BCX4430, an Adenosine Analog, with Potent Activity Against Yellow Fever Virus in a Hamster Model

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Supported in part by NIAID Contract HHSN272201000039I/HHSN27200004, Task A21

Epidemiology of Yellow Fever

- Endemic to Africa and South America
- Cause periodic outbreaks with 20-50% mortality
- Imported cases and vaccine-associated adverse effects in areas outside natural range
- **No approved antiviral therapy**

BCX4430 Characteristics

- Novel adenosine analog
- Efficiently phosphorylated to triphosphate form in cells
- Does not incorporate into mammalian RNA or DNA
- Metabolically stable – not deaminated

Study	Concentration	Result
AMES	5 mg/plate	Negative
hERG	30 μM	Negative
Mammalian DNA incorporation	30 μM	Negative
Mammalian RNA incorporation	30 μM	Negative

Broad-Spectrum Activity of BCX4430

Family	Virus	EC_{50}(µg/mL)	EC_{90}(µg/mL)	In Vivo PoP	Model
Flaviviridae	Yellow Fever	8.3	9.33	Yes	Hamster
	Dengue 2	13	13.05	Yes	Mouse
	West Nile	16	7	Yes	Hamster
	JEV (SA-14)	6.5	n/d	n/d	n/d
Bunyaviridae	Rift Valley Fever	54	37	Yes	Mouse
	Maporal (Hantavirus) (HV97021050)	7.8	n/d	n/d	n/d
Rhabdovirdae	Rabies (Flury LEP)	9.8	n/d	n/d	n/d
Coronaviridae	SARS-CoV	16	n/d	n/d	n/d
Togaviridae	VEE (TC83)	72	60	n/a	n/a
	EEE (FL93-939)	13	n/d	n/d	n/d
Paramyxoviridae	Measles	1.4	0.37	n/a	n/a
	Parainfluenza 3	10	n/d	n/d	n/d
	RSV	13	n/d	n/d	n/d
Picornaviridae	Rhinovirus 2	5.7	19	n/d	n/d
Adenoviridae	Adenovirus	60	25	n/d	n/d

n/d: not determine

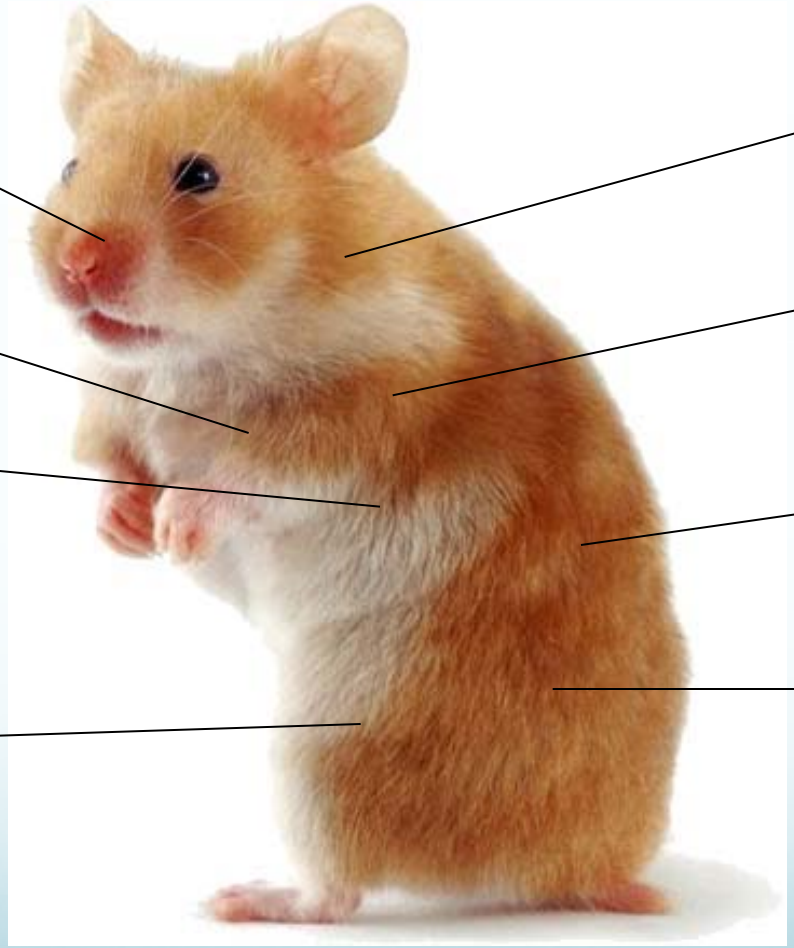
BCX4430 is Active Against YFV

- Broad-spectrum activity against several families, with specific pan-flavivirus activity
- Effective against YFV (17D) in Vero cells:

  EC50: 8.3 µg/ml; EC90: 9.3 µg/ml; CC50: 320 µg/ml
- Potential RNA polymerase inhibitor- results pending
- *In vivo* testing warranted

