animal studies

In this review, 71 studies tested the efficacy of CDV, BCV and/or tecovirimat *in vivo* animals against lethal challenges of OPXV. CDV appeared in 42 studies and was the most studied antiviral. There were 19 BCV and 20 tecovirimat studies. The most commonly used models were VV and ECTV virus. Results were grouped by route of virus inoculation; respiratory (intranasal, aerosol or intratracheal) and systemic (intradermal, subcutaneous, intravenous).

Cidofivir

CDV can be delivered intranasally, intraperitoneally, subcutaneously or via aerosol, and has been tested in various animal models against lethal doses of VV, CPXV, ECTV, rabbitpox virus (RPXV) and MPXV. Of the 42 studies on CDV, most were conducted in CPXV and VV models (Table 4).

Table 3. Summary of in vitro findings in key studies

Virus[a]	Strain[b]	Antiviral[c]	EC_{50} value (µM)	Cell line[d]	Study reference
VV	VV-WR	CDV	59.08±12.38	BSC-40	Pires et al., 2018 (52)
			8.2±2.6	HEL	Duraffour et al., 2014 (42)
			8.1±4.4	HEL	Duraffour et al., 2013 (41)
			33±13	HFF	Quenelle et al., 2007[1&2] (39, 46)
			45.8±16.6	HFF	Kern et al., 2002 (33)
			40	CV-1	Buller et al., 2004 (34)
		BCV	0.013±0.011	HEL	Duraffour et al., 2014 (42)
			0.007±0.009	HEL	Duraffour et al., 2013 (41)
			0.13±0.001	HFF	Quenelle et al., 2007[1] (39)
			1.1±1.0	HFF	Kern et al., 2002 (33)
			0.7	CV-1	Buller et al., 2004 (34)
		Tecovirimat	0.0425±0.0148	BSC-40	Pires et al., 2018 (52)
			0.055±0.003	BSC-40	Santos-Fernandes et al., 2013 (49)
			0.017±0.009	HEL	Duraffour et al., 2013 (41)
			0.1±0.05	HFF	Quenelle et al., 2007[1&2] (39, 46)
	VV-Cop	CDV	6.9±3.0	HEL	Duraffour et al., 2014 (42)
			5.6±2.8	HEL	Duraffour et al., 2013 (41)
			3.7±0.5#	HEL	Duraffour et al., 2007[1] (45)
			29.2±14	HFF	Quenelle et al., 2007[1&2] (39, 46)
			31±5.4	HFF	Keith et al., 2004 (35)
			23±4.1	HFF	Kern et al., 2002 (33)
			46.2±11.9	HFF	Kern et al., 2002 (33)
			2.3±1.0#	PHK	Duraffour et al., 2007[1] (45)
			30±12.6	Vero	Kern et al., 2002 (33)
		BCV	0.005±0.002	HEL	Duraffour et al., 2014 (42)
			0.004±0.002	HEL	Duraffour et al., 2013 (41)
			0.08±0.03	HFF	Ruiz et al., 2011 (53)
			0.14±0.09	HFF	Quenelle et al., 2007[1] (39)
			0.6±0.4	HFF	Keith et al., 2004 (35)
			0.8±0.4	HFF	Kern et al., 2002 (33)
		Tecovirimat	0.008±0.003	HEL	Duraffour et al., 2013 (41)
			0.007±0.003	HEL	Duraffour et al., 2007[1] (45)
			0.05±0.02	HFF	Quenelle et al., 2007[1&2] (39, 46)
			0.003±0.00006#	PHK	Duraffour et al., 2007[1] (45)
	VV-Lister/Elstree	CDV	9.1±6.6	HEL	Duraffour et al., 2014 (42)
			5.9±3.8	HEL	Duraffour et al., 2013 (41)
			41.6±22.4	HFF	Kern et al., 2002 (33)
			1.32±0.47	FLM	Nettleton et al., 2000 (54)
		BCV	0.023±0.021	HEL	Duraffour et al., 2014 (42)
			0.094±0.061	HEL	Duraffour et al., 2013 (41)
			1.2±0.8	HFF	Kern et al., 2002 (33)
		Tecovirimat	0.04±0.06	HEL	Duraffour et al., 2013 (41)
	VV-IHD	CDV	13.4±5.6	HFF	Kern et al., 2002 (33)
		BCV	0.2±0.0	HFF	Kern et al., 2002 (33)
	VV-NYCBH	CDV	10.1±1.3	HFF	Kern et al., 2002 (33)
		BCV	0.4±0.0	HFF	Kern et al., 2002 (33)
		Tecovirimat	0.01	Vero	Yang et al., 2005 (44)
	VV-Wyeth	Tecovirimat	0.046±0.002	BSC-40	Santos-Fernandes et al., 2013 (49)
	WT	CDV	92±8	Vero	Kornbluth et al., 2006 (55)
			61±7	Vero	Smee et al., 2005 (56)
			19±6	Vero 76	Smee et al., 2002[1] (57)
			2.1±0.7	C127I	Kornbluth et al., 2006 (55)
		BCV	0.24±0.1	Vero	Kornbluth et al., 2006 (55)
			0.4±0.2	Vero	Smee et al., 2005 (56)
			0.31±0.2	C127I	Kornbluth et al., 2006 (55)
	Lederle-Chorioallentoic	CDV	2.52±1.45	HEL	Lebeau et al., 2006 (36)
			5.8±4.2	PHK	Lebeau et al., 2006 (36)
		BCV	0.013±0.006	HEL	Lebeau et al., 2006 (36)
			0.48±0.52	PHK	Lebeau et al., 2006 (36)
	Cantagalo (field strain)	CDV	7.68±1.35	BSC-40	Jesus et al., 2009 (58)
		Tecovirimat	0.0086±0.001	BSC-40	Santos-Fernandes et al., 2013 (49)
	IOC	CDV	9.66±0.94	BSC-40	Jesus et al., 2009 (58)
		Tecovirimat	0.034±0.006	BSC-40	Santos-Fernandes et al., 2013 (49)

Virus[a]	Strain[b]	Antiviral[c]	EC_{50} value (μM)	Cell line[d]	Study reference
	Brazilian-GP2V	CDV	27.14±1.04	BSC-40	Pires et al., 2018 (52)
	Brazilian -PSTV		39.42±6.45		
	Brazilian -GP1V		41.68±3.41		
	Brazilian -SH2V		42.40±23.80		
	Brazilian -P1V		62.53±21.37		
	Brazilian-GP2V	Tecovirimat	0.0054±0.0008	BSC-40	Pires et al., 2018 (52)
	Brazilian -PSTV		0.0056±0.0017		
	Brazilian -GP1V		0.0372±0.0002		
	Brazilian -SH2V		0.0381±0.0068		
	Brazilian -P1V		0.0518±0.0168		
	Strain not specified	Tecovirimat	0.04	BSC-40	Bailey et al., 2007 (47)
	Cidofovir resistant	CDV	1030±250	Vero	Kornbluth et al., 2006 (55)
			790±190	Vero	Smee et al., 2005 (56)
			150±36	Vero 76	Smee et al., 2002[1] (57)
			29±6	C127I	Kornbluth et al., 2006 (55)
		BCV	4.6±1.1	Vero	Kornbluth et al., 2006 (55)
			7.0±2.3	Vero	Smee et al., 2005 (56)
	WTp15*	CDV	56±6	Vero	Smee et al., 2005 (56)
		BCV	0.3±0.1	Vero	Smee et al., 2005 (56)
VARV	But	Tecovirimat	0.02	BSC-40	Bailey et al., 2007 (47)
			0.03	Vero	Yang et al., 2005 (44)
	BRZ66	CDV	28.45	Vero	Olson et al., 2014 (43)
		BCV	0.11	Vero	Olson et al., 2014 (43)
		Tecovirimat	0.067±0.0282	BSC-40	Smith et al., 2009 (51)
	BSH74	CDV	6.07	Vero	Olson et al., 2014 (43)
		BCV	0.21	Vero	Olson et al., 2014 (43)
		Tecovirimat	0.028±0.0124	BSC-40	Smith et al., 2009 (51)
			0.05	BSC-40	Bailey et al., 2007 (47)
			0.05	Vero	Yang et al., 2005 (44)
	SOM77	CDV	1.37	Vero	Olson et al., 2014 (43)
		BCV	0.077	Vero	Olson et al., 2014 (43)
		Tecovirimat	0.028±0.0303	BSC-40	Smith et al., 2009 (51)
	JPN51	CDV	10.81	Vero	Olson et al., 2014 (43)
		BCV	0.11	Vero	Olson et al., 2014 (43)
	UNK52	CDV	7.08	Vero	Olson et al., 2014 (43)
		BCV	0.05	Vero	Olson et al., 2014 (43)
	VARV-SLN68-258	Tecovirimat	0.037±0.0063	BSC-40	Smith et al., 2009 (51)
	VARV-SUD47-juba	Tecovirimat	0.019±0.0046	BSC-40	Smith et al., 2009 (51)
	VARV-NEP73-175	Tecovirimat	0.021±0.0139	BSC-40	Smith et al., 2009 (51)
CPXV	CPXV-BR	CDV	13.9±8.3	HEL	Duraffour et al., 2014 (42)
			19.6±9.8	HEL	Duraffour et al., 2013 (41)
			13.3±3.0#	HEL	Duraffour et al., 2007[1] (45)
			6.83±0.34	HEL	Lebeau et al., 2006 (36)
			41.1±4.2	HFF	Quenelle et al., 2007[1&2] (39, 46)
			42±5.4	HFF	Keith et al., 2004 (35)
			48±1.8	HFF	Kern et al., 2002 (33)
			44.7±6.3	HFF	Kern et al., 2002 (33)
			3.2±0.6	PHK	Duraffour et al., 2007[1] (45)
			5.3±2.1	PHK	Lebeau et al., 2006 (36)
			45±7.9	Vero	Kern et al., 2002 (33)
		BCV	0.021±0.026	HEL	Duraffour et al., 2014 (42)
			0.030±0.024	HEL	Duraffour et al., 2013 (41)
			0.035±0.004	HEL	Lebeau et al., 2006 (36)
			0.2±0.1	HFF	Ruiz et al., 2011 (53)
			0.24±0.1	HFF	Quenelle et al., 2007[1] (39)
			0.5±0.3	HFF	Keith et al., 2004 (35)
			0.6±0.3	HFF	Kern et al., 2002 (33)
			0.32±0.19	PHK	Lebeau et al., 2006 (36)
		Tecovirimat	0.33±0.025	BSC-40	Santos-Fernandes et al., 2013 (49)
			0.21±0.15	HEL	Duraffour et al., 2013 (41)
			0.16±0.09#	HEL	Duraffour et al., 2007[1] (45)
			0.48±0.01	HFF	Quenelle et al., 2007[1&2] (39, 46)
			0.013±0.0005#	PHK	Duraffour et al., 2007[1](45)
			0.05	Vero	Yang et al., 2005 (44)
	WT	CDV	45±7	Vero 76	Smee et al., 2002[1] (57)
			53±15	Vero	Smee et al., 2002[2] (59)
			1.0±0.5	C127I	Smee et al., 2002[2] (59)

Virus[a]	Strain[b]	Antiviral[c]	EC_{50} value (µM)	Cell line[d]	Study reference
	WT (SF)**	CDV	58±13	Vero 76	Smee et al., 2002[1] (57)
	CPXV-GER1980-EP4	CDV	20.6±0.5	HEL	Duraffour et al., 2013 (41)
	CPXV-GER1991-3		7.7±2.8		
	CPXV-AUS1999-867		13.1±4.8		
	CPXV-FIN2000-MAN		12.2±6.8		
	CPXV-GER1980-EP4	BCV	0.017±0.000	HEL	Duraffour et al., 2013 (41)
	CPXV-GER1991-3		0.007±0.007		
	CPXV-AUS1999-867		0.01±0.007		
	CPXV-FIN2000-MAN		0.014±0.010		
	CPXV-GER1980-EP4	Tecovirimat	0.03±0.04	HEL	Duraffour et al., 2013 (41)
	CPXV-GER1991-3		0.03±0.02		
	CPXV-AUS1999-867		0.02±0.01		
	CPXV-FIN2000-MAN		0.02±0.01		
	Strain not specified	Tecovirimat	0.6	BSC-40	Bailey et al., 2007 (47)
	Cidofovir resistant	CDV	>1000	Vero 76	Smee et al., 2002[1] (57)
			>1000	Vero	Smee et al., 2002[2] (59)
			230±90	C127I	Smee et al., 2002[2] (59)
		Tecovirimat	0.07	BSC-40	Bailey et al., 2007 (47)
			0.05	Vero	Yang et al., 2005 (44)
	Cidofovir resistant (SF)**	CDV	730±160	Vero 76	Smee et al., 2002[1] (57)
ECTV	ECTV-MOS	CDV	12±2.8	CV-1	Buller et al., 2004 (34)
		BCV	0.125±0.06	BSC-1	Ruiz et al., 2011 (53)
			0.5±0.1	CV-1	Buller et al., 2004 (34)
		Tecovirimat	0.07	Vero	Yang et al., 2005 (44)
	ECTV-7.5E-mIL-4***	CDV	12	CV-1	Buller et al., 2004 (34)
		BCV	0.2	CV-1	Buller et al., 2004 (34)
MPXV	Zaire	Tecovirimat	0.01	Vero	Yang et al., 2005 (44)
	MPXV-V78-I-3945	Tecovirimat	0.023±0.0026	BSC-40	Smith et al., 2009 (51)
	MPXV-V81-I-179		0.032±0.0061		
	MPXV-2003-USA-039		0.036±0.0045		
	MPXV-V77-I-823		0.030±0.0114		
	MPXV-V1979-I-005		0.039±0.0016		
	WT	CDV	27±11	Vero 76	Smee et al., 2002[1] (57)
	Strain not specified	Tecovirimat	0.01	BSC-40	Bailey et al., 2007 (47)
	Cidofovir resistant	CDV	505±50	Vero 76	Smee et al., 2002[1] (57)
	Cidofovir resistant (SF)**	CDV	725±105	Vero 76	Smee et al., 2002[1] (57)
CMLV	CML1	CDV	11.2±4.5	HEL	Duraffour et al., 2014 (42)
			10.8±5.9	HEL	Duraffour et al., 2013 (41)
			2.6±1.2#	HEL	Duraffour et al., 2007[1] (45)
			1.7±0.8#	PHK	Duraffour et al., 2007[1] (45)
		BCV	0.024±0.022	HEL	Duraffour et al., 2014 (42)
			0.021±0.015	HEL	Duraffour et al., 2013 (41)
		Tecovirimat	0.02±0.02	HEL	Duraffour et al., 2013 (41)
			0.03±0.004#	HEL	Duraffour et al., 2007[1] (45)
			0.02±0.01#	PHK	Duraffour et al., 2007[1] (45)
	WT	CDV	2.3±0.5	Vero 76	Smee et al., 2002[1](57)
	Strain not specified	Tecovirimat	0.01	BSC-40	Bailey et al., 2007 (47)
			0.01	Vero	Yang et al., 2005 (44)
	Cidofovir resistant	CDV	22±5	Vero 76	Smee et al., 2002[1](57)
Parapox-viruses	ORF-NZ2	CDV	0.8±0.1	HEL	Duraffour et al., 2014 (42)
			0.28±0.07	FLM	Nettleton et al., 2000 (54)
	PPV-orf-11	CDV	0.27±0.05	FLM	Nettleton et al., 2000 (54)
	PPV-MN	CDV	0.21±0.06	FLM	Nettleton et al., 2000 (54)

