

Supplemental Information

Appendix A: Search strategies

For in vitro, animal and healthy human studies

1. orthopoxvirus/ or cowpox virus/ or ectromelia virus/ or monkeypox virus/ or vaccinia virus/
or variola virus/
2. cowpox/ or ectromelia, infectious/ or monkeypox/ or smallpox/ or vaccinia/
3. orthopox*.mp.
4. 1 OR 2 OR 3
5. cidofovir.mp.
6. hpmpc.mp.
7. vistide.mp.
8. brincidofovir.mp.
9. hdp-bcv.mp.
10. cmx001.mp.
11. tecovirimat.mp.
12. st-246.mp.
13. tpoxx.mp.
14. 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13
15. 4 AND 14

For unhealthy human case reports

1. orthopoxvirus/ or cowpox virus/ or ectromelia virus/ or monkeypox virus/ or vaccinia virus/
or variola virus/
Years: From 1980-2018
Publication type: Case report

Appendix B: In vitro studies

Summary of findings for in vitro studies							
Study type	Study	Antiviral ^a	OPXV ^b	Cell type for propagation ^c	EC ₅₀ value (μM) for strain		Efficacy
					Strain	Value	
Antiviral efficacy as main objective	Olson et al., 2014 (43)	CDV	VARV	Vero cells	BSH74	6.07	BCV EC ₅₀ values averaged 0.11 μM, and therefore nearly 100-fold more potent than CDV
					SOM77	1.37	
					JPN51	10.81	
					UNK52	7.08	
					BRZ66	28.45	
					BCV	0.21	
	Pires et al., 2018 (52)	CDV	Brazilian VV	BSC-40 cells	Br-VV-GP2V	27.14±1.04	
					Br-VV-PSTV	39.42±6.45	
					Br-VV-GP1V	41.68±3.41	
					Br-VV-SH2V	42.40±23.80	
					Br-VV-P1V	62.53±21.37	
					VV-WR	59.08±12.38	
	Duraffour et al., 2014 (42)	CDV	VV CPXV CMLV ORF*	HEL cells	VV-Cop	6.9±3.0	
					VV-Lis	9.1±6.6	
VV-WR					8.2±2.6		
CPXV-BR					13.9±8.3		
CML1					11.2±4.5		
ORF-NZ2					0.8±0.1		
Pires et al., 2018 (52)	Tecovirimat	Brazilian VV	BSC-40 cells	Br-VV-GP2V	0.0054±0.0008		
				Br-VV-PSTV	0.0056±0.0017		
				Br-VV-GP1V	0.0372±0.0002		
				Br-VV-SH2V	0.0381±0.0068		
				Br-VV-P1V	0.0518±0.0168		
				VV-WR	0.0425±0.0148		
Olson et al., 2014 (43)	BCV	VV CPXV	HEL cells	VV-Cop	0.005±0.002		
				VV-Lis	0.023±0.021		
				VV-WR	0.013±0.011		

		CMLV		CPXV-BR	0.021±0.026	
		ORF		CML1	0.024±0.022	
				ORF-NZ2	Not done	
Duraffour et al., 2013 (41)	CDV	CPXV VV CMLV	HEL cells	CPXV-BR CPXV-GER1980-EP4 CPXV-GER1991-3 CPXV-AUS1999-867 CPXV-FIN2000-MAN VV-WR VV-Cop VV-Lis CML1	19.6±9.8 20.6±0.5 7.7±2.8 13.1±4.8 12.2±6.8 8.1±4.4 5.6±2.8 5.9±3.8 10.8±5.9	CDV inhibited replication of all orthopoxviruses in micromolar range. BCV showed 1000-fold lower EC ₅₀ values than CDV and appeared more selective. Toxicity of BCV higher on growing cells than CDV. Tecovirimat demonstrated inhibitory effects in nanomolar range. Due to low toxicity for growing cells, molecule was highly selective.
	BCV	CPXV VV CMLV	HEL cells	CPXV-BR CPXV-GER1980-EP4 CPXV-GER1991-3 CPXV-AUS1999-867 CPXV-FIN2000-MAN VV-WR VV-Cop VV-Lis CML1	0.030±0.024 0.017±0.000 0.007±0.007 0.01±0.007 0.014±0.010 0.007±0.009 0.004±0.002 0.094±0.061 0.021±0.015	Results support that compounds will likely be active in vivo against genetically diverse CPXV.
	Tecovirimat	CPXV VV CMLV	HEL cells	CPXV-BR CPXV-GER1980-EP4 CPXV-GER1991-3 CPXV-AUS1999-867 CPXV-FIN2000-MAN VV-WR VV-Cop VV-Lis CML1	0.21±0.15 0.03±0.04 0.03±0.02 0.02±0.01 0.02±0.01 0.017±0.009 0.008±0.003 0.04±0.06 0.02±0.02	
Santos-Fernandes et al., 2013 (49)	Tecovirimat	CPXV VV	BSC-40 cells	CPXV-BR VV-WR VV-Wyeth VV-IOC Cantagalo	0.33 ± 0.025 0.055 ± 0.003 0.046 ± 0.002 0.034 ± 0.006 0.0086 ± 0.001	Not stated