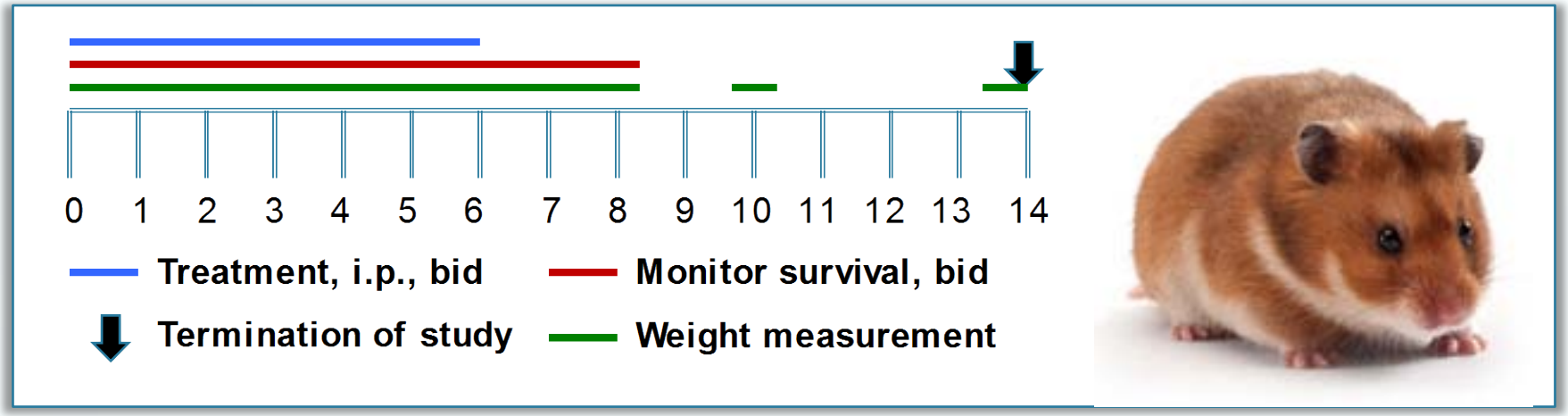
Hamster Model of Yellow Fever

- **Some hemorrhagic manifestations: petechiae, nosebleed**
- **Viremia, 2-5 d**
- **Liver disease w/ microvesicular steatosis**
- **Adapted Jimenez strain, i.p.**



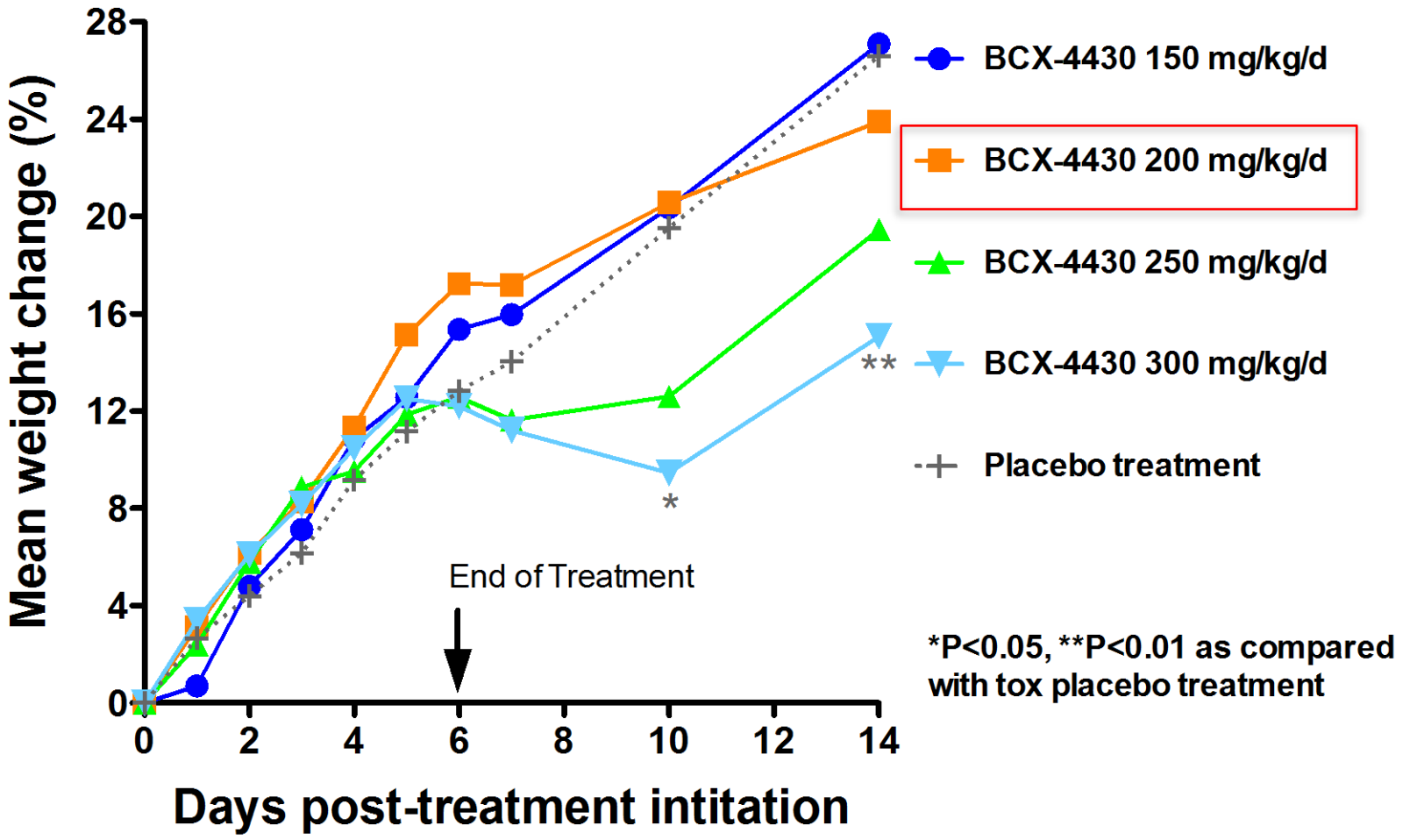
- **80% mortality around 6-9 dpi**
- **Serum AST>ALT**
- **Renal dysfunction**
- **Several measureable blood parameters of disease**