IC_{50} result
*wild type passaged 15 times in cell culture in parallel to CDV resistant strain
** no syncytium-forming [SF] viruses were present
*** ECTV recombinant expressing murine IL-4 from the 7.5 early promoter
[a] VV = vaccinia virus; CPXV = cowpox virus; VARV = variola virus; CPXV = cowpox virus; CMLV = camelpox virus
[b] WR = Western Reserve; Cop = Copenhagen; IHD = International Health Department; NYCBH = New York Board of Health; WT = Wild Type; But = Butler; BR = Brighton
[c] CDV = cidofovir; BCV = brincidofovir
[d] BSC-40, CV-1, Vero African green monkey cells; HEL, human embryonic lung fibroblasts; HFF, human foreskin fibroblasts; PHK, primary human keratinocyte; FLM, fetal lamb muscle; C127I, mouse mammary tumour cells

Cidofivir efficacy in lethal orthopoxvirus respiratory challenges

Intranasal cowpox model (13 studies)

Overall, CDV demonstrated therapeutic efficacy when initiated up to 3-4 days post infection (p.i.) (20, 27, 59-64). Interval dosing was also efficacious, even if as infrequently as every 3 days at 2-6.7mg/kg (20). A single CDV dose was highly protective up to 4 days p.i. (60-100%) (65). CDV delivered intranasally offered higher protection at lower doses vs intraperitoneally (65-67).

Intraperitoneal CDV given prophylactically is protective up to 5 days prior to infection in both single- and multi-dose regimens (20, 64, 68). Delivered via aerosol (14C-cidofovir), CDV may be less nephrotoxic due to greater retention of drug in the lungs vs kidneys. An aerosol dose of 0.5-5mg/kg was highly efficacious (80-100%) up to 2 days prior to challenge, offering protection comparable to subcutaneous delivery (69).

Aerosol cowpox model (2 studies)

Aerosol 0.5-5mg/kg dose of CDV provided prophylactic protection where subcutaneous CDV (1-10mg/kg) did not (70). However, 100mg/kg of subcutaneous CDV was highly efficacious up to 4 days p.i. (90-100%), and moderately efficacious 6 days p.i. (50%)(64).

Intranasal vaccinia model (13 studies)

Western Reserve (WR) strain was commonly used to challenge BALB/c mice. Results indicate that delivery of CDV via intraperitoneal and subcutaneous routes appear equally as efficacious (71). CDV was highly efficacious therapeutically and could be delayed 3-4 days even at low doses (20, 27, 63, 68, 72-74). As expected, antiviral efficacy improved as dosing frequency increased and viral challenge dose decreased (measured as plaque forming units (PFU))(71). A single dose was protective up to 3 days p.i., and prophylactically up to 5 days prior to infection (20, 66, 75). Against International Health Department (IHD) strain, CDV was efficacious in single- and multi-dose regimes up to 3 days p.i. (60, 63, 73, 76, 77).

Aerosol rabbitpox model (1 study)

Powdered CDV leads to higher retention in the lungs, reducing nephrotoxicity; doses of 0.5-1.75mg/kg protected all treatment groups (78).

Intranasal ectromelia model (3 studies)

A single dose of CDV in both BALB/c and A/NCr mice was protective up to 6 days and 3 days respectively (44, 79, 80).

Aerosol ectromelia model (2 studies)

CDV efficacy was dependent on viral challenge dose; at high viral PFU ($2.3x10^4$), CDV was unable to provide protection due to its low bioavailability (34). At lower PFUs, results were more significant and CDV was 50% and 100% protective ($5x10^4$ and $3.3x10^3$ PFU respectively)(80).

Monkeypox model (2 studies)

Intranasally inoculated African dormice were significantly protected by a single dose of CDV (25). Another study found that in an intratracheal inoculation model, a 'humanised' dose 5mg/kg CDV was more protective than traditional vaccination (81).

Cidofivir efficacy in lethal orthopoxvirus systemic challenges

Intraperitoneal vaccinia model (1 study)

CDV (delivered intraperitoneally) was less protective in the intraperitoneal inoculation model compared to intranasal (60% vs. 90% respectively) (72).

Intravenous monkeypox model (1 study)

A dose of 5mg/kg CDV protected non-human primates (NHP) when given 1 day prior to infection (82).

Brincidofovir

BCV is delivered via oral gavage and has been tested in various animal models against lethal doses of CPXV, VV, RPXV, ECTV and MPXV. A total of 19 studies assessed BCV efficacy, the majority in ECTV or RPXV models (Table 5).

Brincidofovir efficacy in lethal orthopoxvirus respiratory challenges

Intranasal cowpox model (1 study)

BCV given as single- or multi-dose regimens offers therapeutic protection efficacy up to 3 days p.i. (68). BCV was also protective when given prophylactically 1-5 days prior to infection.

Intranasal vaccinia model (3 studies)

In a VV-IHD challenge, single doses of BCV (25-100mg/kg) were protective against mortality (76). In comparison, lower doses (2.5-10mg/kg) were only weakly efficacious even if given for a duration of 5 days. BCV could be delayed to 2 days p.i. and protect against both WR and IHD strains (68, 84).

Aerosol rabbitpox model (1 study)

BCV protected 2 of 3 mice when given as one, two or three 20mg/kg doses on observation of secondary lesions (85). Though sample size was small, this suggests BCV may offer some post-lesional protection.

Intranasal ectromelia model (7 studies)

In A/Ncr mice, BCV was protective up to 5 days p.i. in both single- and multi-dose regimens (21, 38, 80, 86, 87). The optimum efficacious loading dose was found to be 20mg/kg (86). Against an escalating ECTV challenge (5-5000 PFU), a minimum dose of 2mg/kg was protective against lowest PFU viral challenge, though 8mg/kg was 100% protective in all groups (80).

In C57BL/6 mice, BCV had a longer therapeutic window and could be delayed up to 6 days p.i. (though this is still in the pre-lesional stage of disease) (21, 88). Against an escalating ECTV challenge (250-6000 PFU), BCV could provide statistically significant protection when treatment was delayed 4-6 days p.i.; at the highest challenge PFU, BCV delay was limited to 4 days p.i.(89). In hairless SKH1 mice challenged with 650-6500 PFU, BCV was >93% protective even when delayed up to 3 days p.i.(88).

Aerosol ectromelia model (3 studies)
Doses of 2-10mg/kg BCV provide 75-100% protection in a dose-dependent relationship (34, 38, 80). A 10mg/kg loading dose initiated immediately p.i. followed by 2.5mg/kg maintenance dose on day 3 was 75% protective from mortality (80).

Intranasal monkeypox model (1 study)
Only 1 study assessed this model using STAT1-deficient C57BL/6 mice, which are particularly sensitive to MPXV (26). It found that 10mg/kg initiated immediately p.i. for a duration of 14 days could provide 100% protection. However, when mice were re-challenged on day 38 p.i., 20% succumbed to infection.

Brincidofovir efficacy in lethal orthopoxvirus systemic challenges
Intradermal rabbitpox model (5 studies)
Studies aimed to assess whether observation of secondary lesions was a sufficient marker to initiate BCV. Pre-lesional treatment (up to 3 days p.i.) was most protective, however post-lesional treatment could provide significant protection up to 4 days p.i. (66-73%)(85, 90-92). A single BCV dose protected 7 of 12 animals (85). BCV was also protective prophylactically, providing 100% protection when given 1 day prior to infection (minimum dose 5mg/kg twice daily)(90, 93).

Tecovirimat
Tecovirimat is delivered via oral gavage and has been tested in various animal models against lethal doses of CPXV, VV, RPXV, ECTV, MPXV and VV. A total of 20 studies involved tecovirimat, the majority using MPXV and VV models (Table 6).

Tecovirimat efficacy in lethal orthopoxvirus respiratory infections
Intranasal cowpox model (1 study)
Only 1 study assessed this model and found that 10-100mg/kg doses of tecovirimat were significantly efficacious up to 3 days p.i. (46). Treatment before 2 days p.i. was 80-93% protective.

Intranasal vaccinia model (3 studies)
Against WR and IHD strains, a 100mg/kg dose was fully protective when given immediately after infection for 14 days (44, 94). Differences were noted in the minimum dosing duration between WR and IHD strains, which were 5 and 2 days respectively (46, 94, 95). Tecovirimat was still efficacious even when delayed 3 days p.i. (94).

Aerosol rabbitpox model (1 study)
Only 1 study assessed this model and found that 40mg/kg of tecovirimat was highly efficacious up to 2 days p.i. (88-100% respectively) (96).

Intranasal ectromelia model (3 studies)
Tecovirimat is highly efficacious, providing full protection even when delayed up to 5 days p.i. (44, 46, 88). Significant protection (73%) from mortality was still seen 6 days p.i., however lesions may not be a reliable marker for treatment initiation as they appear from day 7 (88).

Intranasal monkeypox model (3 studies)
In STAT1-deficient C57BL/6 mice, 100mg/kg initiated immediately p.i. for 10 days was fully protective (26). For prairie dogs, 30mg/kg was 100% protective even when given upon observation of secondary lesions (23). A prophylactic regimen of 40mg/kg starting 1 day prior to infection, followed by doses 2 hours prior infection and daily for 6 days p.i. was 100% protective (97).

Tecovirimat efficacy in lethal orthopoxvirus systemic challenges
Intravenous variola model (2 studies)
In NHP, tecovirimat given at 300mg/kg was fully protective when initiated immediately or 1 day p.i. (16). At a dose of 10mg/kg, tecovirimat could be delayed up to 4 days p.i. (19).

Intravenous vaccinia model (1 study)
A 100mg/kg dose given immediately p.i. for 14 days was fully protective (94).

Intradermal rabbitpox model (1 study)
The minimum efficacious dose was 20-40mg/kg; >90% animals survived when given 40mg/kg for 14 days (98).

Intravenous monkeypox model (2 studies)
Doses between 3-300mg/kg were highly protective up to 5 days p.i. if given for a duration of 14 days; though 3mg/kg was the minimum dose, 10mg/kg also reduced viremia and lesion count (16, 98-100). As lesions appear by 1 day p.i., results suggest tecovirimat can be given post-lesionally (99). Thus, the recommended human therapeutic dose is 400mg/kg, which would provide exposure levels comparable to 10mg/kg in NHP.

Subcutaneous monkeypox model (1 study)
In a ground squirrel model, tecovirimat treatment of 100mg/kg was fully protective up to 4 days p.i. (24).

