Brincidofovir Treatment Efficacy in Two Well-Characterized Orthopoxvirus Infection Models of Smallpox

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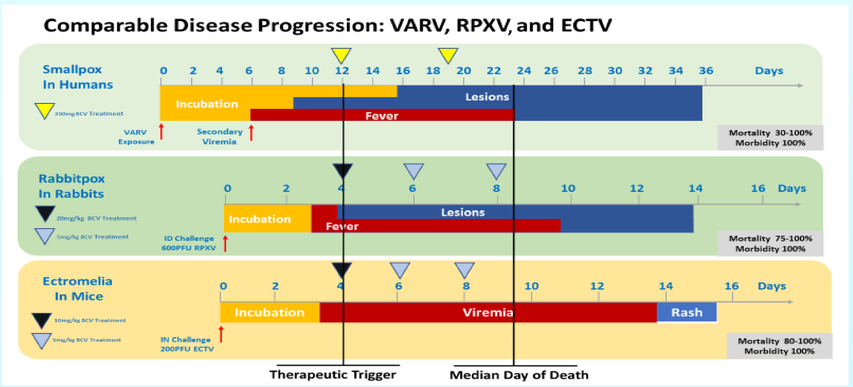
ABSTRACT

The release of variola virus (the etiological agent of smallpox) poses a considerable global health threat. Various countries have stockpiled vaccinia virus-based smallpox vaccines as their primary countermeasure. However, given the limited efficacy of vaccination when used following viral exposure and substantial side effects of replicating smallpox vaccines, the availability of antiviral drugs provides treatment options post viral exposure as well as a remedy for vaccine-associated complications. Tecovirimat was recently approved as an antiviral for the treatment of smallpox under the FDA Animal Rule; a second antiviral with a different mechanism of action is needed due to the potential for viral resistance to develop, or is engineered. Brincidofovir (BCV) is in development as a medical countermeasure against smallpox. Confirmatory, placebo-controlled, blinded, GLP efficacy studies in rabbit and mouse models of smallpox utilizing rabbitpox (RPXV) and ectromelia (ECTV) virus have been executed using fixed-treatment regimens initiated following onset of clinical signs [Days 3, 4, 5, or 6 and Days 4, 5, 6 or 7 post inoculation (PID), respectively]. The primary efficacy endpoint in both models was survival; secondary assessments included the reduction in the severity and progression of clinical events associated with disease. Following lethal viral challenge, all animals were qPCR positive for viral load by PID 3 (RPXV) or PID 4 (ECTV). Compared to placebo, both rabbit and mouse models showed a statistically significant increase in survival in BCV-treatment groups, including BCV treatment initiation as late as PID 6 (rabbit) and PID 7 (mouse). Further, as the median day of death for placebo treated rabbits and mice was PID 9.4 and PID 8.6, respectively, BCV efficacy was demonstrated beyond the midpoint of both RPXV and ECTV disease. Significant improvements in clinical and virology parameters were also noted and all animals surviving through study completion demonstrated an immune response. Collectively, these studies show that treatment with BCV post exposure results in a statistically significant survival benefit in two well-characterized orthopoxvirus infection models for smallpox. These studies also provide a scientific rationale for therapeutic intervention with BCV in event of a smallpox outbreak when vaccination is contraindicated or when diagnosis follows the appearance of clinical signs and symptoms. BARDA contract HHSO100201100013C

Orthopoxvirus Infection Model Features:

- Rabbit and mouse models of smallpox are naturally permissive, robust, reproducible, and lethal at low inoculums
- Both models develop severe disease with an incubation phase preceding generalized viremia
- The antiviral target of BCV (viral DNA polymerase) in both models is approximately 98% identical to variola virus