Tolerated dose

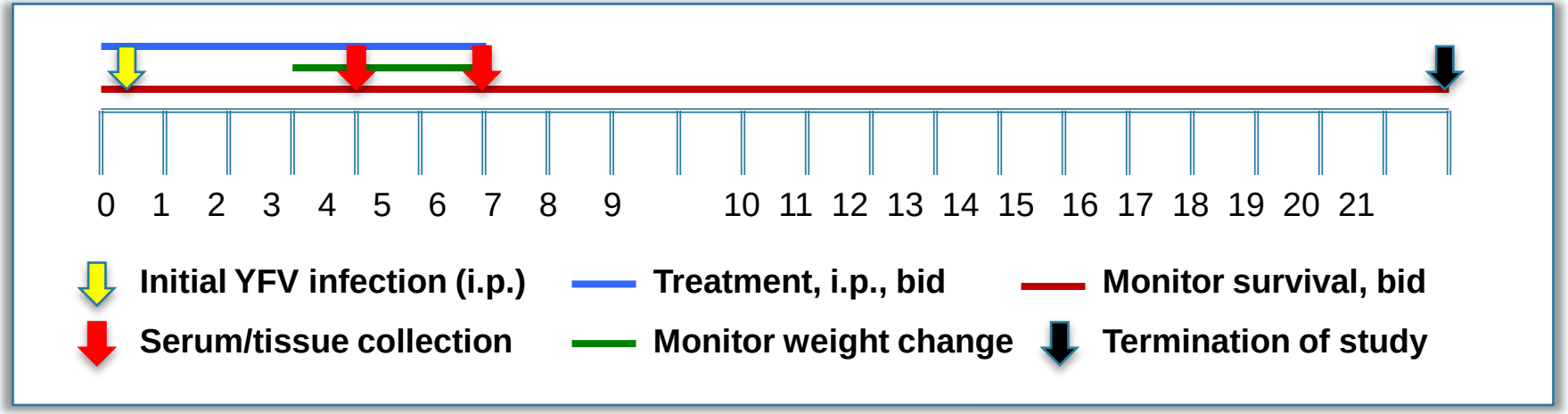


- Golden Syrian hamsters, Charles River Labs
- Uninfected animals, 3/group
- BCX4430 doses from 150-300 mg/kg/d tested
- Weights and survival monitored