Table 4. Summary of cidofovir efficacy in lethal challenge animal models

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
Intranasal CPX (BR) in BALB/c mice	Smee et al., 2013 (83)	Intraperitoneal	100mg/kg CDV given immediately p.i. for duration of 2 days	100% protective
	Smee et al., 2008 (62)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for duration of 2 days	100% protective
	Quenelle et al., 2007[3] (61)	Intraperitoneal	10mg/kg CDV given 1, 2 or 3 days p.i. for duration of 5 days	100% protective
	Quenelle et al., 2006 (60)	Intraperitoneal	15mg/kg CDV given 1 day p.i. for duration of 5 days	100% protective
	Prichard et al., 2006[1](63)	Intraperitoneal	15mg/kg CDV given 1 day p.i. for duration of 5 days	100% protective
	Smee et al., 2004[1] (67)	Intraperitoneal	Single dose of 100mg/kg CDV given 1 day p.i.	4 experiments of this regime lead to survival rate of 80-100%
	Quenelle et al., 2004 (68)	Intraperitoneal	5 or 10mg/kg CDV given 5, 3 or 1 days prior to infection until the day of infection.	Protective even when 5 days prior to infection.
		Intraperitoneal	Single dose of 30mg/kg CDV given 5, 3 or 1 days prior to infection, and 1 or 3 days p.i.	Protective even when 5 days prior to infection.
	Quenelle et al., 2003 (20)	Intraperitoneal	2 or 6.7mg/kg CDV given at 1, 2 or 3 days p.i. daily, every other day, or every 3rd day for 7 days	Interval dosing clearly efficacious, even when dosing was as infrequent as every 3 days at 2-6.7mg/kg
		Intraperitoneal	6.7-60mg/kg CDV given at 1, 2 or 3 days p.i. for duration of 7 days	Although placebo treated mice only had 47% mortality, all regimens significantly reduced mortality even with delay of 3 days p.i.
		Intraperitoneal	0.7-6.7mg/kg CDV given at 1, 2 or 3 days p.i. for duration of 7 days	At higher PFU (compared to above experiment), only 6.7mg/kg dose was protective, but it significantly reduced mortality up to 4 days p.i.
		Intraperitoneal	Single doses of 3, 10, 30, 100mg/kg CDV given 5, 3 or 1 days prior to infection, and 1 or 3 days p.i.	CDV efficacy could be retained for 5 days in a dose-dependent manner. 3 or 10mg/kg were only efficacious therapeutically; 30mg/kg was efficacious from 3 days prior infection to 3 days p.i.; 100mg/kg provided significant protection even when given 5 days prior to infection.
	Smee et al., 2003 (66)	Intraperitoneal	Single doses of 20, 40, 80, 160mg/kg CDV given 1 day p.i.	For intraperitoneal CDV delivery, only doses 40-160mg/kg were fully protective; at 20mg/kg all mice died.
		Intranasal	Single doses of 5, 10, 20, 40mg/kg CDV given 1 day p.i.	In comparison, intranasal delivery requires a lower dose of CDV and gave protection of 80-100% for all cases.

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
	Roy et al., 2003 (69)	Aerosol	Single doses of 0.06-0.5 (low), 0.5-5.0 (high)mg/kg CDV given 2 or 1 days prior to infection, or 0, 1, or 2 days p.i.	High dose of aerosolised CDV resulted in similar survival to 100mg/kg delivered subcutaneously and was efficacious when given both before and after infection (80-100% survival).
		Subcutaneous	Single dose of 100mg/kg CDV given 2 or 1 days prior to infection, or 0, 1, or 2 days p.i.	
	Smee et al., 2002[2](59)	Intraperitoneal	30mg/kg CDV given at 1 day p.i. for duration of 5 or 10 days	100% protective
	Smee et al., 2000 (65)	Intranasal	Single doses of 2.5, 5, 10, 20, 40mg/kg CDV given 1 day p.i.	Intranasal delivery requires a lower dose of CDV and gave protection of 60-100% for all cases
		Intranasal	Single doses of 20, 40mg/kg CDV given 1-5 days p.i.	Treatment up to 3 days was most protective in both doses (80-90%), though 40mg/kg did provide some protection up to 4 days (60%).
		Intranasal	Single doses of 10mg/kg CDV were given at different volumes (5-40µl) 1 day p.i.	A larger treatment volume had greater efficacy - 40 µl and 20 µl had 100% and 50% efficacy respectively.
Aerosol CPX (BR) in BALB/c mice	Bray et al., 2002 (70)	Aerosol	Single doses of 0.06-0.5 (low), 0.5-5.0 (high)mg/kg CDV given 1 days prior to infection, or 0, 1 day p.i.	The high dose of CDV was fully protective when given 1 day prior or the same day as infection, whereas a 10mg/kg subcutaneous dose of CDV did not prevent death at all. The high dose of CDV was always more efficacious than 25mg/kg of subcutaneous CDV, and sometimes more efficacious than 100mg/kg.
		Subcutaneous	Single dose of 10, 25, 50, 75, 100mg/kg CDV given 1 days prior to infection	
	Bray et al., 2000 (64)	Subcutaneous	Single dose of 100mg/kg CDV given immediately, 2, 4 or 6 days p.i.	Treatment highly protective (90-100%) up to 4 days p.i. and moderately protective (50%) 6 days p.i.
Intranasal VV (WR) in BALB/c mice	Smee et al., 2013 (83)	Intraperitoneal	100mg/kg CDV given immediately p.i. for 2 days	100% protective
	Smee et al., 2007[1] (73)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for 2 days	100% protective
	Smee et al., 2007[2] (74)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for 2 days	100% protective
	Quenelle et al., 2007[3] (61)	Intraperitoneal	10-15mg/kg CDV given 1, 2 or 3 days p.i. for 5 days	100% protective
	Knorr et al., 2006 (72)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for 2 days	Significantly improved survival
	Prichard et al., 2006[1](63)	Intraperitoneal	15mg/kg CDV given 1 day p.i. for 5 days	100% protective
	Quenelle et al., 2004 (68)	Intraperitoneal	5mg/kg CDV given 1, 2 or 3 days p.i. for duration 5 days	Significant protection (73-100%) despite delay

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
	Quenelle et al., 2003 (20)	Intraperitoneal	0.7, 2.2, 6.7mg/kg CDV given 2, 3 or 4 days p.i. for duration 7 days	Significant protection despite delay and low dose
		Intraperitoneal	Single doses of 3, 10, 30, 100mg/kg CDV given 5, 3 or 1 day prior to infection, or 1, 3 days p.i.	Single dose of CDV at 3-100mg/kg was efficacious when given as early as 5 days prior to infection, and as late as 3 days p.i.
	Smee et al., 2003 (66)	Intraperitoneal	Single doses of 20, 40, 80, 160mg/kg CDV given 1 day p.i.	Doses of 40, 80, 160mg/kg prevented mortality by 70%, while no mice survived at 20mg/kg.
		Intranasal	Single doses of 5, 10, 20, 40mg/kg CDV given 1 day p.i.	All doses prevented mortality by 70-80%
	Smee et al., 2001[1] (71)	Subcutaneous	3, 10, 30, 100mg/kg CDV given 1 and 4 days p.i.	Doses of 30 and 100mg/kg protected 80-100% of mice.
		Subcutaneous	30mg/kg CDV given 1 day p.i. once every 3 days or every day for 5 days	A moderate improvement in survival was seen for 30mg/kg when the dosing frequency increased from once every three days to daily (60% to 90% survival rates).
		Subcutaneous and intraperitoneal	Against decreasing virus challenge doses, 10, 30, 100mg/kg CDV given on days 1 and 4 p.i.	CDV efficacy increased as virus challenge dose decreased. Subcutaneous and intraperitoneal delivery of CDV produced comparable results and appear to have equal efficacy.
	Smee et al., 2001[2] (75)	Intraperitoneal	Single dose of 100mg/kg CDV given 1 day p.i.	100% protective
Intraperitoneal VV (WR) in BALB/c mice	Knorr et al., 2006 (72)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for 2 days	CDV in an intraperitoneal model was not as preventive compared to intranasal inoculation (60% vs. 90% respectively)
Intranasal VV (IHD) in BALB/c mice	Smee et al., 2010 (77)	Intraperitoneal	100mg/kg CDV given 1 day p.i. for duration of 2 days	100% protective
	Smee et al., 2007[1] (73)	Intraperitoneal	100mg/kg CDV given 1, 2 or 3 days p.i. for duration of 2 days	Provided significant protection against mortality (70-100%)
	Quenelle et al., 2006 (60)	Intraperitoneal	15mg/kg CDV given 1, 2 or 3 days p.i. for duration of 5 days	Provided significant protection against mortality (93-100%)
	Prichard et al., 2006[1](63)	Intraperitoneal	15mg/kg CDV given 1 day p.i. for 5 days	100% protective
	Smee et al., 2004[2] (76)	Intraperitoneal	Single doses of 10, 30, 100mg/kg CDV given 1 day p.i.	Doses 30 and 100 mg/kg resulted in 90% and 100% survival respectively, whereas the 10mg/kg dose did not prevent death.
Aerosol RPXV (Utrecht) in NZ	Verreault et al., 2012 (78)	Powdered CDV (NanoFOVIRTM)	0.5, 1, 1.75mg/kg CDV given immediately p.i. for 3 days	All treatment groups were protected from mortality. Results demonstrate that powdered CDV delivered directly to the lung could avoid the need to increase the dose and is a promising anti-orthopoxvirus agent.
		Intravenous	10mg/kg CDV given immediately p.i. for 3 days	

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
White Rabbits				
Intranasal ECTV in BALB/c mice	Israely et al., 2012 (79)	Intraperitoneal	Single doses of 2.5, 5, 10, 25, 50, 100mg/kg CDV given 1-7 days p.i.	Demonstrates even a single dose of just 5mg/kg can be efficacious up to day 6 (late stage of disease). Higher doses of 100mg/kg were fully protective at day 6, and 50% at day 7.
Intranasal ECTV in A/NCr mice	Yang et al., 2005 (44)	Intraperitoneal	Single dose of 100mg/kg CDV given 4h p.i.	Single dose was 100% protective
	Parker et al., 2008[1] (80)	Intraperitoneal	Single dose of 100mg/kg CDV given immediately or 3 days p.i.	Interestingly, CDV given 3 days p.i. was more protective (100%) compared to when given immediately p.i.
Aerosol ECTV in A/NCr mice	Parker et al., 2008[1] (80)	Intraperitoneal	5mg/kg CDV given immediately p.i. followed by a maintenance dose on day 3 of 1.25mg/kg	CDV gave significant protection and was 50% and 100% protective ($5x10^4$ and $3.3x10^3$ viral PFU respectively).
	Buller et al., 2004 (34)	Intraperitoneal	1.25, 2.5, 5, 10mg/kg CDV given 4h p.i. for 5 days	Not able to provide any protection due to low bioavailability of CDV
Intranasal MPXV (Zaire) in African dormice	Schultz et al., 2009 (25)	Intraperitoneal	Single dose of 100mg/kg CDV given 4h p.i.	Significantly protected from mortality (81%)
Intratracheal MPXV (MSF#6) in macaquees	Stittelaar et al., 2006 (81)	Intraperitoneal	5mg/kg CDV given 1 day p.i. and repeated 1, 3, 7, 10 and 13 days after initial treatment	This was a 'humanised' dose, i.e. equivalent to those recommended for humans. CDV demonstrated between 67-83% protection, which was greater than that provided by vaccination.
Intravenous MPXV (Zaire) in NHP*	Song et al., 2013 (82)	N/A	5mg/kg given 1 day prior to infection for 14 days	100% protective

[a] CPXV = cowpox virus; BR = Brighton; VV = vaccinia virus; WR = Western Reserve; IHD = International Health Department; RPXV = rabbitpox virus; ECTV = ectromelia virus; MPXV = monkeypox virus; NHP = non-human primates

[b] CDV = cidofovir; p.i. = post infection

Table 5. Summary of brincidofovir efficacy in lethal challenge animal models

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
Intranasal CPX (BR) in BALB/c mice	Quenelle et al., 2004 (68)	Oral gavage	6.7mg/kg BCV given 1, 2 or 3 days p.i. for 5 days	All doses significantly reduced mortality, even when initiated 3 days p.i.
		Oral gavage	5 or 10mg/kg BCV given 5, 3 or 1 days prior to infection, until day of infection	BCV highly protective when given 1-5 days prior to infection
		Oral gavage	Single dose of 12.5mg/kg BCV given 5, 3 or 1 days prior to infection, or 1, 3 days p.i.	Single dose of 12.5mg/kg was protective at all different times of initiation
Intranasal VV (IHD) in BALB/c mice	Smee et al., 2004[2] (76)	Oral gavage	2.5, 5, 10mg/kg BCV given 1 day p.i. for 5 days	Low doses are only weakly efficacious
		Oral gavage	Single dose of 25, 50, 100mg/kg BCV given 1 day p.i.	Single doses highly protective (80-100%)
	Zaitseva et al., 2015 (84)	Oral gavage	2.5, 5, 20mg/kg BCV given 1 day p.i. followed by maintenance doses on days 3 and 5	5 and 20mg/kg doses were 100% protective
		Oral gavage	Above regime, re-challenged on day 41	All mice survived re-challenge, BCV does not impair generation of protective immunity
		Oral gavage	5, 20mg/kg BCV given 2 days p.i. followed by maintenance doses on days 4 and 6	20mg/kg doses were 100% protective, 5mg/kg not efficacious
Intranasal VV (WR) in BALB/c mice	Quenelle et al., 2004 (68)	Oral gavage	5mg/kg given 1, 2 or 3 days p.i. for 5 days	BCV protective (33-87%) up to 2 days p.i.
Aerosol RPXV (Utrecht) in NZ White Rabbits	Rice et al., 2011[1] (85)	Oral gavage	Sentinel animals co-housed with index animals inoculated with RPXV. 1, 2 or 3 doses of 20mg/kg BCV beginning the day secondary lesions are seen (~day 7)	2 of 3 animals in each treatment group survived
Intradermal RPXV (Utrecht) in NZ White Rabbits	Grossi et al., 2017 (91)	Oral gavage	20mg/kg BCV given 0-3 days p.i., followed by a maintenance dose of 5mg/kg 2 and 4 days later.	BCV highly protective (93-100%) up to 2 days p.i. (prior to fever). BCV initiated day 3 improved mortality, but was not statistically significant.
	Trost et al., 2015 (92)	Oral gavage	Loading doses of 5 or 20mg/kg given upon first observation of secondary lesions. Maintenance doses of 5 or 20mg/kg given 2 and 4 days later.	20mg/kg doses had a significantly higher rate of survival. Loading dose 20mg/kg followed by two 5mg/kg maintenance doses was concluded to be the optimised BCV regime.
	Rice et al., 2011[2] (90)	Oral gavage	1-10mg/kg (bid) or 20mg/kg (daily) BCV given 1 day prior to infection for 5 days	BCV 100% protective
	Rice et al., 2011[1] (85)	Oral gavage	1, 2 or 3 doses of 20mg/kg BCV given every other day beginning 3 or 4 days p.i.	BCV 100% protective when treatment delayed 3 days p.i., and 66% for 4 days p.i.