EFFICACY STUDIES - EXPERIMENTAL DESIGNS

RABBITPOX VIRUS (RPXV) IN NEW ZEALAND WHITE (NZW) RABBITS

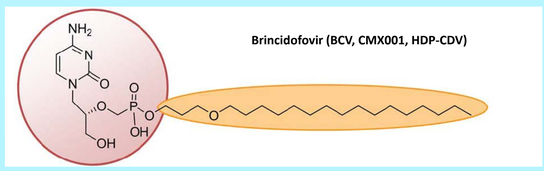
BLINDED GROUP	NUMBER OF ANIMALS	RPXV CHALLENGE	TIMING OF TREATMENT ADMINISTERATION PER BLINDED KIT*							
		DAY 0	PID 3	PID 4	PID5	PID6	PID7	PID8	PID9	PID10
		TARGET INOCULUM DOSE	BLINDED DOSING KIT DOSE VIAL #1 KIT	BLINDED DOSING KIT DOSE VIAL #2 KIT	BLINDED DOSING KIT DOSE VIAL #3 KIT	BLINDED DOSING KIT DOSE VIAL #4 KIT	BLINDED DOSING KIT DOSE VIAL #5 KIT	BLINDED DOSING KIT DOSE VIAL #6 KIT	BLINDED DOSING KIT DOSE VIAL #7 KIT	BLINDED DOSING KIT DOSE VIAL #8
1	30 (15/SEX)	600 PFU	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo	Placebo
2	30 (15/SEX)		Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo
3	30 (15/SEX)		Placebo	Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo
4	30 (15/SEX)		Placebo	Placebo	Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)
5	30 (15/SEX)		Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo

*Blinded dosing kits were randomly assigned to individual rabbits at challenge. The first treatment dose of each 8-dose blinded treatment kit was administered on Post Inoculation Day 3 (PID 3), with each subsequent treatment dose administered on PID 4 through PID 10.
PFU = Plaque Forming Unit

ECTROMELIA VIRUS (ECTV) in BALB/C MICE

ARM	GROUP	NUMBER OF ANIMALS	ECTV CHALLENGE	TIMING OF TREATMENT ADMINISTRATION PER BLINDED KIT*								Collection Days (Blood, Liver, Spleen)	Number of Mice per Collection Time Point
			Day 0	PID 4	PID 5	PID 6	PID 7	PID 8	PID 9	PID 10	PID 11		
			TARGET INOCULUM DOSE	BLINDED DOSING KIT DOSE VIAL #1	BLINDED DOSING KIT DOSE VIAL #2	BLINDED DOSING KIT DOSE VIAL #3	BLINDED DOSING KIT DOSE VIAL #4	BLINDED DOSING KIT DOSE VIAL #5	BLINDED DOSING KIT DOSE VIAL #6	BLINDED DOSING KIT DOSE VIAL #7	BLINDED DOSING KIT DOSE VIAL #8		
Efficacy Evaluation	1	32 (16/sex)	200 PFU	BCV (10mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo	Placebo	T	16/sex
	2	32 (16/sex)		Placebo	BCV (10mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo	T	16/sex
	3	32 (16/sex)		Placebo	Placebo	BCV (10mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	T	16/sex
	4	32 (16/sex)		BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo	Placebo	T	16/sex
	5	32 (16/sex)		Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	Placebo	T	16/sex
	6	32 (16/sex)		Placebo	Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	T	16/sex
	7	32 (16/sex)		Placebo	Placebo		BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	T	16/sex
	8	32 (16/sex)		Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	T	16/sex
VIRAL LOAD**	9	40 (20/sex)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	PID 2, 3, 4, 5, 6, & T	3/sex
Resistance**	10	14 (7/sex)	200 PFU	N/A	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	Placebo	N/A	PID 7, 8, 9, 10, 11, 12, 15 & T	1/sex
	11	14 (7/sex)		N/A	Placebo	BCV (20mg/kg)	Placebo	BCV (5mg/kg)	Placebo	BCV (5mg/kg)	N/A		1/sex
	12	14 (7/sex)		N/A	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	N/A		1/sex