Tolerated Dose in Uninfected Hamsters

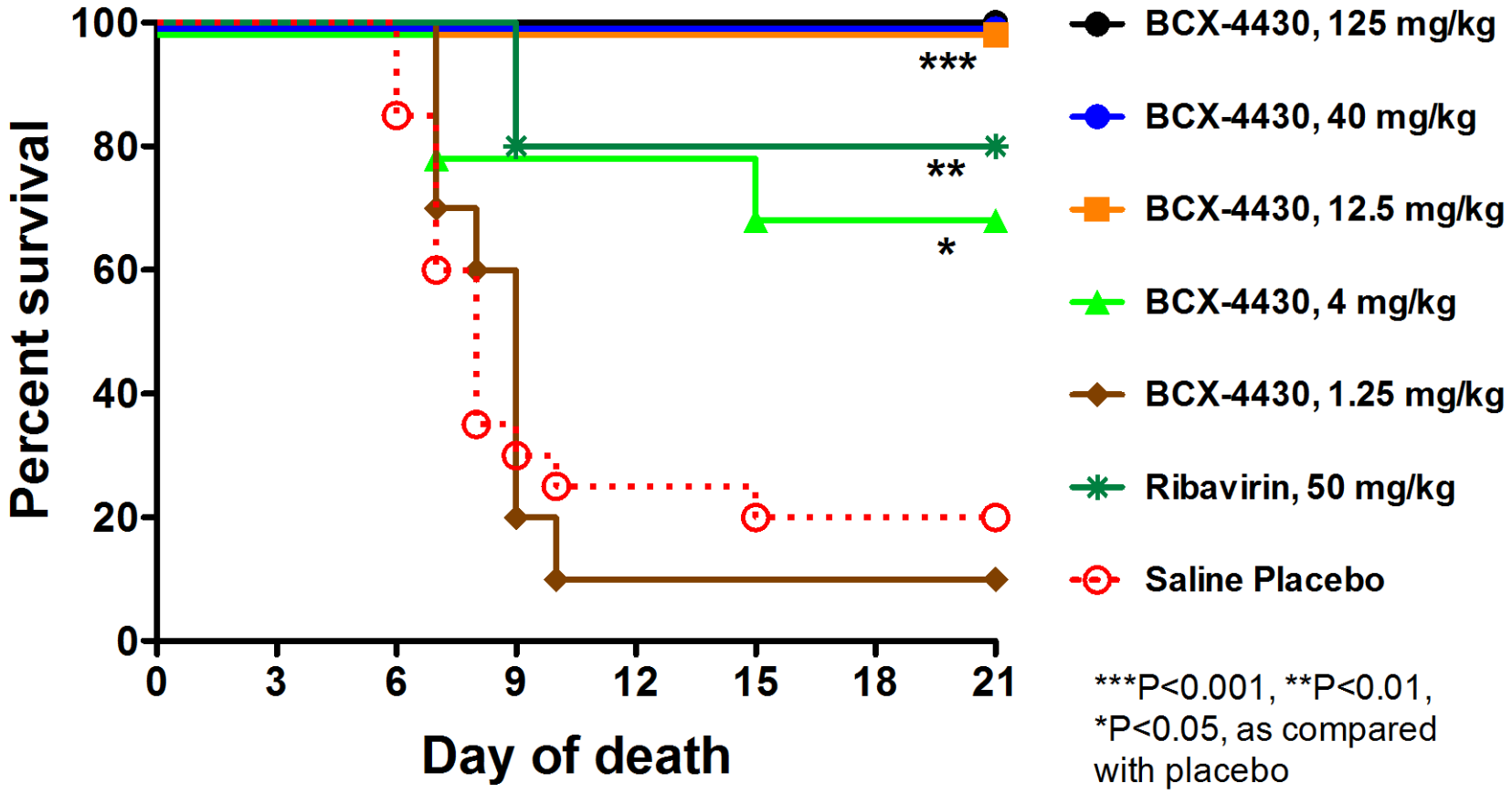


Dose Response in YFV Infected Hamsters

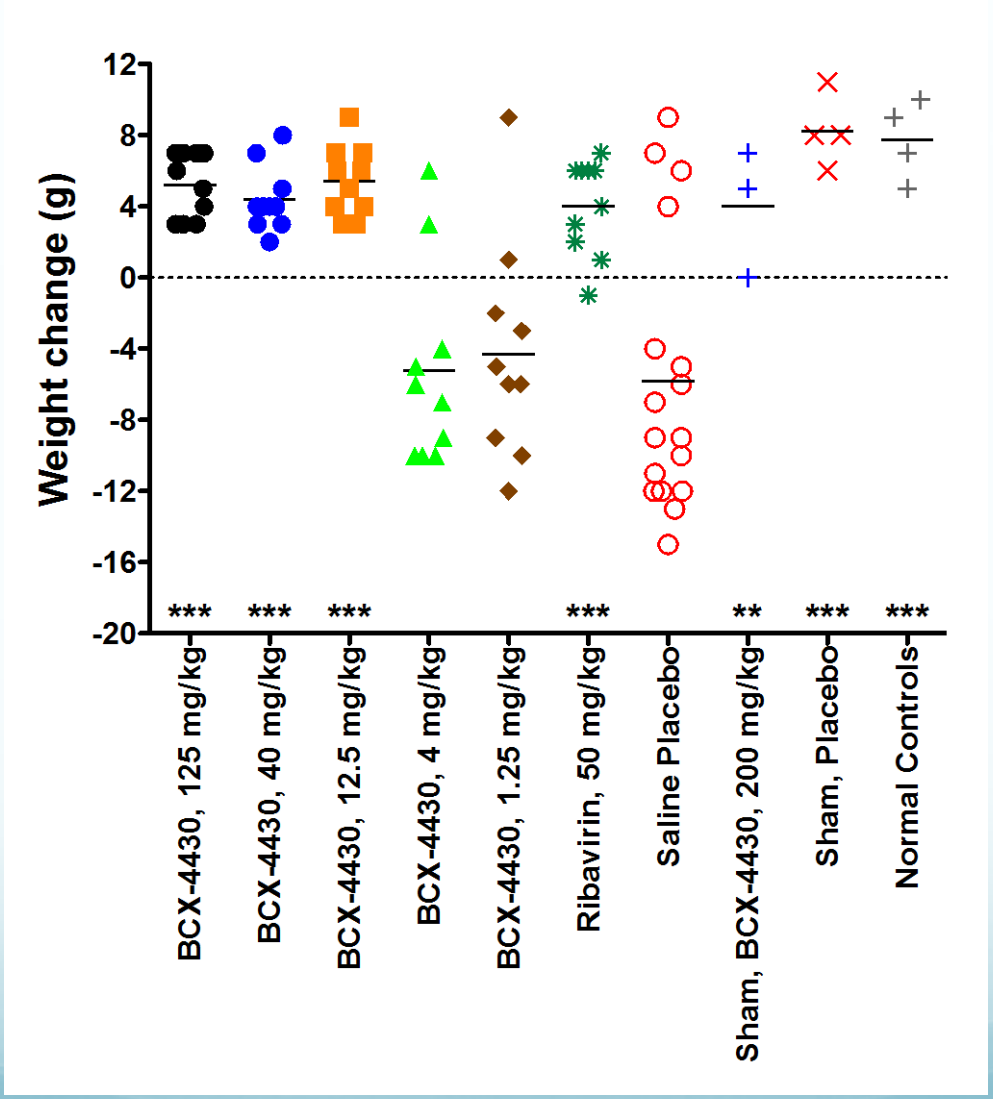


- 10 hamsters/group infected, 5/group tox
- Test doses of 1.25, 4.0, 12.5, 40 and 125 mg/kg/d
- Administered i.p., bid X 7 days beginning -4 h
- Disease parameters: survival, Δ weight, serum ALT (day 6), viremia (d 4)