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
		Oral gavage	1, 2 or 3 doses of 20mg/kg BCV beginning the day symptoms observed (~day 3)	Single dose BCV protected 7 of 12; 2 doses protected 8 of 12; 3 doses protected 11 of 12.
	Adams et al., 2007 (93)	Oral gavage	1 or 5mg/kg (bid) BCV given 1 day prior to infection for 5 days	5mg/kg dose was 100% protective given prophylactically
Intranasal ECTV (MOS) in A/NCr mice	Hostetler et al., 2007 (38)	Oral gavage	2 or 8mg/kg BCV given immediately p.i. for 5 days	BCV significantly protective (80-100%)
	Parker et al., 2014 (87)	Oral gavage	10mg/kg BCV given 4 days prior to infection, immediately, or 2, 4 days p.i. Maintenance dose of 2.5mg/kg given every other day until day 14.	BCV protective therapeutically up to 2 days p.i. (80-100%), and prophylactically 4 days prior to infection (100%).
	Parker et al., 2009 (21)	Oral gavage	Loading doses of 10mg/kg BCV given 0-6 days p.i. followed by a maintenance dose of 2.5mg/kg every other day	BCV 80-100% protective up to 4 days p.i.
	Parker et al., 2008[2] (86)	Oral gavage	Loading doses of 2.5, 5, 10, 20mg/kg BCV given 5 days p.i. followed by a maintenance dose of 2.5mg/kg every other day	Loading dose of 20mg/kg provided highest level of delayed protection to mice treated 5 days p.i. (90%)
		Oral gavage	Loading doses of 20mg/kg BCV given 5 days p.i. followed by a maintenance doses of 0.31-2.5mg/kg every other day	BCV was 90-100% protective for all regimes, indicating that maintenance dose is of limited benefit when mice received loading dose of 20mg/kg
		Oral gavage	Singe doses of 20, 25, 30mg/kg given 4-7 days p.i.	BCV was >90% protective when initiated within 4 days p.i.
	Parker et al., 2008[1] (80)	Oral gavage	Against escalating viral challenge doses (0.012-5000 PFU), 1-8mg/kg BCV given immediately p.i. for 5 days	A minimum of 2mg/kg BCV every day for 5 days is required to protect mice from low dose (<5 PFU) infection. 8mg/kg protected all mice from mortality.
		Oral gavage	0.3-5mg/kg BCV given immediately p.i. for 14 days	Doses >1.25mg/kg significantly protected mice from lethal infections (90-100%)
		Oral gavage	1.25 or 2.5mg/kg BCV given immediately p.i. every day or every 2, 3 or 4 days	Minimum treatment of 2.5mg/kg every 2 days
Intranasal ECTV (MOS) in C57BL/6 mice	Crump et al., 2017 (89)	Oral gavage	Against escalating viral challenge doses (250-600 PFU), a loading dose of 20mg/kg is given 4, 5 or 6 days p.i. Maintenance dose of 5mg/kg is given 2 days later, and 4mg/kg given 4 days later.	BCV provided significant protection at all 3 viral challenge doses when initiated day 4 p.i.. At day 5, only mice challenged with lower viral doses survived.
	Parker et al., 2014 (87)	Oral gavage	20mg/kg BCV given immediately p.i. followed by a maintenance dose of 2.5mg/kg given every other day until day 14.	BCV was not protective (10%)
	Parker et al., 2012 (88)	Oral gavage	20mg/kg BCV given 0-9 days p.i. for 12 days	BCV protective up to day 6 p.i.

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
	Parker et al., 2009 (21)	Oral gavage	Loading doses of 10mg/kg BCV given 0-6 days p.i. followed by a maintenance dose of 2.5mg/kg every other day	BCV 100% protective up to 6 days p.i. (though placebo survival rate was 60%)
Intranasal ECTV (MOS) in hairless SKH1 mice	Parker et al., 2012 (88)	Oral gavage	Against escalating viral challenge doses (650-6500 PFU), 25mg/kg given 3, 6 or 9 days p.i. for 14 days	BCV intervention at day 3 afforded >93% protection at all doses. No significant protection when delayed to day 6 or 9.
Aerosol ECTV (MOS) in A/NCr mice	Parker et al., 2008[1] (80)	Oral gavage	Loading doses of 10mg/kg BCV given immediately p.i. followed by a maintenance dose of 2.5mg/kg every other day	BCV 75% protective
	Hostetler et al., 2007 (38)	Oral gavage	2 or 8mg/kg BCV given immediately p.i. for 5 days	BCV 100% protective
	Buller et al., 2004 (34)	Oral gavage	1.25-10mg/kg BCV given immediately p.i. for 5 days	5-10mg/kg doses were 80-100% protective
Intranasal MPXV (Zaire) in STAT1 deficient C57BL/6 mice	Stabenow et al., 2010 (26)	Oral gavage	10mg/kg BCV given immediately p.i. for 14 days	BCV 100% protective. At re-challenge 38 days p.i., 20% died.

[a] CPXV = cowpox virus; BR = Brighton; VV = vaccinia virus; IHD = International Health Department; WR = Western Reserve; RPXV = rabbitpox virus; ECTV = ectromelia virus; MOS = Moscow; MPXV = monkeypox virus

[b] BCV = brincidofovir; p.i. = post infection; bid = twice daily

Table 6. Summary of tecovirimat efficacy in lethal challenge animal models

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
Intranasal CPX (BR) in BALB/c mice	Quenelle et al., 2007[2] (46)	Oral gavage	100mg/kg tecovirimat given 0 or 1 day p.i. for 5, 7, 10 or 14 days	All dosing regimens of duration greater than 7 days significantly decreased mortality
		Oral gavage	10, 30 or 100mg/kg tecovirimat given 0-3 days p.i. for 14 days	Tecovirimat highly protective even when delayed to 3 days p.i.
Intranasal VV (WR) in BALB/c mice	Berhanu et al., 2009 (94)	Oral gavage	100mg/kg tecovirimat given immediately p.i. for 14 days	Tecovirimat 100% protective
		Oral gavage	100mg/kg tecovirimat given immediately p.i. for 1-14 days	A minimum dosing duration of 5 days is required to prevent mortality. Tecovirimat provided 100% protection.
		Oral gavage	100mg/kg tecovirimat given 0-4 days p.i. for 14 days	Tecovirimat 100% protective up to 4 days p.i.
	Quenelle et al., 2007[2] (46)	Oral gavage	100mg/kg tecovirimat given 0 or 1 day p.i. for 5-14 days	Dosing duration beyond 5 days does not seem to be an important factor. Tecovirimat initiated 1 day p.i. yielded better results than same day as challenge.
Intranasal VV (IHD) in BALB/c mice	Zaitseva et al., 2013 (95)	Oral gavage	30 or 100mg/kg tecovirimat given 1 day p.i. for 1-5 days	At 100mg/kg dose, a minimum of 2 days dosing duration is required for protection.
	Yang et al., 2005 (44)	Oral gavage	50mg/kg tecovirimat given immediately p.i. for 14 days	Tecovirimat 100% protective
Intravenous VV (WR) in BALB/c mice	Berhanu et al., 2009 (94)	Oral gavage	100mg/kg tecovirimat given immediately p.i. for 14 days	Tecovirimat 100% protective
Aerosol RPXV (Utrecht) in NZ White Rabbits	Nalca et al., 2008 (96)	Oral gavage	40mg/kg tecovirimat given 0-4 days p.i. for 14 days	Tecovirimat highly protective up to 2 days p.i.
Intradermal RPXV (Utrecht) in NZ White Rabbits	Grosenbach et al., 2018 (98)	Oral gavage	20-120mg/kg tecovirimat given 4 days p.i. for 14 days	The minimum efficacious dose of tecovirimat to achieve >90% survival was 20-40mg/kg
Intranasal ECTV (MOS) in A/NCr mice	Quenelle et al., 2007[2] (46)	Oral gavage	100mg/kg tecovirimat given 0-3 days p.i. for 10 days	Tecovirimat 100% protective
	Yang et al., 2005 (44)	Oral gavage	50mg/kg tecovirimat given immediately p.i. for 14 days	Tecovirimat 100% protective

Model[a]	Study	Delivery route	Dose Regime[b]	Findings
Intranasal ECTV (MOS) in C57BL/6 mice	Parker et al., 2012 (88)	Oral gavage	100mg/kg tecovirimat given 0-9 days p.i. for 14 days	Tecovirimat highly protective up to 6 days p.i.
Intranasal MPXV (Zaire) in STAT1 deficient C57BL/6 mice	Stabenow et al., 2010 (26)	Oral gavage	100mg/kg tecovirimat given immediately p.i. for 10 days	Tecovirimat 100% protective
Intranasal MPXV (Zaire) in prairie dogs	Smith et al., 2011 (23)	Oral gavage	30mg/kg tecovirimat given 0 or 3 days p.i., or upon first observation of secondary lesions. Dosing duration of 14 days.	Tecovirimat 100% protective
Intranasal MPXV (V79- 1-005) in marmots	Mazurkov et al., 2016 (97)	Oral gavage	40mg/kg tecovirimat given 1 day prior to infection, 2 hours p.i. and then daily for 6 days p.i.	Tecovirimat 100% protective
Intravenous MPXV (Zaire) in NHP	Grosenbach et al., 2018 (98)	Oral gavage	0.3-20mg/kg tecovirimat given upon first observation of clinical signs (~day 4) for 14 days	Minimum efficacious dose 3-10mg/kg providing ~95% protection.
	Berhanu et al., 2015 (100)	Oral gavage	10mg/kg tecovirimat given immediately p.i. for 14 days. Mice were re-challenged on day 63.	Tecovirimat 100% protective. All mice survived re-challenge.
		Oral gavage	10mg/kg tecovirimat given 4-6 days p.i. for 14 days	Tecovirimat highly protective up to 5 days p.i.
	Huggins et al., 2009 (16)	Oral gavage	300mg/kg tecovirimat given 0 or 3 days p.i. for 14 days	Tecovirimat 100% protective
	Jordan et al., 2009 (99)	Oral gavage	3-100mg/kg tecovirimat given 3 days p.i. for 14 days	Tecovirimat 100% protective. 3mg/kg was minimum efficacious dose, but 10mg/kg also reduced levels of viremia and lesion count.
Subcutaneous MPXV (Zaire) in ground squirrels	Sbrana et al., 2007 (24)	Oral gavage	100mg/kg tecovirimat given 0-4 days p.i. for 14 days	Tecovirimat 100% protective
Intravenous VARV (Harper) in NHP	Mucker et al., 2013 (19)	Oral gavage	10mg/kg tecovirimat given 2 or 4 days p.i. for 14 days	Tecovirimat 100% protective
	Huggins et al., 2009 (16)	Oral gavage	300mg/kg tecovirimat given 0 or 1 day p.i. for 14 days	Tecovirimat 100% protective

[a] CPXV = cowpox virus; BR = Brighton; VV = vaccinia virus; WR = Western Reserve; IHD = International Health Department; RPXV = rabbitpox virus; ECTV = ectromelia virus; MOS = Moscow; MPXV = monkeypox virus; NHP = non-human primates; VARV = variola virus

[b] p.i. = post infection

Resistance studies

Despite the potential of these antivirals, a major risk is the development of resistant OPXV (101). However, we found only 3 studies assessing antiviral efficacy against CDV-resistant strains.

Against A314T and A684V cidofovir-resistant VV strains, 50mg/kg of CDV for 5 days significantly protected mice from challenge (102). However, 100mg/kg of CDV could not protect against CDV-resistant CPXV (though the same dose provided 80-100% protection in wild-type (WT) CPXV (57). Single doses of BCV (50 or 100mg/kg) was partially protective (80-90%) against marker-rescued CDV-resistant VV (55).

Synergistic efficacy of BCV and tecovirimat

Combination therapy is an important consideration as it could reduce risk of developing treatment-resistant OPXV strains (22). Coadministration of BCV and tecovirimat were only discussed in 2 studies (Table 7).

In different dose combinations, BCV and tecovirimat coadministration consistently provided high levels of protection where monotherapy did not; no evidence of toxicity was observed (39, 103). Significantly, lower doses of each antiviral are required in a co-administration regime, which minimises the risk of AEs without reducing the therapeutic effect. Coadministration therapy could also be delayed up to 6 days p.i. (39).

One study assessed BCV and tecovirimat coadministration against a model of vaccine resistance, using ECTV recombinant strain encoding murine IL-4 gene, which is lethal to immunised mice (103). Combination therapy protected 75% of mice vs. antiviral monotherapy which lead to complete fatality.