*Blinded dosing kits for groups 1 through 8 were randomly assigned at challenge. The first treatment dose of each 8-dose blinded treatment kit was administered on Post Inoculation Day 4 (PID 4), with each subsequent treatment dose administered on PID 5 through PID 11.
**Groups 9 through 12 were not blinded. Group 9 animals were not treated, but solely had samples collected for viral load assessments. Group 10, 11 and 12 animals all initiated unblinded treatment on PID 5 and completed on PID 10
T Refers to "Terminal" for survivors on last day of study (PID 42),unscheduled euthanasia or animals found dead. Blood was not collected from animals found dead; only liver and spleen collected. Note: Last day on study for Group 9 animals was PID 6. Last day on study for Groups 10, 11 and 12 animals was PID 15.
PFU=Plaque Forming Unit

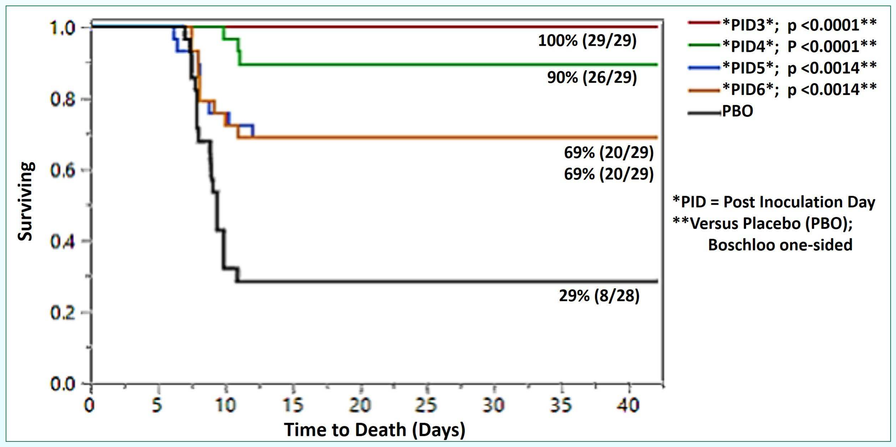


- Brincidofovir is anabolized intracellularly to the active antiviral, Cidofovir-diphosphate (CDV-PP)
- CDV-PP inhibits viral replication by selectively inhibiting viral DNA polymerases, blocking viral DNA replication and thereby reducing viral burden

RESULTS

Rabbitpox Virus Model:

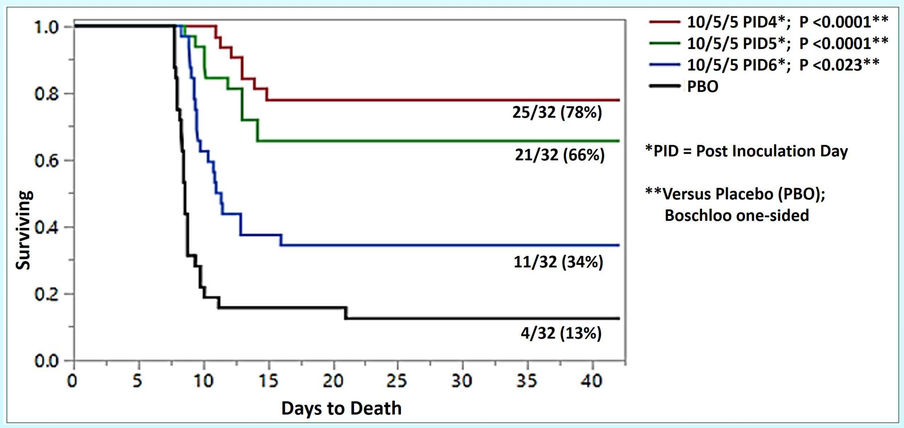
Survival plot comparing BCV treatment to placebo. BCV treatment at 20/5/5 mg/kg (q48h) was initiated at 3, 4, 5, or 6 days post lethal intradermal challenge of rabbitpox virus