BCX-4430 Protected Animals at Doses Ranging from 4 to 125 mg/kg

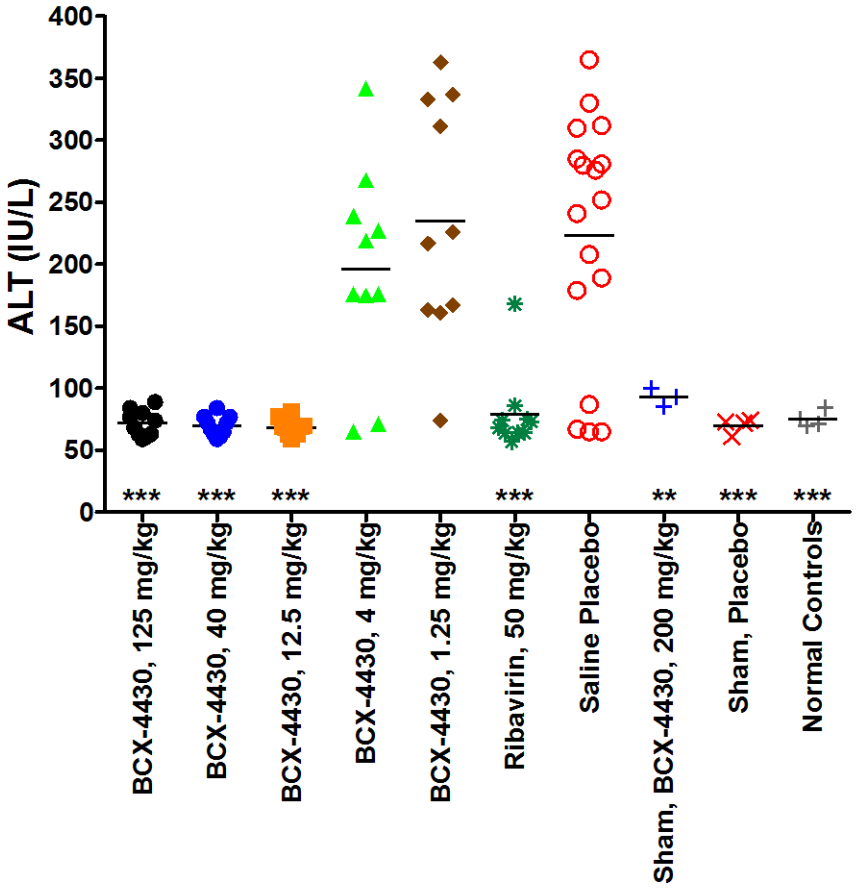


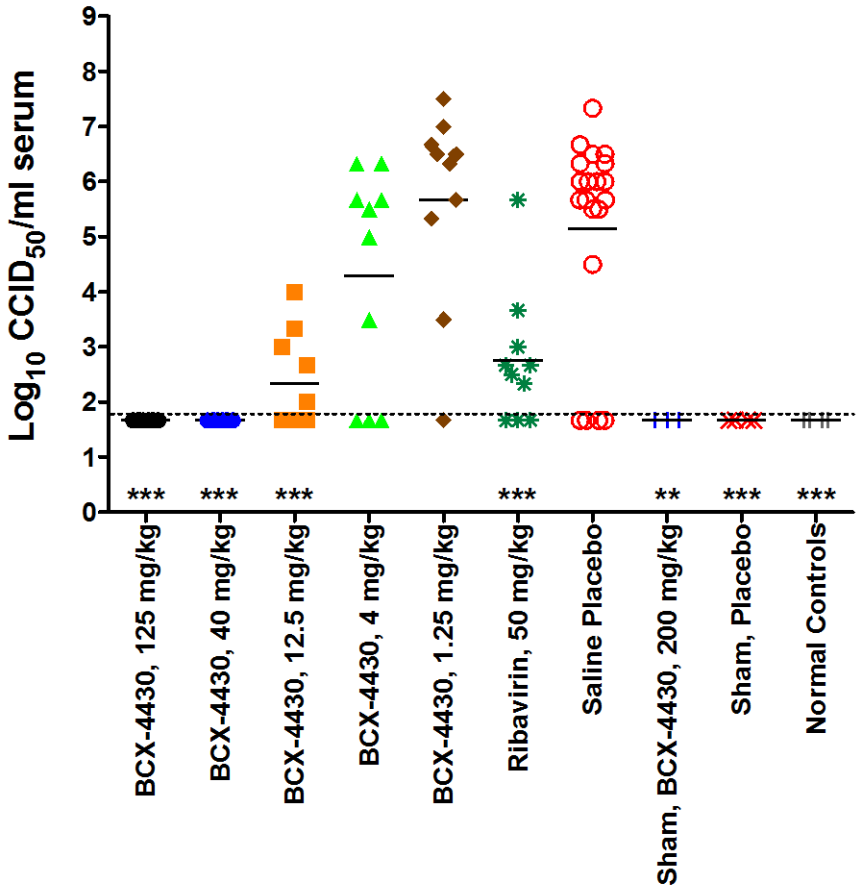
BCX-4430 Treatment Improves Weight Change