Table 7. Synergistic brincidofovir and tecovirimat efficacy in lethal challenge mice models

Study	Model[a]	Antiviral[b]	Dose Regime	Findings
Quenelle et al., 2007[1] (39)	Intranasal CPXV (BR) in BALB/c mice	Oral gavage both tecovirimat + BCV	1, 3 or 10mg/kg tecovirimat and 0.3, 1 or 3mg/kg of BCV used individually or together beginning 1 day p.i. and continued for 5 days.	Alone, tecovirimat at 3mg/kg (80%) and BCV at 1 and 3mg/kg (80% and 100% respectively) were highly protective. In combination, treatment was highly efficacious in all groups except for regime with lowest doses of both antivirals. No adverse reactions observed.
	Intranasal CPXV (BR) in BALB/c mice	Oral gavage both tecovirimat + BCV	1, 3 or 10mg/kg tecovirimat and 0.3, 1 or 3mg/kg of BCV used individually or together beginning 3 days p.i. and continued for 5 days.	Alone, tecovirimat at 3 and 10 (67% and 87% respectively) and BCV at 3mg/kg (73%) were highly protective. All doses of either extended mean time to death. In combination, treatment was highly efficacious in 7 of 9 groups, including animals receiving 1mg/kg of both compounds. As protection was not offered for 1mg/kg of either antiviral alone, this indicates combination therapy could give improved efficacy.
	Intranasal CPXV (BR) in BALB/c mice	Oral gavage both tecovirimat + BCV	1, 3 or 10mg/kg tecovirimat and 0.3, 1 or 3mg/kg of BCV used individually or together beginning 6 days p.i. and continued for 5 days.	Alone, neither tecovirimat or BCV significantly reduced mortality. In combination, treatment demonstrated efficacy in 3 of 9 groups, particularly in 2 of the 3. Like the above experiment, no mice survived when given these doses alone. Therefore, combination therapy provides synergistic efficacy against lethal CPX virus.
Chen et al., 2011 (104)	Intranasal recombinant (ECTV-11KM-IL-4) ECTV in A/Ncr mice	Oral gavage both tecovirimat + BCV	100mg/kg tecovirimat and 4mg/kg BCV used individually or together beginning immediately p.i. and continued for 14 days	Alone, neither tecovirimat or BCV protected any mice from mortality. In combination, treatment was 75% protective against mortality.

[a] CPXV = cowpox virus; BR = Brighton; ECTV = ectromelia virus
[b] BCV = brincidofovir
[c] p.i. = post infection

In vivo findings in immunodeficient animal studies

In this review, 11 studies assessed antiviral efficacy in immunodeficient mice; 8 on CDV, 1 on BCV and 2 in tecovirimat (Table 8).

Cidofovir

Cutaneous vaccinia model (5 studies)

Immunodeficiency can be modelled using SKH-1 hairless mice immunosuppressed with cyclophosphamide 1 day prior to infection. Topical CDV (1% cream) twice daily for 7 days protected 10-40% of these mice but was not efficacious when given in longer 92h intervals (105-107). CDV delivered intraperitoneally was not protective from mortality (106, 107). Though CDV (delivered topically or intraperitoneally) did not offer high efficacy, it consistently delayed time to death, and reduced primary lesion size and satellite lesion number. A triple therapy combination of 0.5% topical CDV, 50mg/kg peritoneal CDV and VIG was most efficacious in delaying time to death compared to mono- or double therapy combinations (107).

Another model used athymic nude mice, which lack a thymus and are therefore T cell deficient. Topical CDV (1% cream) was 75-100% protective up to 2 days p.i., which is prior to viral spread to organs (108). When treatment was initiated after onset of disseminated infection (approx. day 15), a subcutaneous dose of 100mg/kg CDV for minimum 3 weeks protected 80-100% of mice.

Severe combined immunodeficiency (SCID) mice lack both B and T cells. In this model, all mice succumbed to VV, however mean time to death was significantly extended (20, 109).

Intranasal cowpox model (1 study)

CDV dose of 100mg/kg (that was protective in immunocompetent mice) could not protect SCID mice even with repeated therapy (64).

Intraperitoneal cowpox model (1 study)

CDV doses between 2.2-20mg/kg could not protect SCID mice (20). However, treatment delayed time to death and reduced viral organ replication.

Intranasal camelpox model (1 study)

A 100mg/kg CDV dose delivered immediately p.i. for 3 days could provide full protection in athymic nude mice (28).

Brincidofovir

Only 1 study assessed BCV efficacy in an immunodeficient animal model. To model severe immunodeficiency, BALB/c mice lacking T cells were challenged with VV-IHD. Although all mice succumbed to disease, time to death was significantly delayed (84).

Less severe immunodeficiencies were modelled by partially reconstituting mice with T cells from healthy mice 1 day prior to infection (84). BCV was significantly protective (57-100%) and facilitated the development of strong adaptive immune responses that protected mice from re-challenge without further treatment.

Tecovirimat

In this review, 2 studies assessed the efficacy of tecovirimat in an intranasal lethal vaccinia model. In Nude and SCID mice, tecovirimat was not protective, but could delay disease progression (110). In BALB/c mice lacking CD4+ or CD8+ T cells, 100mg/kg of tecovirimat was 100% protective when administered up to 3 days p.i.(110). In the same experiment, Jh mice (genetic condition causing lack of B cells) with or without additional CD4+, CD8+ or CD4+ and CD8+ T cell depletion were modelled. 100mg/kg of tecovirimat was 100% protective when administered immediately p.i. for Jh, Jh/CD4- and Jh/CD8- mice. When administered 3 days p.i., Jh/CD8- and Jh/CD4- mice were protected 100 and 80% respectively; Jh mice all succumbed to disease. Jh mice lacking both CD4+ and CD8+ T cells did not survive any treatment.

In Nude BALB/c mice, tecovirimat is not protective unless mice are reconstituted with T cells, in which case full protection was conferred (95).

In vivo synergistic treatment with antiviral and vaccination

The antivirals CDV, BCV and tecovirimat do not inhibit the development of protective immunity when co-administered with vaccination (Table 9).

Cidofovir

Synergistic effect of CDV and vaccination were tested by 2 studies. In an intranasal ectromelia model with immunocompetent BALB/c mice, CDV and vaccination (Lister and ACAM3000) were shown to demonstrate synergistic efficacy (79). This was efficacious even when the regime was given pre-exposure to ECTV, and up to 4 days post-exposure. In contrast, in an NHP monkeypox model, 1 study found that a single dose of CDV and Dryvax coadministration significantly reduced vaccine-related immune responses (112). Though coadministration regime was still efficacious compared to naïve controls, it resulted in a higher lesion count and reduced survival rates compared to Dryvax alone; CDV's ability to inhibit viral replication appears to compromise Dryvax-induced immunity.

Brincidofovir

Only 1 study assessed BCV and vaccination; using an intranasal ectromelia model with immunocompetent A and C57BL/6 mice, the study found that BCV could be co-administered with Dryvax, ACAM2000 and ACAM3000, reducing the severity of vaccination-related lesions without preventing the development of protective immunity (87). This was supported by comparing results of ACAM2000 and ACAM3000, which are replicating and non-replicating vaccines respectively, which indicated that BCV's mechanism of action is likely through limiting viral replication rather than inhibition of the immune system.

Table 8. Antiviral efficacy in immunodeficient mice against lethal orthopoxvirus challenge

Study	Model[a]	Antiviral(s)	Dose Regime[b]	Findings
Smee et al., 2015 (107)	Cutaneous VV (WR strain) in SKH-1 mice	Topical CDV	0.5% CDV twice per day on days 2, 5, 8 and 11 p.i.	No effect on delaying time to death
		Double combination topical CDV + VIG	0.5% CDV twice per day on days 2, 5, 8 and 11 p.i. + VIG once daily parenterally on days 2, 6, 10	Significant delay in time to death
		Triple combination 0.5% topical CDV + VIG	0.5% topical CDV twice per day on days 2, 5, 8 and 11 p.i. + parenteral 50mg/kg CDV once daily on days 2, 5, 8, 11 p.i. + VIG once daily parenterally on days 2, 6, 10	Most significant delay in time to death compared to above regimes
Tarbet et al., 2011 (105)	Cutaneous VV (WR strain) in SKH-1 mice	Topical CDV	1% CDV twice daily beginning 1 day p.i. for 7 days	Demonstrated significant antiviral efficacy and delayed time to death
Smee et al., 2011 (111)	Cutaneous VV (WR strain) in SKH-1 mice	Topical CDV	1% CDV twice daily beginning 5 days p.i. for 7 days	All mice died, although CDV group lived the longest. CDV reduced primary lesions size and number of satellite lesions.
Smee et al., 2004[3] (98)	Cutaneous VV (WR strain) in SKH-1 mice	Topical CDV	1% CDV twice daily beginning 1 day p.i. for 7 days	Significantly delayed time to death, 90% mice died
		Intraperitoneal CDV	100mg/kg CDV once daily beginning 1 day p.i. every 3 days till day 21	Significantly delayed time to death, 90% mice died
		Topical CDV	1% CDV twice daily beginning 1, 3 or 5 days p.i. for 7 days	Treatment given 1 day p.i. was most efficacious (40% survival). Delay until day 3 or 5 still demonstrated significantly reduced severity of lesions.
		Intraperitoneal CDV	100mg/kg CDV daily beginning 1, 3 or 5 days p.i. for 7 days	Treatment given 1 day p.i. was most efficacious (10% survival). Delay until day 3 or 5 still demonstrated significantly reduced severity of lesions.
Quenelle et al., 2003 (20)	Intraperitoneal VV (WR strain) in SCID mice	Intraperitoneal CDV	2.2, 6.7 or 20mg/kg CDV daily beginning 2, 3 or 4 days p.i. for 7 days	All mice died, however time to death was significantly delayed in most groups
Neyts et al., 2004 (108)	Cutaneous VV (Lister) in athymic nude (nu/nu) mice	Topical CDV	1% CDV once daily beginning immediately, 1, 2, 3 or 4 days p.i. for 4 days	Treatment initiated immediately or 1 day p.i. resulted in full protection. At day 2 p.i. 75% protection provided. Delay to days 3 and 4 did not protect from mortality, but reduced the severity of lesions and delayed time to death.
		Subcutaneous CDV	100mg/kg CDV daily beginning day 15 p.i. for 21 days over 25 days	CDV was fully protective, and able to cause healing of disseminated vaccinia lesions
		Subcutaneous CDV	100mg/kg CDV daily beginning day 14 p.i. one, two, three or five times a week for 6 weeks	CDV 5 or 3 times a week was 80-100% protective from mortality
Neyts et al., 1993 (109)	Intravenous VV in SCID mice	Subcutaneous CDV	1, 5 or 20mg/kg CDV daily beginning immediately p.i. for 5 days	All mice died, however time to death was significantly delayed
		Subcutaneous CDV	20mg/kg CDV once daily beginning immediately p.i. twice a week until week 20	All mice died, however time to death was significantly delayed

Study	Model[a]	Antiviral(s)	Dose Regime[b]	Findings
		Subcutaneous CDV	Single dose of 100mg/kg CDV beginning 7 or 1 day prior to infection	All mice died, however time to death was significantly delayed even when CDV given 1 week prior to infection
		Subcutaneous CDV	20mg/kg CDV once daily beginning immediately, 2, 4, 6 or 8 days p.i. for 5 days	All mice died, however time to death was significantly delayed when CDV was delayed 2, 4 or 6 days
Quenelle et al., 2003 (20)	Intraperitoneal CPXV (BR) in SCID mice	Intraperitoneal CDV	2.2, 6.7 or 20mg/kg CDV daily beginning 2, 3 or 4 days p.i. for 7 days	All mice died, however time to death was significantly delayed in most groups
Bray et al., 2000 (64)	Intraperitoneal CPXV (BR) in SCID mice	Subcutaneous CDV	100mg/kg CDV beginning immediately p.i. as a single dose, or repeated every 3 or 6 days.	Single dose was not protective from mortality. Repeated dose every 3 days was 30% protective, and repeated dose every 6 days was 10-20% protective.
Duraffour et al., 2011 (28)	Intranasal CMLV (Iran) in athymic nude (nu/nu) mice	Intraperitoneal CDV	50mg/kg CDV daily beginning immediately p.i. for 3 days	CDV afforded full protection from morbidity
Zaitseva et al., 2015 (84)	Intranasal VV (IHD-J-Luc) in nude BALB/c mice	Oral gavage BCV	20mg/kg BCV given 1, 3 and 5 days p.i.	All mice died, however time to death was significantly delayed
		Oral gavage BCV	20mg/kg BCV given 1, 3, 5, 7, 10, 14, 17, 21, and 24 days p.i.	All mice died, however time to death was significantly delayed
	intranasal VV (IHD-J-Luc) in nude BALB/c mice with reconstituted T cells	Oral gavage BCV	20mg/kg BCV given 1, 3 and 5 days p.i. + 10^5 T cells	BCV was fully protective
		Oral gavage BCV	20mg/kg BCV given 1, 3 and 5 days p.i. + 10^4 T cells	BCV was 57% protective. BCV does not impair development of immunity, all mice that survived initial challenge also survived re-challenge on day 55.
Zaitseva et al., 2013 (95)	Intranasal VV (IHD-J-Luc) in nude BALB/c mice with reconstituted T cells	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning day 1 p.i. for 3, 5 or 7 days	Tecovirimat was 100% protective in a 7 day treatment, reducing viral dissemination and lesion development. All mice treated with tecovirimat without T cell reconstitution died.
	Intranasal VV (IHD-J-Luc) in nude BALB/c mice with reconstituted T cells (CD4+ or CD8+)	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning day 1 p.i. for 7 days	Tecovirimat was 100% protective in a 7 day treatment of mice partially reconstituted with either CD4+ or CD8+ T cells. 10^5 cells was identified as the lowest cell number required for full protection from lethality.
Grosenbach et al., 2010 (110)	Intranasal VV (WR) in nude BALB/c mice	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately p.i. for 21 days in various viral doses	No protection, but delayed disease progression. Significantly extended survival at all challenge doses - essentially inhibited acute-onset disease caused by the higher challenge doses, resulting in prolonged disease characteristic of the low challenge dose
	Intranasal VV (WR) in SCID mice	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately p.i. for 21 days in various viral doses	No protection, but delayed disease progression. Significantly extended survival at higher but not lower challenge doses.