Ruiz et al., 2011 (53)	BCV	ECTV CPXV VV	BSC-1 cells (ECTV) HFF cells (CPXV, VV)	ECTV-MOS CPX-BR VV-Cop	0.125±0.06 0.2±0.1 0.08±0.03	Study demonstrated that BCV was not as metabolically stable in monkey liver, suggesting in vivo testing is unlikely to produce levels of intact drug sufficient for successful treatment. However, they noted BCV was more stable in human liver S9.
Smith et al., 2009 (51)	Tecovirimat	VARV MPXV	BSC-40 cells	VARV-BRZ66-39 VARV-SLN68-258 VARV-SUD47-juba VARV-BSH74-sol VARV-NEP73-175 VARV-SOM77-ali MPXV-V78-I-3945 MPXV-V81-I-179 MPXV-2003-USA-039 MPXV-V77-I-823 MPXV-V1979-I-005	0.067±0.0282 0.037±0.0063 0.019±0.0046 0.028±0.0124 0.021±0.0139 0.028±0.0303 0.023±0.0026 0.032±0.0061 0.036±0.0045 0.030±0.0114 0.039±0.0016	Tecovirimat protects cells and inhibits variola and monkeypox viruses similarly. This study also found that tecovirimat reduces viral plaque size and comet tail formation in a dose-dependent manner.
Jesus et al., 2009 (58)	CDV	VV	BSC-40 cells	Cantagalo virus IOC	7.68±1.35 9.66±0.94	Results demonstrate that CDV has potent antiviral activity against smallpox vaccine strain IOC and VV field strain Cantagalo virus.
Bailey et al., 2007 (47)	Tecovirimat	VV CPXV MPXV CMLV VARV	BSC-40 cells	VV CPXV CDVrCowpox* MPXV CML VARV-But VARV-BSH	0.04 0.6 0.07 0.01 0.01 0.02 0.05	Demonstrates orthopoxvirus selectivity for tecovirimat, results are consistent with F13L being target of antiviral activity. Tecovirimat was found to have high metabolic stability compared to other compounds. *CDV resistant strain of cowpox
Quenelle et al., 2007 ² (46)	Tecovirimat	VV CPXV	HFF cells	VACV-Cop VACV-WR CPXV-BR	0.05±0.02 0.1±0.05 0.48±0.01	In vitro activity of tecovirimat was significantly greater than CDV
	CDV	VV CPXV	HFF cells	VACV-Cop VACV-WR CPXV-BR	29.2±14 33±13 41.1±4.2	