***P<0.001, **P<0.01, as compared with placebo

BCX-4430 Treatment Significantly Reduces Serum ALT and Viremia



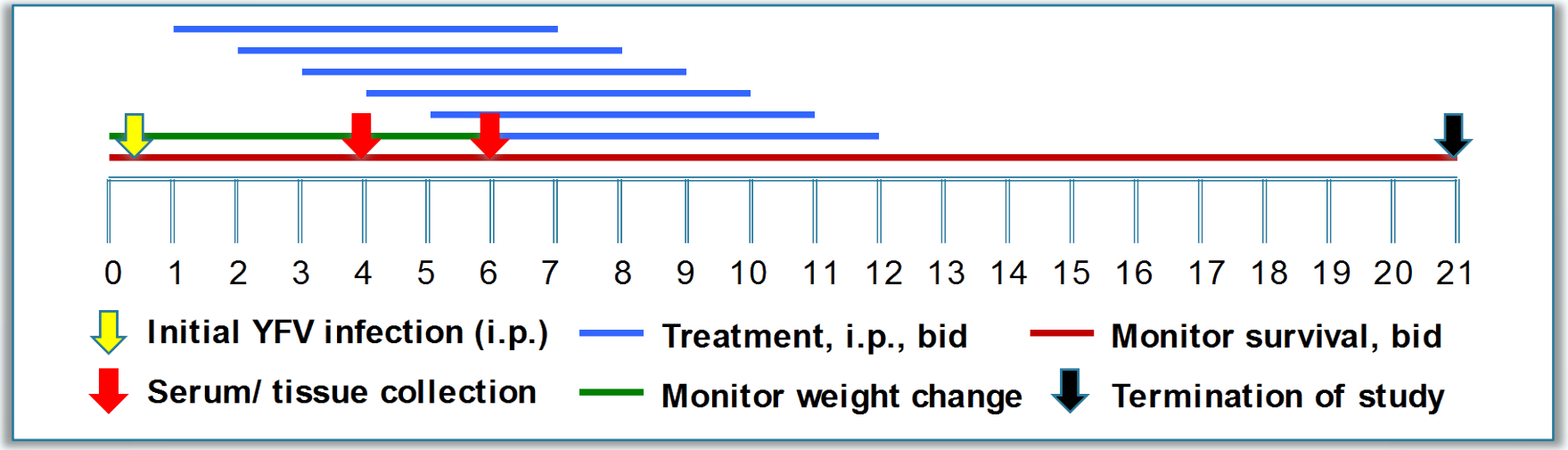


***P<0.001, **P<0.01, as compared with placebo treatment

Dose Response- Key Findings

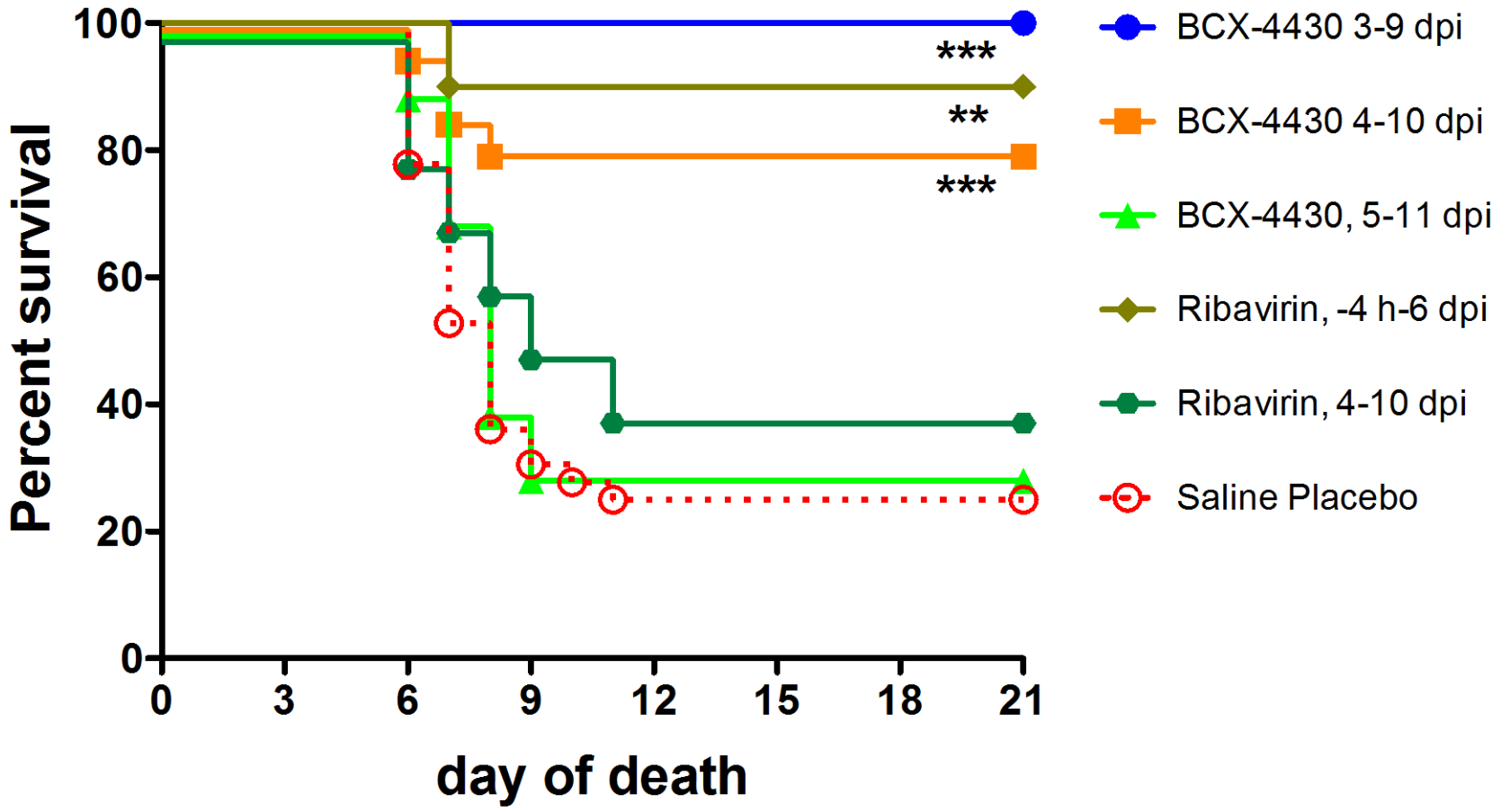
- Maximum tolerated dose 200 mg/kg/d administered i.p., bid for 7 days
- Minimum effective dose 4 mg/kg/d
- 12.5 mg/kg/d required for significant improvement of all disease parameters
- Broad therapeutic index ~50 with i.p. administration