Study	Model[a]	Antiviral(s)	Dose Regime[b]	Findings
	Intranasal VV (WR) in BALB/c mice lacking CD4+ or CD8+ cells	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately or 3 days p.i. for 14 days	100% protection for all mice in all regimens
	Intranasal VV (WR) in Jh mice (lacking mature B cells)	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately or 3 days p.i. for 14 days	100% protection when treatment began immediately. When initiated at 3 days p.i., all mice died.
	Intranasal VV (WR) in Jh mice (lacking mature B cells) with an additional lack of CD4+ OR CD8+ T cells	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately or 3 days p.i. for 14 days	100% protection when treatment began immediately in both mice groups. When initiated at 3 days p.i., 80% survival in Jh/CD4-, and 100% survival in Jh/CD8- mice.
	Intranasal VV (WR) in Jh mice (lacking mature B cells) with an additional lack of CD4+ AND CD8+ T cells	Oral gavage tecovirimat	100mg/kg tecovirimat daily beginning immediately or 3 days p.i. for 14 days	All mice died regardless of when treatment began.

[a] VV = vaccinia virus; WR = Western Reserve; SCID = severe combined immunodeficiency; CPXV = cowpox virus; BR = Brighton; CLMV = camelpox virus; IHD = International Health Department

[b] CDV = cidofovir; BCV = brincidofovir; p.i. = post infection

Table 9. Summary of studies assessing efficacy of synergistic antiviral and vaccination treatment

Study	Model	Vaccination	Antiviral	Dose Regime + orthopoxvirus challenge	Re-challenge (Y/N; Day)	Findings
Israely et al., 2012 (79)	Intranasal ECTV (Moscow) in BALB/c mice	Lister ($1x10^6$ PFU)	Intraperitoneal CDV	5, 25 or 100mg/kg CDV was given 4h or 1 day prior to Lister vaccination. Lethal challenge on day 31 of 70PFU of ECTV.	N	All animals treated with CDV and Lister vaccination were fully protected from lethal ECTV challenge. CDV did not affect development of protective immunity even when given at high dose 4h before vaccination.
	Intranasal ECTV (Moscow) in BALB/c mice	Lister ($1x10^6$ PFU)	Intraperitoneal CDV	Lethal challenge on day 0 with 70-100PFU of ECTV. 5mg/kg CDV was given 3, 4 or 5 days p.i. followed 4h later with Lister vaccination.	N	Treatment with both CDV alone or combined with vaccination afforded significant protection when given up to 4 days p.i., though there was no significant difference between the two regimes.
	Intranasal ECTV (Moscow) in BALB/c mice	ACAM3000 ($1x10^8$ PFU)	Intraperitoneal CDV	Lethal challenge on day 0 with 70-100PFU of ECTV. 5mg/kg CDV was given 3, 4 or 5 days p.i. followed 4h later with ACAM3000 vaccination.	N	
Wei et al., 2009 (112)	Intravenous MPX (Zaire) in cynomolgus monkeys	Dryvax ($2x10^5$ PFU)	Intravenous CDV	Single dose of Dryvax co-administered with 20mg/kg CDV on day 0. Lethal challenge on day 55 of $5x10^7$ PFU of ECTV.	N	Monkeys treated with vaccination and CDV had little/no skin rashes, while those treated with only vaccination had significant lesions that were slow-healing. 83% of monkeys survived from the coadministration group compared to 100% of the vaccination-only group; the coadministration group showed significantly longer survival times, though not survival rate versus control.
Parker et al., 2014 (87)	Intranasal ECTV (Moscow) in A strain mice	Dryvax (ranging $2.5x10^5$-400 PFU) *	Oral gavage BCV	Dryvax was co-administered with 10mg/kg BCV on day 0 followed by 2.5mg/kg every other day until day 14. ECTV challenge on day 50.	N	All mice vaccinated with Dryvax were protected from lethal challenge. Indicates Dryvax can be diluted to 1:625 (400PFU) and still provide significant protection against death. No significant difference between mice given BCV or vehicle, though BCV treatment appeared inferior due to greater weight change observed as a measure of morbidity. *approx. 3.5-35 fold higher than Dryvax dose given to humans based on PFU/ bodyweight
	Intranasal ECTV (Moscow) in A strain mice	Dryvax ($2.5x10^5$ PFU)	Oral gavage BCV	Lethal challenge on day 0 with 40PFU of ECTV. Dryvax was co-administered with 10mg/kg BCV beginning 4 days prior to challenge or 0, 2 or 4 days p.i. followed by 2.5mg/kg every other day until day 14	Y; Day 91	Co-administration of Dryvax and BCV significantly protective (50-100%) when given up to 2 days post challenge. All re-challenged mice survived, indicating BCV does not prevent development of protective immunity.
	Intranasal ECTV (Moscow) in	ACAM2000 ($2.5x10^5$ PFU)	Oral gavage BCV	ACAM2000 vaccination was given Day 0. 20mg/kg BCV beginning 1 day prior to challenge or 0, 1 days p.i. followed	N	Co-administration of ACAM2000 and BCV does not affect mortality or morbidity when BCV initiated day 1. BCV does not affect mortality at Day 0 and 1, and only

Study	Model	Vaccination	Antiviral	Dose Regime + orthopoxvirus challenge	Re-challenge (Y/N; Day)	Findings
	C57BL/6 strain mice			by 4 more doses of 20mg/kg every 3rd day. Lethal challenge on day 52 of 4000PFU of ECTV.		slightly affects morbidity. BCV does not impede vaccination efficacy, though it may slightly diminish immune response as ACAM2000 is a replicating vaccine.
	Intranasal ECTV (Moscow strain) in C57BL/6 strain mice	ACAM3000 ($1x10^7$, $2x10^6$, $4x10^5$, and $8x10^4$ PFU)	Oral gavage BCV	Dryvax was co-administered with 20mg/kg BCV on day 0 followed by 3 more doses of 20mg/kg on days 2, 4 and 6. Lethal challenge on day 52 of 4000PFU of ECTV.	N	A single dose of $4x10^5$PFU ACAM3000 is required to provide protection against lethal challenge. Since ACAM3000 is non-replicating, BCV does not alter the immune response following vaccination.
Berhanu et al., 2015 (100)	Intravenous MPX (Zaire) in cynomolgus macaques	ACAM2000 ($2.5x10^5$ - $12.5x10^5$ PFU)	Oral gavage tecovirimat	Lethal challenge on day 0 with $5x10^7$ PFU of MPX. ACAM2000 was co-administered with 10mg/kg tecovirimat beginning 3 days p.i. for 14 days.	Y; Day 63	All animals treated with vaccination alone succumbed to disease. All animals treated with tecovirimat (with or without vaccination) survived both initial MPX challenge, and re-challenge 2 months later. There was no clear ACAM2000-induced efficacy, unlike tecovirimat.
Grosenbach et al., 2008 (113)	Dermal scarification VV (WR) in BALB/c mice	VV-WR used as vaccination ($1x10^6$ PFU*) *approximates human dose and is 10 x the mean lethal dose $10LD_{50}$ when mice challenged intranasally	Oral gavage tecovirimat	VV-WR as vaccination was co-administered with 20mg/kg tecovirimat on day 0 for a duration of 7 or 14 days.	N	None of the mice died due to VV-WR, however control groups experienced symptoms of systemic disease and formation of satellite lesions. Tecovirimat treated groups had less severe lesion development, and no signs of systemic disease. This indicates tecovirimat given orally is present in sufficient concentrations to arrest viral dissemination and prevent severe lesion development.
	Dermal scarification Dryvax in BALB/c mice	Dryvax ($5x10^5$ PFU)	Oral gavage tecovirimat	Dryvax was co-administered with 20mg/kg tecovirimat on day 0 for a duration of 7 or 14 days.	N	None of the mice died due to Dryvax vaccination, and no signs of systemic disease were seen. Severity of lesions was less than that of VV vaccination in the study above. Due to the reduced virulence of Dryvax, the efficacy of tecovirimat was less obvious compared to VV.
	Intranasal VV (WR) in BALB/c mice	Dryvax ($5x10^5$ PFU)	Oral gavage tecovirimat	Dryvax was co-administered with 20mg/kg tecovirimat on day 0 for a duration of 7 or 14 days. Lethal challenge on day 21 with doses 10, 100 or 1000 LD_{50} of VV.	N	All vaccinated mice survived the challenge with or without tecovirimat coadministration. No other signs of disease were observed, regardless of challenge dose. Vaccine efficacy is not compromised by treatment with tecovirimat.

Study	Model	Vaccination	Antiviral	Dose Regime + orthopoxvirus challenge	Re-challenge (Y/N; Day)	Findings
	Intranasal VV (WR) in BALB/c mice	Dryvax ($5x10^5$ PFU)	Oral gavage tecovirimat	Dryvax was co-administered with 20mg/kg tecovirimat on day 0 for a duration of 7 or 14 days. Lethal challenge on day 180 with dose 10 LD_{50} of VV.		All vaccinated mice survived challenge with or without tecovirimat coadministration, while 3/5 naïve mice died (the surviving 2 showed severe disease). Vaccinated mice were able to recover from weight loss and maintain body temperature. Therefore, tecovirimat does not impair the long-term development of protective immunity.
Berhanu et al., 2010 (114)	Intranasal VV (WR) in BALB/c mice with depleted CD4- and/or CD8- T cells	ACAM2000 ($7.91x10^5$ PFU)	Oral gavage tecovirimat	ACAM2000 was co-administered with 100mg/kg tecovirimat on day 0, for a duration of 14 days. Lethal challenge of 4000PFU of VV on day 30 (1 month) post vaccination.	N	At 1 month, treatment of CD4- / CD8- deficient BALB/c and JH-KO mice was 100% protective against lethal challenge. 60% and 0% CD4-CD8- deficient BALB/c and JH-KO mice were protected respectively. There were no differences in survival between vaccine + tecovirimat or vehicle, indicating that tecovirimat does not interfere with development of short-term protective immunity.
	Intranasal VV (WR) in B-cell deficient (JH-KO) mice with depleted CD4- and/or CD8- T cells	ACAM2000 ($7.91x10^5$ PFU)	Oral gavage tecovirimat	ACAM2000 was co-administered with 100mg/kg tecovirimat on day 0, for a duration of 14 days. Lethal challenge of 4000PFU of VV on day 30 (1 month) post vaccination.	N	
	Intranasal VV (WR) in BALB/c mice with depleted CD4- and/or CD8- T cells	ACAM2000 ($1.26x10^8$ PFU) * *older mice more resistant to VV-WR disease	Oral gavage tecovirimat	ACAM2000 was co-administered with 100mg/kg tecovirimat on day 0, for a duration of 14 days. Lethal challenge of 4000PFU of VV on day 184 (6 months) post vaccination.	N	At 6 months, all BALB/c mice groups (CD4- / CD8- / CD4-CD8-) were protected against lethal challenge. In CD4- and CD8- JH-KO mice, treatment was 100% and 80% protective respectively. For CD4-CD8- JH-KO mice, only 20% were protected. There were no differences in survival between vaccine + tecovirimat or vehicle, indicating that tecovirimat does not interfere with development of long-term protective immunity.
	Intranasal VV (WR) in B-cell deficient (JH-KO) mice with depleted CD4- and/or CD8- T cells	ACAM2000 ($1.26x10^8$ PFU at day)	Oral gavage tecovirimat	ACAM2000 was co-administered with 100mg/kg tecovirimat on day 0, for a duration of 14 days. Lethal challenge of 4000PFU of VV on day 184 (6 months) post vaccination.	N	

Tecovirimat

Efficacy of tecovirimat and vaccination was assessed by 3 studies. Coadministration with ACAM2000 was tested in healthy cynomolgus macaques in an intravenous monkeypox model (100). Where ACAM2000 given alone was not efficacious, all animals treated with tecovirimat, with or without ACAM2000 were fully protected from initial challenge, and re-challenge 2 months later. Further, tecovirimat does not inhibit the development of short- and long-term protective immunity in a lethal intranasal vaccinia model against BALB/c mice (113). Tecovirimat was shown to reduce the severity of lesion formation in vaccination with VV (WR) but did not affect the formation of less severe lesions from Dryvax vaccination. This indicates that tecovirimat co-administered with vaccination will not inhibit the "take" lesion, used as evidence of vaccine protection.

To assess the prospect of tecovirimat coadministration with ACAM2000 for immunodeficient individuals, BALB/c and B cell deficient (JH-KO) mice with varying degrees of T cell deficiency were challenged in a lethal intranasal vaccinia model (114). In these studies, mice treated with coadministration achieved similar survival rates to mice with vaccination alone, indicating that tecovirimat does not impair development of short or long-term protective immunity. Tecovirimat reduced the severity of vaccination lesions in all mice except those lacking both CD4- and CD8- T cells.

Human trials

In this review, 9 studies reported on human trials of BCV and tecovirimat safety (Table 10). One review, Lanier et al. was also included as it contained information of trials not found in an individual paper.

Brincidofovir

BCV has been studied in Phase I, II and III human trials for the prophylaxis and treatment of various dsDNA viral infections including smallpox, prophylaxis/pre-emption of cytomegalovirus disease in human stem cell transplant (HSCT) recipients, and pre-emption treatment of adenovirus disease in paediatric HSCT recipients (115). These results are useful in supporting BCV as a treatment candidate for smallpox; the recommended dose is 200mg (22, 116).

Two Phase I trials indicate BCV is well tolerated in both adults and children (116). The most common AEs were gastrointestinal, usually diarrhoea. Laboratory AE of elevated serum transaminases was the main reason for treatment discontinuation, though elevations were later found to be non-symptomatic and transient.

BCV was also tested in immunocompromised and haematopoietic cell transplant recipients in Phase II and III trials to prevent/treat cytomegalovirus and adenovirus infections (115-120). Though an increased frequency of AEs were seen, this must be considered relative to the comorbidities in this population. The 200mg once weekly (QW) dose had fewer AEs relative to the 200mg twice weekly (BIW) dose (116, 119). Acute graft-versus-host disease (aGVHD) was an AE specific to this population and lead to death (2.3% vs. 1.9% in placebo) (116, 117). Overall, BCV is safe and well tolerated in the general population, including children and immunosuppressed groups. No dose-limiting toxicity has been observed, and humans have been tested with doses higher than that suggested for treating smallpox (115).