Quenelle et al., 2007 ¹ (39)	CDV	VV CPXV	HFF cells	VV-Cop VV-WR CPXV-BR	29.2±14 33±13 41.1±4.2	BCV and tecovirimat were more efficacious than CDV when used against orthopoxviruses. Using CellTitre-Glo assays, this study also determined that synergistic combination of BCV and tecovirimat was more efficacious than the antivirals alone. No toxicity was detected.
	BCV	VV CPXV	HFF cells	VV-Cop VV-WR CPXV-BR	0.14±0.09 0.13±0.001 0.24±0.1	
	Tecovirimat	VV CPXV	HFF cells	VV-Cop VV-WR CPXV-BR	0.05±0.02 0.1±0.05 0.48±0.01	
Duraffour et al., 2007 ¹ (45)	Tecovirimat	VV CPXV CMLV	HEL cells	VV-Cop CPXV-BR CML1	0.007±0.003# 0.16±0.09 0.03±0.004	In both cell lines, tecovirimat exhibited more potent activity against VV, CPXV and CLMV than CDV. The results further support F13L as the target of tecovirimat, and the study provides several hypotheses on the mechanism of action of tecovirimat.
	CDV	VV CPXV CMLV	HEL cells	VV-Cop CPXV-BR CML1	3.7±0.5 13.3±3.0 2.6±1.2	
	Tecovirimat	VV CPXV CMLV	PHK cells	VV-Cop CPXV-BR CML1	0.003±0.00006 0.013±0.0005 0.02±0.01	
	CDV	VV CPXV CMLV	PHK cells	VV-Cop CPXV-BR CML1	2.3±1.0 3.2±0.6 1.7±0.8	
Kornbluth et al., 2006 (55)	CDV	VV	C127I cells	WT CDV-R ^{mr*}	2.1±0.7 29±6	CDV was more active in C127I cells than Vero. CDV resistant virus was also resistant to BCV. *marker-rescued CDV resistant vaccinia WR viruses
	BCV	VV	C127I cells	WT CDV-R ^{mr}	0.31±0.2 -	
	CDV	VV	Vero cells	WT CDV-R ^{mr*}	92±8 1030±250	
	BCV	VV	Vero cells	WT CDV-R ^{mr*}	0.24±0.1 4.6±1.1	
Lebeau et al., 2006 (36)	BCV	VV CPXV	HEL cells	VV-Lederle- Chorioallantoic CPXV-BR	0.013±0.006 0.035±0.004	In vitro activity of BCV was significantly greater than CDV
	CDV	VV CPXV	HEL cells	VV-Lederle- Chorioallantoic CPXV-BR	2.52±1.45 6.83±0.34	

	BCV	VV CPXV	PHK cells	VV-Lederle- Chorioallentoi CPXV-BR	0.48±0.52 0.32±0.19	
	CDV	VV CPXV	PHK cells	VV-Lederle- Chorioallentoi CPXV-BR	5.8±4.2 5.3±2.1	
Yang et al., 2005 (44)	Tecovirimat	VV VARV CPXV ECTV MPXV CML	Vero cells	VV-NYCBH VARV-But VARV-BSH CPXV-BR ECTV-MOS MPXV-Zaire CML CDVrCowpox	0.01 0.02 0.05 0.05 0.07 0.01 0.01 0.05	Tecovirimat is a potent and specific inhibitor of poxvirus replication in cell culture. The study also found that tecovirimat inhibits plaque formation, extracellular virus production, and the mechanism of action corresponds to the V061 gene in CPXV, and the F13L gene in vaccinia. Tecovirimat was also well absorbed in pharmacokinetic studies and had good oral availability. Tecovirimat was also active against a CDV-resistance strain of CPXV.
Smee et al., 2005 (56)	BCV	VV	Vero cells	WT WTp15* CDV-R**	0.4±0.2 0.3±0.1 7.0±2.3	The CDV resistant virus was 13- to 14-fold more resistant to CDV than the two forms of WT virus, and similar results were seen with BCV. *Virus passaged 15 times in cell culture in parallel with the CDV-R virus. **CDV resistant
	CDV	VV	Vero cells	WT WTp15* CDV-R**	61±7 56±6 790±190	
Keith et al., 2004 (35)	BCV	VV CPXV	HFF cells	VV-Cop CPXV-BR	0.6±0.4 0.5±0.3	BCV was more active than CDV.
	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	31±5.4 42±5.4	
Buller et al., 2004 (34)	BCV	ECTV VV	CV-1 cells	ECTV-MOS ECTV-7.5E-mIL-4* VV-WR	0.5±0.1 0.2 0.7	In vitro activity of BCV was significantly greater than CDV *An ECTV recombinant expressing murine IL-4 from the 7.5 early promoter.
	CDV	ECTV VV	CV-1 cells	ECTV-MOS ECTV-7.5E-mIL-4* VV-WR	12±2.8 12 40	
Kern et al., 2002 (33)	CDV	VV CPXV	Vero cells	VV-Cop CPXV-BR	30±12.6 45±7.9	