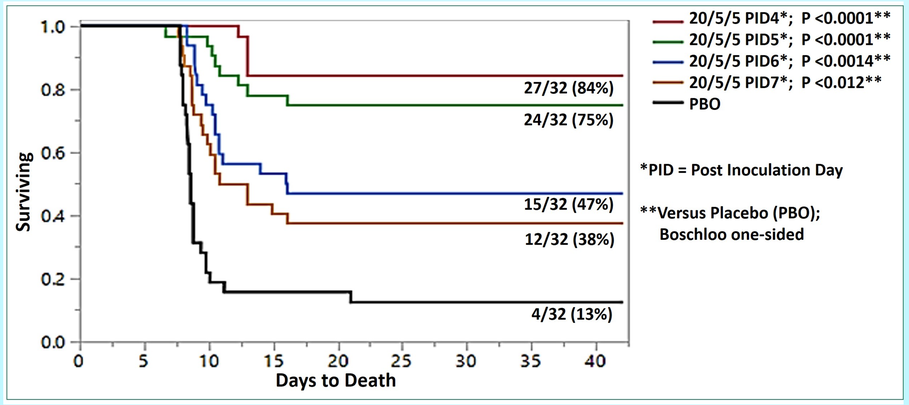
- BCV treatment provided a statistically significant survival benefit compared to placebo regardless of day post challenge BCV treatment was initiated (up to Day 6)
- The median day of death for placebo treatment was Day 9.4; therefore, BCV treatment was efficacious even when treatment was initiated beyond the midpoint of disease progression

Ectromelia Virus Model:

Survival plot comparing BCV treatment to placebo. BCV treatment at 10/5/5 mg/kg (q48hr) was initiated 4, 5, or 6 days post lethal intranasal challenge of ectromelia virus



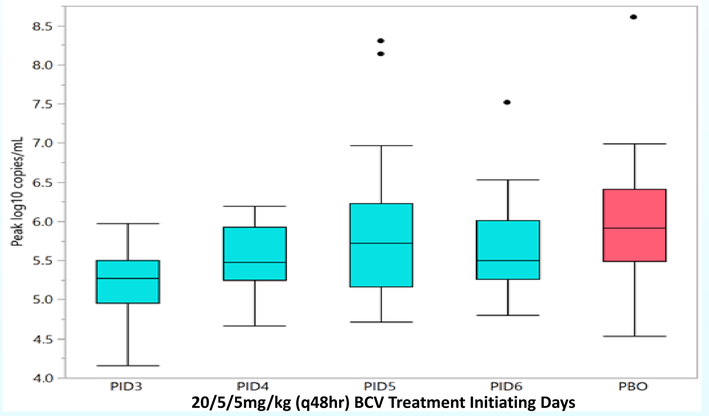
Survival plot comparing BCV treatment to placebo. BCV treatment at 20/5/5mg/kg (q48hr) was initiated 4, 5, 6 or 7 days post lethal intranasal challenge of ectromelia virus



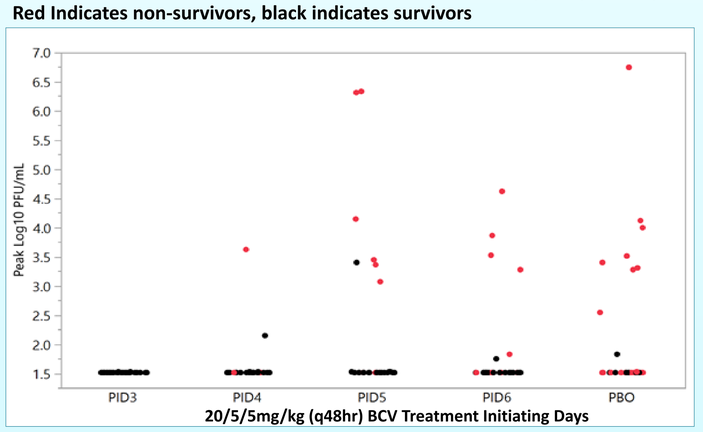
- BCV treatment provided a statistically significant survival benefit compared to placebo, regardless of day post challenge BCV treatment was initiated (up to Day 7)
- The median day of death for placebo treatment was Day 8.6; therefore, BCV treatment was efficacious even when treatment was initiated beyond the midpoint of disease progression
- The efficacies of the 10/5/5 and 20/5/5mg/kg (q48hr) BCV treatment regimens were comparable