Post-Virus Treatment Initiation



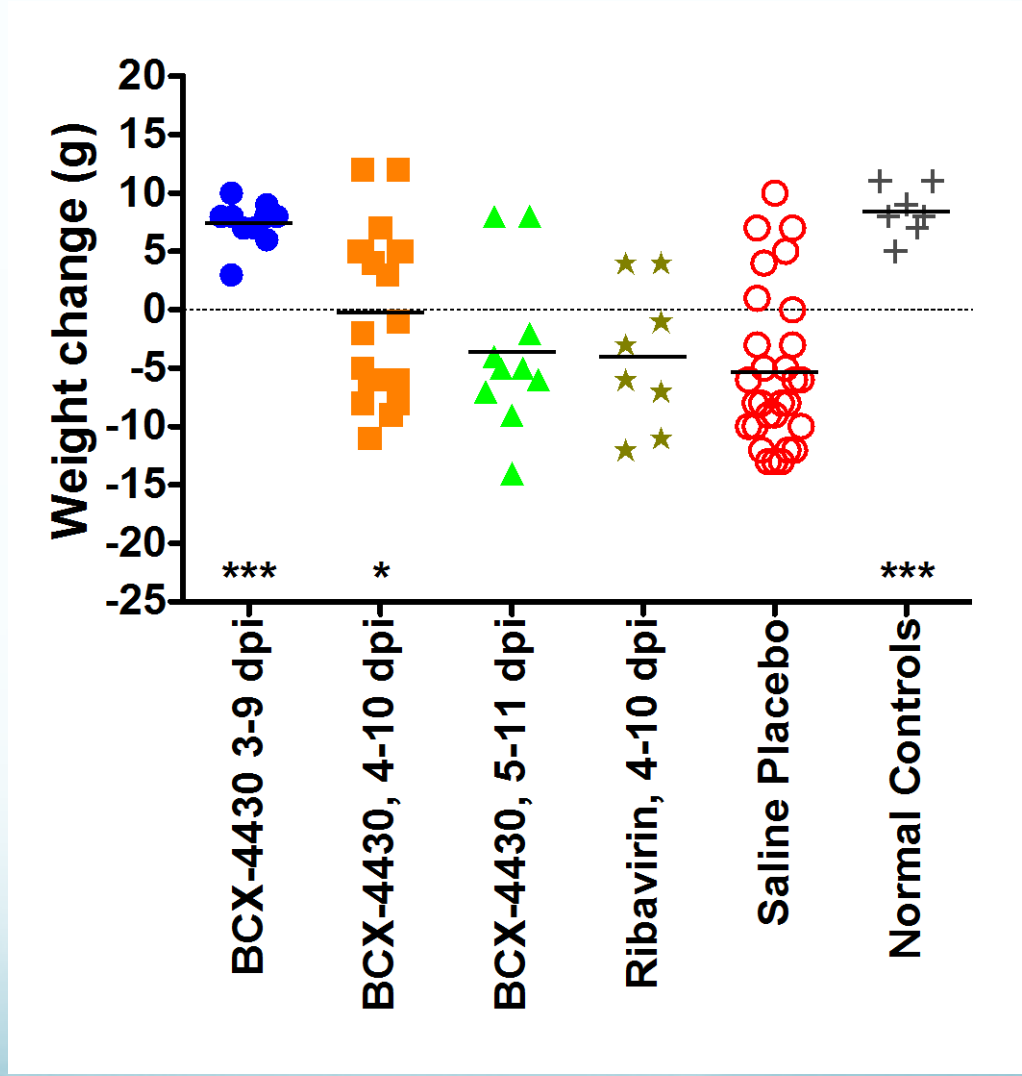
- BCX-4430 200 mg/kg/d, bid X 7
- Treatment initiated daily (0-6 dpi) 2 separate studies
- Disease parameters include survival, Δ weight, ALT (day 6), and viremia (day 4)