	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	23±4.1 48±1.8	Though CDV demonstrated high activity against orthopoxviruses, BCV had higher potency.	
	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	46.2±11.9 44.7±6.3		
	BCV	VV CPXV	HFF cells	VV-Cop CPXV-BR	0.8±0.4 0.6±0.3		
	CDV	VV CPXV	HFF cells	VV-Cop VV-WR VV-Elstree VV-IHD VV-NYC	46.2±11.9 45.8±16.6 41.6±22.4 13.4±5.6 10.1±1.3		
	BCV	VV CPXV	HFF cells	VV-Cop VV-WR VV-Elstree VV-IHD VV-NYC	0.8±0.4 1.1±1.0 1.2±0.8 0.2±0.0 0.4±0.0		
Smee et al., 2002 ¹ (57)	CDV	VV CPXV CMLV MPXV	Vero 76 cells	VV-WT VV-R** CPXV-WT CPXV-WT (SF)* CPXV-R** CPXV-R (SF)* CMLV-WT CMLV-R** MPXV-WT MPXV-R** MPXV-R (SF)*	19±6 150±36 45±7 58±13 >1000 730±160 2.3±0.5 22±5 27±11 505±50 725±105	In plaque reduction assays, 16- to 27-fold-higher concentrations of CDV were required to inhibit the CPXV-R and MPXV-R viruses than were required to inhibit WT viruses. The degrees of resistance were less for CMLX and VV viruses. * (no syncytium-forming [SF] viruses were present) **R=resistant	
Nettleton et al., 2000 (54)	CDV	VV	FLM cells	VV-Lister PPV-orf-11 PPV-NZ-2 PPV-MN	1.32±0.47 0.27±0.05 0.28±0.07 0.21±0.06	CDV has activity against VV consistent with the literature. Sensitivity of parapoxviruses (PPV) to CDV not previously recorded.	
CDV as reference drug	Carcelli et al., 2018 (154)	CDV	VV	HEL cells	VV HSV-1 ADV2	16 0.95 7.5	Not stated

Kang et al., 2016 (155)	CDV	VV	HEL cells	VV Adenovirus-2	25 10	Consistent with literature
Rogolino et al., 2015 (156)	CDV	VV	HEL cells	VV-Lederle	16	Not stated
Kokosza et al., 2015 (157)	CDV	VV	HEL cells	VV-Lederle	22	Not stated
Daghigh et al., 2015 (158)	CDV	VV	E ₆ SM cells	VV	25	Not stated
Patel et al., 2014 (159)	CDV	VV	HEL cells	VV	13±5.1	Not stated
Loddo et al., 2014 (160)	CDV	VV	Vero cells	VV-Lis	19	Not stated
Gangarapu et al., 2014 (161)	CDV	VV	HEL cells	VV	22	Not stated
McGuigan et al., 2013 (162)	CDV	VV	BSC-1 cells	VV	11.5#	Not stated
Tichy et al., 2012 (163)	CDV	VV	HEL cells	VV-Lederle	14.0±6.2	Not stated
Flusin et al., 2012 (164)	CDV	VV	Vero cells	VV-Lis	23±3.1	CDV displays low but significant inhibition of VV DNA synthesis.
Zakharova et al., 2011 (165)	CDV	CPXV VV	KB Cells	CPXV-BR VACV-Cop	30# 20#	Not stated
Schormann et al., 2011 (166)	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	1.8 4.3	CDV has higher selectivity index due to low cytotoxicity
Kesel et al., 2011 (167)	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	6.7 9.8	Not stated
Kumar et al., 2010 (168)	CDV	VV	HEL cells	VV	8	Not stated
Torres et al., 2010 (48)	CDV	VV	HEL cells	VV	5.7	Not stated
Sharma et al., 2009 (169)	CDV	VV	HEL cells	VV	7	Not stated
El-Sabbagh et al., 2009 (170)	CDV	VV	HEL cells	VV-Lederle	10	Not stated