Tecovirimat

Tecovirimat has recently been approved as the first drug for smallpox treatment (15). Three Phase I trials demonstrated that tecovirimat is generally safe and well tolerated (121-123). No serious adverse events (SAEs) were observed and the most common drug-related AE was headache. Across all subjects, only 1 subject withdrew from drug-related AE (headache), and they were in a high dose group of 800mg/day (123). Absorption was faster in non-fasting volunteers and form I was chosen to be used in further treatment (121, 122).

One Phase II trial was completed in a generally healthy population with mild comorbidities (124). There were no SAEs or deaths, but 44.9% of subjects reported at least 1 AE, which were mild and commonly headache or nausea. Withdrawals from the study were not drug-related.

One expanded safety Phase III trial was done in healthy volunteers (98). The dose tested (600mg twice daily for 14 days) provided greater exposure than that considered efficacious. 19.8% of subjects experienced drug-related AE, commonly headache, osteoarthritis and hidradenitis. One death occurred, however was not deemed drug-related; pulmonary embolism was reported in the patient with significant history 1 week after treatment completed.

Human case studies

This review found 26 human cases of OPXV infection treated with antivirals since 1980 (Table 11). Humans can become infected through several routes: contact with infected animal vectors (cats or rats), tampered vaccinia-rabies baits or military vaccination against smallpox causing adverse reaction or transmission to immediate contact (125). In healthy humans, OPXV infections are usually mild; it is only on rare, serious occasions where antivirals may be used. Systemic OPXV infections are treated with CDV, BCV, tecovirimat or VIG, and ocular infection is treated with CDV or trifluride drops.

CDV was administered in 3 cases; a dose of 5mg/kg in a baby with eczema vaccinatum contributed to improvement (126, 127). CDV appears to lack effectiveness in ocular CPXV infection and when the patient is severely immunosuppressed (128, 129).

Table 10. Human trials assessing BCV and tecovirimat safety

Trial	Description[a]	Antiviral dose regime[b]	No. Participants	Adverse events and findings[c]	Study reference
CMX001–102	Phase I, dose-escalation, PK, FTIH study of the safety and tolerability of BCV in healthy human volunteers	Single ascending dose BCV – 9 cohorts of 0.025-2 mg/kg Multiple ascending dose BCV – 5 cohorts of 0.1-1.0 mg/kg. Total of 3 doses, 1 every 6 days.	36	No SAEs or AEs that prevented dose escalation. No evidence of GI toxicity. AEs reported were mild, most frequently diarrhoea, nausea and vomiting. 9/36 reported at least 1 AE in those treated with BCV, 7/18 reported 1 at least 1 AE in placebo group. Elevated serum transaminases were the most common laboratory AEs, though they were asymptomatic and transient.	Lanier et al., 2010 (115) Chittick et al., 2017 (116)
CMX001–103	Phase I comparative bioavailability study of BCV solution versus tablets, plus a comparison of PK parameters for BCV and CDV in subjects administered BCV after fasting overnight versus having eaten a high fat meal within 30 minutes of dosing	3 single doses of BCV- 40 mg solution, fasted; 40 mg tablet following a high fat breakfast; and 40 mg tablet fasted	24	Safe and well tolerated. Most frequently reported AEs: headache (17%), increased blood CPK (17%), increased ALT (13%), nausea (8%), and oropharyngeal pain (8%). Elevated serum transaminases were the most common laboratory AEs, though they were asymptomatic and transient. Significant food effect found, BCV given as tablet in fed state decreased various PK values.	Lanier et al., 2010 (115)
CMX001–104	Phase II study of the safety, tolerability, and preliminary antiviral activity of BCV in renal transplant and HSCT recipients with BK viruria, is nearing completion	BCV 10, 20 or 40mg/kg BIW up to 28 days BCV 10, 20 or 40mg/kg QW up to 28 days	28	Safe and well tolerated, no SAEs attributable to study drug reported.	Lanier et al., 2010 (115)
CMX001–108	Two-part, randomized, blinded, four-period crossover study	Single dose of BCV 350 mg	8	Safe and well tolerated, no SAEs. AEs reported were mild, most frequently diarrhoea, nausea and vomiting. As 350mg is supra-therapeutic, it was associated with a higher frequency of GI AEs (30% of subjects, diarrhoea in 20%). Elevated serum transaminases were the most common laboratory AEs, though they were asymptomatic and transient.	Chittick et al., 2017 (116)
CMX001-201 (NCT00942305)*	Phase II randomized, double-blind, placebo- controlled, dose-escalation study evaluating the safety, tolerability, and ability of BCV to prevent or control CMV	BCV 100mg BIW BCV 200mg QW	230	Safe and well tolerated. >75% subjects in all treatment cohorts (including placebo) experienced at least 1 AE during first 3 weeks of study; AE frequency was similar in treatment and placebo groups, but more common in BW than QW regime.	Chittick et al., 2017 (116) Marty et al., 2013 (119)

Trial	Description[a]	Antiviral dose regime[b]	No. Participants	Adverse events and findings[c]	Study reference
	infection in adult CMV seropositive HCT recipients			AEs reported were mild, most frequently diarrhoea, nausea, vomiting, abdominal pain and decreased appetite in both treatment and placebo groups.	
CMX001-301 (NCT01769170)*	Phase III, randomized, double-blind, placebo- controlled, parallel-group, multicentre study of the safety, tolerability, and efficacy of BCV for the prevention of CMV infection in 458 adult CMV- seropositive HCT recipients	BCV 100mg BIW	452	A HCT population specific AE is aGVHD. No deaths reported in QW treatment; similar incidence of AEs leading to death in BIW group to placebo (2.3% vs. 1.9%). Deaths due to aGVHD (2%), acute recurrent myeloid leukaemia (<1%), and veno-occlusive liver disease (<1%), which were considered not related to BCV.	Chittick et al., 2017 (116) Marty et al., 2016 (118)
CMX001-202 (NCT01241344)	Phase II, randomized, multicentre, placebo- controlled study to evaluate the safety and efficacy of BCV pre-emptive treatment for the prevention of AdV disease in paediatric and adult HCT recipients with asymptomatic AdV viremia	BCV 100 mg BIW (2 mg/kg BIW for subjects weighing <50 kg) BCV 200 mg QW (4 mg/kg QW for subjects weighing <50 kg)	48	>75% subjects in all treatment cohorts (including placebo) experienced at least 1 AE during first 3 weeks of study; AE frequency was similar in treatment and placebo groups, but more common in BW than QW regime. AEs reported were mild, most frequently diarrhoea, nausea, vomiting, abdominal pain and decreased appetite in both treatment and placebo groups. A HCT population specific AE is aGVHD. No deaths in the BIW and placebo groups. 2 subjects in QW group died from fatal AEs (metachromatic leukodystrophy and respiratory failure) though neither were considered related to BCV.	Chittick et al., 2017 (116) Grimley et al., 2017 (117)
CMX001-304 (NCT02087306)	Open-label, multicentre study to evaluate safety, tolerability, and efficacy of BCV when administered for the treatment of adult and paediatric subjects with AdV infection or disease	BCV 100 mg BIW (2 mg/kg BIW for subjects weighing <50 kg)	201	Ratio of AEs experienced by HCT and non-HCT studies was similar between adult and paediatric subjects; though paediatric subjects experienced fewer treatment-limiting AEs. Most common AEs were reported were diarrhoea, nausea, vomiting and abdominal pain. A HCT population specific AE is aGVHD.	Chittick et al., 2017 (116)
CMX001-350 (NCT01143181)	Open-label registry study conducted in 210 subjects with life-threatening conditions caused by dsDNA viral infections	BCV ≤200mg/week (≤4 mg/kg/week for subjects weighing <50 kg)	210	Lab AEs included elevations in alanine aminotransferase and aspartate aminotransferase.	Chittick et al., 2017 (116) Florescu et al., 2012 (120)
Tecovirimat	This phase I, double-blind, randomized, placebo-controlled single ascending dose study (FTIH) was conducted to determine the safety, tolerability, and pharmacokinetics of ST-246 in healthy human volunteers.	Single ascending dose tecovirimat – 500, 1000 or 2000mg in fasting state Single ascending dose tecovirimat –1000mg in non-fasting state	38	Safe and well tolerated, no SAEs. No subject withdrawn due to tecovirimat. Neutropenia was most commonly reported AE, though considered not treatment-related. Absorption was greater in nonfasting volunteers than fasting.	Jordan et al., 2008 (121)

Trial	Description[a]	Antiviral dose regime[b]	No. Participants	Adverse events and findings[c]	Study reference
NCT00431951	This phase I, double-blind, randomized, placebo- controlled, escalating multiple-dose study was conducted to determine the safety, tolerability, and PK of ST-246 administered as a single daily oral dose of 250, 400, or 800 mg for 21 days to nonfasting healthy human volunteers.	Oral tecovirimat 250, 400 or 800mg/day for 21 days.	30	Safe and well tolerated, no SAEs. Most commonly reported AE was headache; 1 subject from 800mg group discontinued study as result. PK analysis indicated 400mg/day dose can provide plasma concentrations of efficacious dose.	Jordan et al., 2010 (123)
NCT00728689	Phase I, double-blind, randomized, crossover, exploratory study was conducted to compare the PK of a single daily 400-mg oral dose of ST-246 polymorph form I versus polymorph form V administered to fed, healthy human volunteers.	Single dose oral tecovirimat form I Single dose oral tecovirimat form V	12	Both forms are well tolerated, no SAE. AEs included headache and underarm tenderness. Since form I is more thermostable, it was selected for further development and use.	Chinsangaram et al., 2012[1] (122)
NCT00907803	Phase II, double-blind, randomized, placebo-controlled, multicentre trial was conducted to assess the safety, tolerability, and PK of tecovirimat in fed relatively healthy adult volunteers	Single dose oral tecovirimat - 400 mg or 600 mg for 14 days	107	Safe, well tolerated. No deaths or SAEs. 44.9% of subjects reported at least 1 AE, which were most commonly mild nausea and headache. 2 subjects withdrew from AEs (upper respiratory tract infection and post-procedural haematoma), neither which were related to study medication. PK were predictable.	Chinsangaram et al., 2012[2] (124)
NCT02474589	Phase III, double-blind, randomized, multicentre trial was conducted as expanded safety trial to assess tecovirimat in healthy volunteers 18-79 years.	Tecovirimat 600mg twice daily for 14 days	449	This dose was expected to provide exposure in excess of that provided by efficacious doses in animals. 19.8% of subjects experience an AE related to trial agent; AE of grade 3 or higher occurred or worsened during treatment at a frequency of both 1.1% in both treatment and placebo groups. AEs included headache, osteoarthritis and hidradenitis. 1 death was reported due to pulmonary embolism that occurred in a patient 1 week after treatment completed; patient had a history of recurrent deep-vein thromboses untreated with anticoagulants. This was deemed to be unrelated to tecovirimat.	Grosenbach et al., 2018 (98)

[a] PK = pharmacokinetics; FTIH = First Time in Humans; BCV = brincidofovir; CMV = cytomegalovirus; HCT = haematopoietic cell transplant; AdV = adenovirus

[b] BIW = twice weekly; QW = once weekly

[c] SAEs = serious adverse events; AEs = adverse events; GI = gastrointestinal; CPK = creatine phosphokinase; ALT = alanine transaminase; aGVHD = acute graft-versus-host disease

* data presented together as pooled analysis done by Chittick et al., 2017

Adapted from Chittick et al., 2017

Table 11. Human cases of orthopoxvirus infection treated with antiviral (1980-current)

Study	Case	Patient history[a]	Route of infection	Antiviral treatment[b]	Did treatment improve condition? (Y/N; detail)
Gazzani et al., 2017 **(129)**	Disseminated cowpox infection	17 yo boy immunosuppressed due to renal transplantation and history of chronic kidney disease	Secondary transmission via pet cat which had contracted infection from wild animal vectors	CDV, BCV, VIG (doses unknown) initiated day 9 after admission.	N; lesions worsened despite treatment. Patient died 4 weeks after hospital admission due to septic shock and intractable multi-organ failure.
Said et al., 2013 **(132)**	Vaccinia infection	23 yo female, history of atopic dermatitis	Secondary transmission via contact with military smallpox vaccinee	Only VIG	Y; no new lesions within 5 days of treatment, lesions on thigh, toe and back almost or completely resolved
Graef et al., 2013 **(128)**	Persistent corneal cowpox infection necessitating corneal transplant	49 yo female, history DMII	Primary transmission via contact with rat suspected of cowpox infection	Cidofovir 350g IV once weekly – probably inhibited viral replication	N; conditions seemed to improve, however regressed by 5 weeks after continuous therapy
CDC et al., 2013 **(133)**	Vaccinia infection	N/A	Secondary transmission via contact military smallpox vaccinee	VIGIV	Y; man discharged 2 days after treatment, at follow-up lesions had healed completely
	Vaccinia infection	N/A	Tertiary transmission via contact military smallpox vaccinee	VIGIV	Y; man discharged 4 days after treatment, at follow-up lesions had healed completely
Lederman et al., 2012 **(130)**	Progressive vaccinia	US Marine Corps member with unknown acute myelogenous leukemia	Primary transmission via smallpox vaccination	Total treatment: 241 vials of VIGIV, 73 days of oral tecovirimat (nearly 75g), 68 days topical tecovirimat, 6 weekly doses of BCV (totalling 700mg) VIGIV: more than any patient required to date. Doses were 6000, 18000, 24000 IU/kg Tecovirimat: first patient to receive topical ST-246. Resistance noted to develop late in disease. Escalation: 400 mg – 800 mg – 1200 mg BCV: first time given to orthopoxvirus infected patient. Doses 100mg or 200mg.	Y; discharged 5 months after vaccination with ACAM2000. -
Young et al., 2011 **(134)**	Vaccinia infection	24yo male	Secondary transmission via contact military smallpox vaccinee	Trifluridine ophthalmic solution	Y; blepharitis and eyelid erythema resolves with 48h of initiation
	Vaccinia infection	29yo female	Tertiary transmission via military smallpox vaccinee	VIG	Y; lesion resolved 3 days after treatment