RPXV Viral Load in Blood by qPCR and Plaque Assay Assessments

Whisker Box Plot of peak blood RPXV viral loads in BCV vs PBO treated rabbits by qPCR

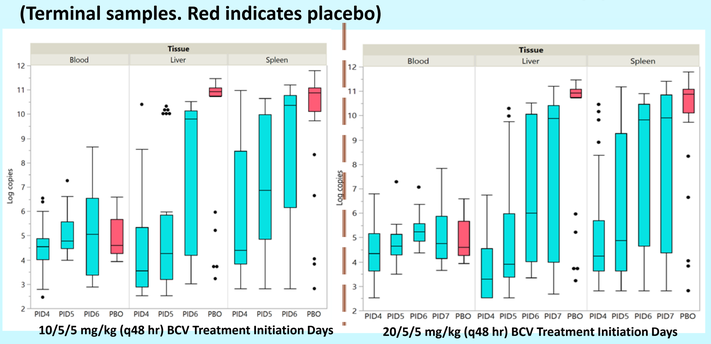


Peak blood RPXV viral loads in BCV vs PBO treated rabbits by Plaque Assay; Red Indicates non-survivors, black indicates survivors

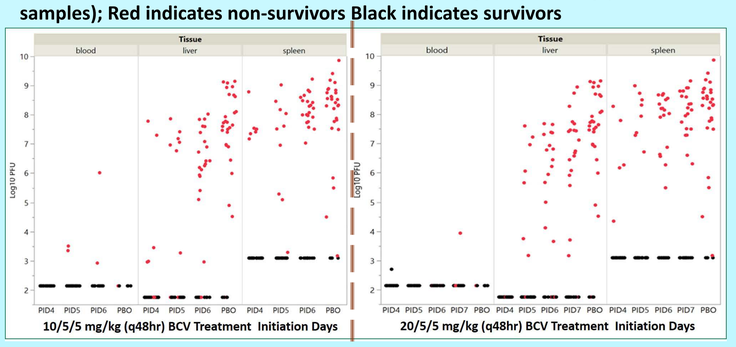


ECTV Viral Load in Blood, Liver and Spleen Tissues by qPCR and Plaque Assay Assessments

Whisker Box Plots of ECTV vial load in BCV vs PBO treated mice by qPCR (Terminal samples. Red indicates placebo)



ECTV viral loads in BCV vs PBO treated Balb/c mice by Plaque Assay (Terminal samples); Red indicates non-survivors Black indicates survivors



- Consistent with BCV's antiviral mechanism of action, earlier BCV treatment following viral challenge resulted in lower peak blood viral loads in the RPXV model. BCV treatment also resulted in lower viral loads in terminal tissue samples in the ECTV model
- Delayed treatment reduced the effect on viral load

CONCLUSIONS

- Brincidofovir demonstrated a survival benefit in two well-characterized animal models of lethal orthopoxvirus infection
- Initiating treatment at fixed intervals after infection mimics a wide range of conditions from early diagnosis and mild disease to late diagnosis associated with severe disease, under which the drug may be used in the clinical setting of a smallpox outbreak.
- BCV treatment was efficacious even when started beyond the midpoint of disease progression in both models
- Efficacy of BCV treatment on viral load was consistent with survival results
- An immune response was demonstrated in surviving animals (data not shown)
- The antiviral effect and survival benefit demonstrated by BCV treatment in animal models of orthopoxvirus infection are believed to be predictive of similar benefit in humans and supports the potential for use in the event of a smallpox release

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