Vigne et al., 2009 (171)	CDV	VV	HEL cells	WR	34±5	Single mutation viruses were more resistant than wild-type virus to CDV, but less resistant than double mutation viruses. *Single mutations of cidofovir resistant VV-WR strains *Double mutations
				WR-A314T*	139±17	
				WR-A684V	151±13	
				WR-S851Y	151±8	
				WR-A314T+A684V**	>159±0	
WR- A684V+S851Y	>159±0					
McSharry et al., 2009 (172)	CDV	VV	HeLa-S3 cells	WR	30.85±8.78	Results for CDV are consistent with the literature. This study also found that the currently licensed dose of CDV is unlikely to provide protection for human patients exposed to VARV.
				IHD-J	18.74±6.02	
				IHD-W	20.61±4.21	
Prichard et al., 2008 (173)	CDV	VV	HFF cells	VV-FL2-gfp (recombinant)	11±4.2	Deletion of F2L gene and replacement with gfp gene does not seem to change CDV efficacy in the recombinant VV.
				VV-WR	10±0.3	
Eriksson et al., 2008 (174)	CDV	VV CPXV	HFF cells	VV	27	Results for CDV are consistent with the literature.
				CPXV	30	
Quenelle et al., 2007 ³ (61)	CDV	VV CPXV	HFF cells	VV-WR	29±3.1	Not stated
				VV-Cop	26±5.2	
				CPXV-BR	35±4.3	
Duraffour et al., 2007 ² (175)	CDV	CMLV	HEL cells	CML1	8.2±3.7	Not stated
				CML14	6.6±1.9	
	CDV(175)	CMLV	PHK cells	CML1	5.3±2.6	
				CML14	8.8±4.7	
Smee et al., 2007 ² (74)	CDV	VV	C127I cells	VACV-WT	4.9±1.7	CDV demonstrated activity against the three viruses, though potency was greater in mouse cells than monkey cells (expected from literature).
				VACV-S-variant	3.7±2.5	
				VACV-WR	1.7±0.2	
	CDV	VV	Vero cells	VACV-WT	79±9	
				VACV-S-variant	46±5.3	
				VACV-WR	61±8	
	CDV	VV	A549 cells	VACV-WT	65±9	
				VACV-S-variant	35±9	
				VACV-WR	45±8	
Quenelle et al., 2006 (60)	CDV	VV CPXV	HFF cells	VV-Cop	32±0.5	Not stated
				VV-WR	19±11	
				VV-NYC	8.9±3.5	
				VV-Lis	22±4.9	

				VV-IHD	28±8.1	
				VV-IHD-W	23±6.0	
				CPXV-BR	39±5.2	
Prichard et al., 2006 ² (176)	CDV	CPXV	HFF cells	CPXV-TK ⁺	6.1±0.3	Both strains of CPXV were fully sensitive to CDV.
				CPXV-TK ⁻	3.3±2.3	
Prichard et al., 2006 ¹ (63)	CDV	CPXV	HFF cells	VV-Cop	3.3±0.3	CDV was efficacious, and no difference was seen between TK ⁺ and TK ⁻ strains of CPXV.
				CPXV-BR	4.4±0.6	
				CPXV-TK ⁺	3.5±3.3	
				CPXV-TK ⁻	4.2±3.9	
Fan et al., 2006 ¹ (177)	CDV	VV CPXV	HFF cells	VV-Cop	24±12	CDV does not require phosphorylation to be active as it is a monophosphate analogue. Therefore, its activity is similar in TK ⁺ and TK ⁻ strains. *thymidine kinase (TK) deficient strain
				CPXV-BR	3.3±1.1	
				CPXV-TK ⁺ lacZ*	40±6.1	
				CPXV-TK ⁻ lacZ*	5.2±3.9	
Fan et al., 2006 ² (178)	CDV	VV CPXV	HFF cells	VV-Cop	20±11	Not stated
				CPXV-BR	32±10	
Fan et al., 2006 ³ (179)	CDV	VV CPXV	HFF cells	VV-Cop	24±12	Not stated
				CPXV-BR	40±6.1	
Selvam et al., 2006 (180)	CDV	VV CPXV	HFF cells	VV-Cop	20	Not stated
				CPXV-BR	32	
Arumugham et al., 2006 (181)	CDV	VV CPXV	HFF cells	VV	4.8	Not stated
				CPXV	5.0	
Beadle et al., 2006 (182)	CDV	VV CPXV	HFF cells	VV-Cop	25.3±6.1	Not stated
				CPXV-BR	30.7±6.3	
Remichkova et al., 2006 (183)	CDV	VV	CEF cells	RIIPD (10 000*)	26.5±0.19#	CDV approximately has the same effect on both virus strains, and antiviral effect is dependent on size of virus inoculum. *virus inoculum CCID ₅₀
				RIIPD (1 000)	21.3±0.25#	
				RIIPD (100)	8.5±0.14#	
				RIIPD (10)	8.5±0.29#	
				Bratislava (10 000*)	13.6±0.23#	
				Bratislava (1 000)	17.5±0.16#	
				Bratislava (100)	8.5±0.17#	
				Bratislava (10)	7.1±0.25#	
Roy et al., 2005 (184)	CDV	VV CPXV	HFF cells	VV	30	Not stated
				CPXV	36	
Smee et al., 2004 ¹ (67)	CDV	CPXV	C127I cells	CPXV-BR	1.1±0.6	CDV emerged as the most viable antiviral for further investigation and demonstrated