Study	Case	Patient history[a]	Route of infection	Antiviral treatment[b]	Did treatment improve condition? (Y/N; detail)
CDC et al., 2009 **(131)**	Vaccinia infection of the hand	35 yo female, taking immunosuppressive medication for IBD	Primary transmission via contact with raccoon rabies vaccine bait	VIGIV: 2 doses given each 6000 IU/kg Tecovirimat: Unknown dose given for 14 days	Y; discharged day 19 after admission, lesions healed 22 days after first dose of VIGIV and 16 days after tecovirimat
Becker et al., 2009 **(146)**	Cowpox infection of eye	17 yo boy	Primary transmission via contact with infected pet rat	CDV	N/A*
Van Dam et al., 2009 **(135)**	Post vaccinia encephalitis	19yo male, military	Primary transmission via smallpox vaccination	VIGIV: 1 dose of 400 000 units for 5 days	Y; discharged 27 days after admission, 23 days after treatment
Vora et al., 2008 **(126)** CDC et al., 2007 **(127)**	Eczema Vaccinatum	28-month baby, history of refractory atopic dermatitis and failure to thrive.	Secondary transmission via contact military smallpox vaccinee (father)	VIGIV: Total of 3.96 g/kg of vaccinia IgG in 11 doses (more than double maximum dose administered in severe cases of progressive vaccinia/ eczema vaccinatum in era of smallpox vaccination) CDV: 1 dose 5mg/kg Tecovirimat: 5mg/kg for 14 days Trifluridine: unknown	Y; discharged 48 days after hospitalisation March 3 admitted
Vora et al., 2008 **(126)** CDC et al., 2007 **(127)**	Eczema Vaccinatum	Mother of baby with eczema vaccinatum	Tertiary transmission via contact military smallpox vaccinee	VIGIV: Single dose 6000 IU/kg	Y; lesions resolved
Lewis et al., 2006 **(136)**	Ocular vaccinia	Age unknown, unvaccinated lab worker	Primary transmission via accidental laboratory accident	VIGIV: 6000 IU/kg Trifluridine: unknown	Y; Discharged day 9 after admission. Ocular symptoms improved 24h after VIGIV treatment.
Fillmore et al., 2004 **(145)**	Ocular vaccinia	21yo male, military	Primary transmission via smallpox vaccination	Trifluridine 1% drops: 5 or 9 times daily	Y; lesions resolved without sequelae
	Ocular vaccinia	26yo male	Primary transmission via smallpox vaccination	Trifluridine: unknown	Y; lesions resolved without sequelae
	Ocular vaccinia	47yo male	Primary transmission via smallpox vaccination	Trifluridine 1% drops: 5 times daily for 2 weeks	Y; lesions resolved without sequelae
CDC et al., 2003 **(137)** Hu et al., 2004 **(147)**	Ocular vaccinia	26yo female	Secondary transmission via contact military smallpox	VIGIV: single dose, 6000 U/kg Trifluridine: unknown	Y; lesions improved 24h after treatment
	Ocular vaccinia	18yo female	Secondary transmission via contact military smallpox	Trifluridine: unknown	Y; lesions improved 24h after treatment

Study	Case	Patient history[a]	Route of infection	Antiviral treatment[b]	Did treatment improve condition? (Y/N; detail)
Wills et al., 2000 **(138)** Kesson et al., 1997 **(139)**	Vaccinia necrosum/ Progressive vaccinia	66yo male, history of metastatic melanoma and chronic lymphocytic leukemia	Primary transmission via intradermal vaccinia melanoma cell lysate inoculation for treatment of metastatic melanoma	VIG: 0.25mL/kg given intramuscularly	Y; Discharged 8 days after treatment, no new lesions (NB: man died 2 months later due to progressive metastatic melanoma)
Czerny et al., 1991 **(148)** Eis-Hubinger et al., 1990 **(149)**	Generalised cowpox-like infection	18yo male, history of atopic dermatitis	Secondary transmission via cat	Homologous vaccinia antiserum: unknown	N; spread of lesions halted, however patient died 2 weeks later due to acute heart failure due to massive pulmonary thromboembolism.
Redfield et al., 1987 **(140)**	Disseminated vaccinia	19yo male, military member, unknown history of HIV	Primary transmission via smallpox vaccination	VIG: 50ml weekly for 12 weeks given intramuscularly	Y; lesions resolved 2 months after treatment (NB: man died 1 year later due to HIV complications)
CDC et al., 1985 **(143)**	Vaccinia infection	15yo female	Secondary transmission via contact military smallpox	VIG: 30ml for 2 days given intramuscularly Trifluridine: unknown	Y; lesions resolved without sequelae
Keane et al., 1983 **(142)**	Vaccinia necrosum/ Progressive vaccinia	56yo female, previously vaccinated with successful take	Primary transmission via smallpox vaccination as travel precaution	VIG: 0.6 mL/kg (NB: case occurred 1976)	Y; discharged 14 days after treatment, lesions resolved without sequelae
CDC et al., 1982 **(150)**	Disseminated vaccinia	19yo male	Primary transmission via smallpox vaccination	VIG: 25ml (half indicated dose) given intramuscularly	Y; lesions resolved within 5 days
Funk et al., 1981 **(141)**	Vaccinia necrosum/ Progressive vaccinia	50yo female, previously vaccinated with successful take	Primary transmission via smallpox vaccination	VIG: 5 courses with dose 0.6 mL/kg	Y; lesions completely resolved 9 months after initial symptom onset without sequelae
Chudwin et al., 1981 **(144)**	Vaccinia necrosum/ Progressive vaccinia	7-month old boy, undiagnosed combined immunodeficiency	Primary transmission via smallpox vaccination	VIG: 5 injections totalling 35 ml	N; patient dies of acute respiratory failure 51 days after admission, lesions did not heal
Olding-Stenkvist et al., 1980 **(151)**	Vaccinia necrosum/ Progressive vaccinia	3-month old female, undiagnosed immunodeficiency	Primary transmission via smallpox vaccination	VIG: 35ml Transfer factor from young healthy adults: 3 occasions	N; patient did not improve, died of pneumonia at age 22 weeks
	Vaccinia necrosum/ Progressive vaccinia	4-month old male, undiagnosed immunodeficiency	Primary transmission via smallpox vaccination	VIG: 24ml Transfer factor: unknown	N; patient did not improve, died of pneumonia

* incomplete report

[a] DMII = diabetes mellitus type 2; IBD = inflammatory bowel disease; HIV = human immunodeficiency virus

[b] CDV = cidofovir; BCV = brincidofovir; VIG = vaccinia immune globulin; VIGIV = vaccinia immune globulin intravenously

BCV and tecovirimat were used in 2 and 3 cases respectively. In a case of severe immunosuppression, BCV was not able to provide protection (129). Tecovirimat contributed to the successful resolution of symptoms in all 3 cases (126, 127, 130, 131). BCV, oral and topical tecovirimat were used together in a case of progressive vaccinia in a military vaccinee who had unknown underlying acute myelogenous leukaemia (AML)(130). After several months of antiviral treatment and chemotherapy, the man recovered. However, tecovirimat-resistant VV was detected late in disease, indicating that BCV may have played an important role in recovery.

VIG has been used in 22 human cases and appears to demonstrate some protective effect (126, 127, 129-144). However, there is limited supply as it must be synthesised from blood drawn from smallpox vaccinees. The above case used 241 vials of intravenous VIG (VIGIV), which placed unanticipated strain on the US national stockpile (130). Topical trifluride was used in 9 cases, all of which successfully resolved (126, 127, 134, 136, 137, 143, 145).

Discussion

Though re-emergence of smallpox is hypothetical, there is an imperative for continued research as the consequences would be disastrous. Since its eradication, many compounds have been considered as anti-smallpox agents with varying, but limited, levels of efficacy. They include methisazone, M&B7714, cytosine arabinoside (ara-C), adenine arabinoside, ribavirin, CSA-13 cera-genin, imiquimod, idoxuridine, interferon and phosphonoacetic acid (27, 50, 152, 153).

CDV, BCV and tecovirimat are considered the most viable antivirals in the event of smallpox re-emergence or vaccine AEs. This systematic review reviewed 230 articles on their efficacy *in vitro, in vivo* animal studies, in healthy humans and in human case reports to provide a holistic understanding of their potential use.

In vitro, CDV demonstrated consistently high potency; however, it is limited by its poor bioavailability and nephrotoxicity when administered intravenously. BCV, a bioavailable derivate of CDV, is both safer and more efficacious *in vitro*. Likewise, tecovirimat is also more efficacious than CDV and demonstrates specific activity against multiple VARV and MPXV clades, the two OPXV of greatest concern to human health (44).

In vivo studies in various animal models support the use of these antivirals therapeutically. Both BCV and tecovirimat were efficacious when given in single- and multi-dose regimens and were efficacious in most animal models when delayed several days p.i.. One model suggested BCV treatment could be initiated after observation of secondary lesions, though there was a small sample size of animals and further study is required to substantiate this (85). In immunodeficiency studies, BCV provided partial protection to mice with moderate immunodeficiency (57-100% survived). However, it could only extend time to death in mice with severe immunodeficiency (84). Tecovirimat was protective in moderately immunocompromised mice but could only delay death when mice lacked both B and T cell immunity (95, 110).

CDV and BCV demonstrate strong potential for prophylactic therapy and both were shown to be efficacious when given up to 5 days prior to lethal challenge, depending on the animal model (20, 64, 68). No studies assessed prophylactic effect of tecovirimat despite its recent FDA approval, which is a significant gap that should be addressed.

BCV and tecovirimat has been shown to be safe and well tolerated in both adult and paediatric populations in Phase I, II and III trials. Most common BCV-related AEs were gastrointestinal (diarrhoea or nausea); tecovirimat-related AEs were neurological (headache) and gastrointestinal (diarrhoea or nausea) (98, 115-117, 119, 120, 122-124). These AEs were in a dose-dependent relationship and were mild at recommended therapeutic doses. Tecovirimat has been successfully approved under FDA Animal Rule and is available as 200mg capsules, of which 2 million courses have been delivered to the Strategic National Stockpile. It is now undergoing Phase I development for IV formulation (15).

Though these antivirals demonstrate promise, a major limitation is the potential for antiviral-resistant strains of OPXV, particularly in a bioterrorism context. This is already possible through selective cell culture in the lab. More concerningly, tecovirimat-resistant VV was detected in a human case of progressive vaccinia after tecovirimat treatment (130). Viral DNA can also be manipulated via synthetic biology. BCV demonstrates a high barrier to resistance, but only a few mutations are required at the F13L gene for VV to become tecovirimat-resistant. Research into antiviral efficacy on resistant OPXV strains is very limited. Only CDV-resistant OPXV was investigated; CDV was weakly protective, while BCV was partially protective (55, 59, 102). However, the studies are not conclusive, and given the likelihood of tecovirimat-resistance, more research needs to be done in this area.

A proposed way to reduce the risk of antiviral resistance is through combination therapy. BCV and tecovirimat have strong synergistic efficacy due to their differing mechanisms of action and provide protection against lethal challenge when antivirals used alone could not (39, 103). This protection also extended to an ECTV model of vaccination resistance, suggesting combination therapy may be effective against more virulent stains of OPXV. However, data is limited to two studies in this review and therefore a definitive conclusion cannot be made. There is a need for further research into the promising results in this area.

Conclusion

The achievements in antiviral research for OPXV treatment has greatly changed the landscape of bioterrorism preparedness post-smallpox eradication. Use of antivirals could alleviate the risks of vaccination and extend protection to immunocompromised populations in the event of a smallpox outbreak. Future research should look beyond antiviral monotherapy, as the limited research on combination therapy is promising. Given that antivirals would provide the most benefit for immunodeficient populations, more focus should be given to developing relevant models. Finally, with the risk of antiviral-resistance, more robust models to test antiviral efficacy against more virulent strains should be developed.

Abbreviations

AEs	Adverse Events
aGVHD	Acute Graft Vs Host Disease
AML	Acute Myelogenous Leukaemia
BCV	Brincidofovir
BIW	Twice weekly
CDV	Cidofovir
CMLV	Camelpox virus
CMV	Cytomegalovirus
CPXV	Cowpox virus
EC_{50}	Effective concentration capable of inhibiting 50% of cytopathic effect
ECTV	Ectromelia virus
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
HCT	Haematopoietic Cell Transplantation
HSCT	Human stem cell transplant
IC_{50}	50% inhibitory concentration
IHD	International Health Department
IL-4	Interleukin 4
IND	Investigational New Drug
MeSH	Medical Subject Headings
MPXV	Monkeypox virus
NHP	Non-human primates
OPXV	Orthopoxvirus
p.i.	Post infection
PFU	Plaque Forming Units
PRISMA	Preferred Reporting Items for Systematic Review
QW	Once weekly
RPXV	Rabbitpox virus
SAEs	Severe Adverse Events
SCID	Severe combined immunodeficiency
VARV	Variola Virus
VIG	Vaccinia immune globulin
VIGIV	Intravenous vaccinia immune globulin
VV	Vaccinia virus
WHO	World Health Organisation
WR	Western Reserve
WT	Wild type

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