	CDV	CPXV	Vero cells	CPXV-BR	53±15	high selectivity and potency against CPXV.	
Quenelle et al., 2004 (68)	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	31±5.4 42±5.4	Not stated	
Keith et al., 2003 (31)	CDV	VV CPXV	HFF cells	VV-Cop CPXV-BR	33±9.1 43±2.5	Not stated	
Baker et al., 2003 (30)	CDV	VARV	Vero cells	Average result for 35 VARV isolates	12±1#	CDV demonstrate particularly high activity against orthopoxviruses.	
	CDV	VARV MPXV CPXV VV	Vero cells	VAR-BSH VAR-YAM VAR-GAR MPXV-Zaire CPXV-BR VV-Cop	9.5 12.1 4.5 6.7 7.5 8.1		
Snoeck et al., 2002 (185)	CDV	VV	PHK cells	VV-Lederle-Chorioallentoi	4.0±2.6#	CDV demonstrated particularly high activity against orthopoxviruses.	
Smee et al., 2002 ² (59)	CDV	VV	Vero 76 cells	VV-WT* VV-R**	19±6 150±36	*WT=Wild type **R=Resistant	
Smee et al., 2001 ³ (186)	CDV	VV CPXV MPXV CML	Vero cells	VV-Cop CPXV-BR MPXV-Zaire CML-Somalia	37±4 92±4 96±12 33±8	CDV demonstrated higher activity in 3T3 (mouse) cells compared to Vero (monkey) cells.	
	CDV	VV CPXV MPXV CML	3T3 cells	VV-Cop CPXV-BR MPXV-Zaire CML-Somalia	3.9±2.3 3.2±2.7 8.0±4.6 2.5±1.1		
BCV as reference drug	Hostetler et al., 2007 (38)	BCV	ECTV	BSC-1 cells	ECTV	0.22	Not stated
Tecovirimat as reference drug	Selivanov et al., 2017 (187)	Tecovirimat	VV	Vero cells	VV-Cop	0.001	Not stated
	Mazurkov et al., 2016 (97)	Tecovirimat	ECTV MPXV	Vero cells	ECTV-K-1 MPXV-V79-1-005	0.003# 0.003	Tecovirimat displayed high efficacy
	Torres et al., 2010 (48)	Tecovirimat	VV	HEL cells	VV	0.065	Not stated

IC₅₀ result

^a CDV = cidofovir; BCV = brincidofovir

^b VV = vaccinia virus; VARV = variola virus; CPXV = cowpox virus; CMLV = camelpox virus

^c Vero, BSC-40, CV-1 African green monkey cells; HEL, human embryonic lung fibroblasts; HFF, human foreskin fibroblasts; PHK, primary human keratinocyte; C127I, mouse mammary tumour cells; FLM, fetal lamb muscle; E₆SM, human cells; KB, human carcinoma of nasopharynx; HeLa S3, human cervix carcinoma cells; A549, human lung carcinoma