BCX-4430 Protected Animals When Initiated up to 4 Days Post-Infection



Data combined from 2 studies

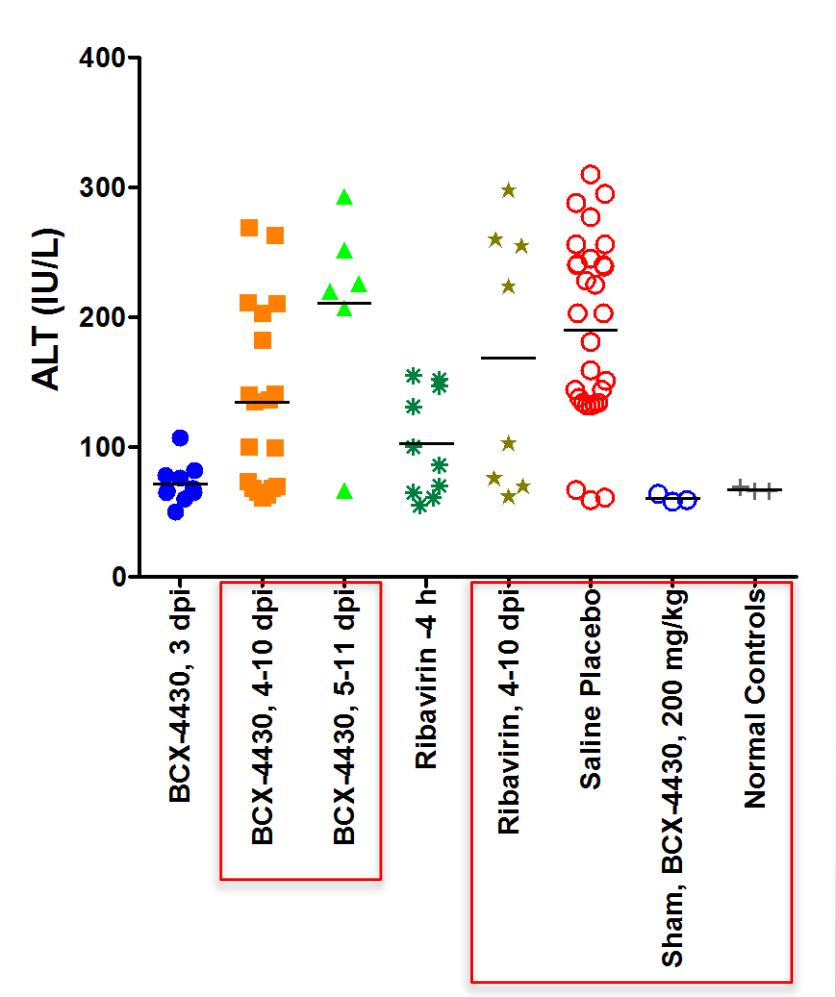
Treatment Ameliorated Weight Loss When Delayed 3 or 4 Days Post-Infection

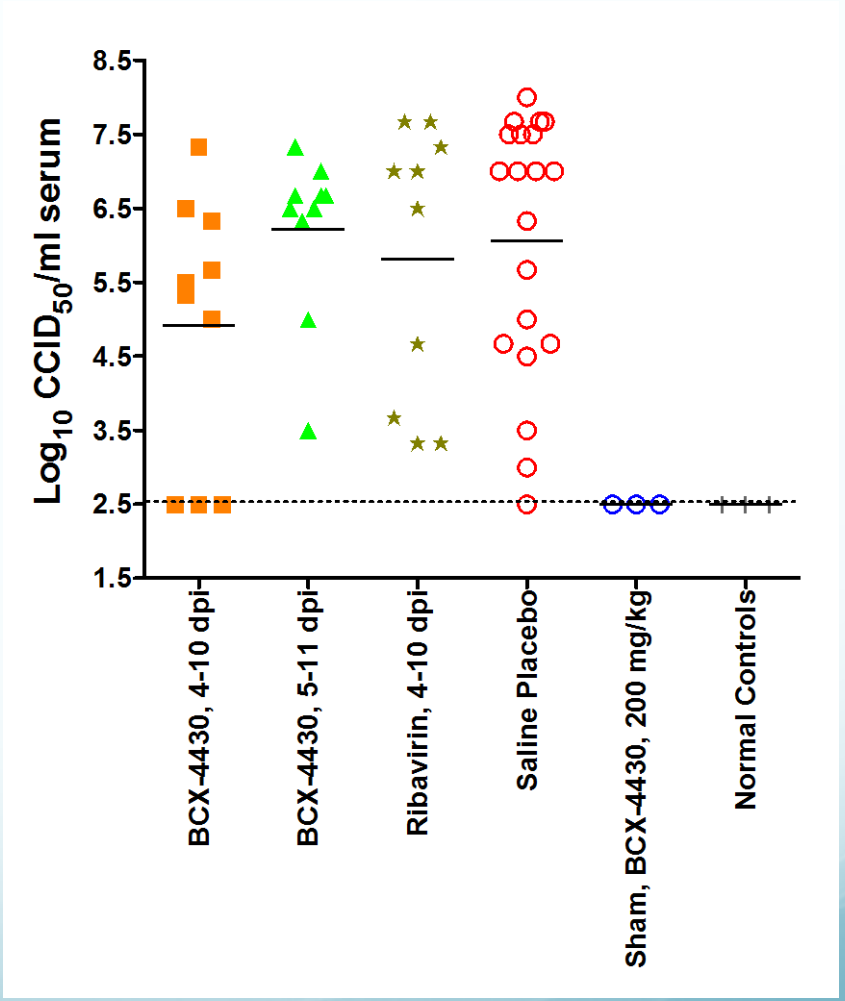


Data combined from 2 studies, weight change between 3 and 6 dpi

***P<0.001, *P<0.05, as compared with placebo

Effect of Therapeutic BCX-4430 Treatment on Serum ALT and Viremia



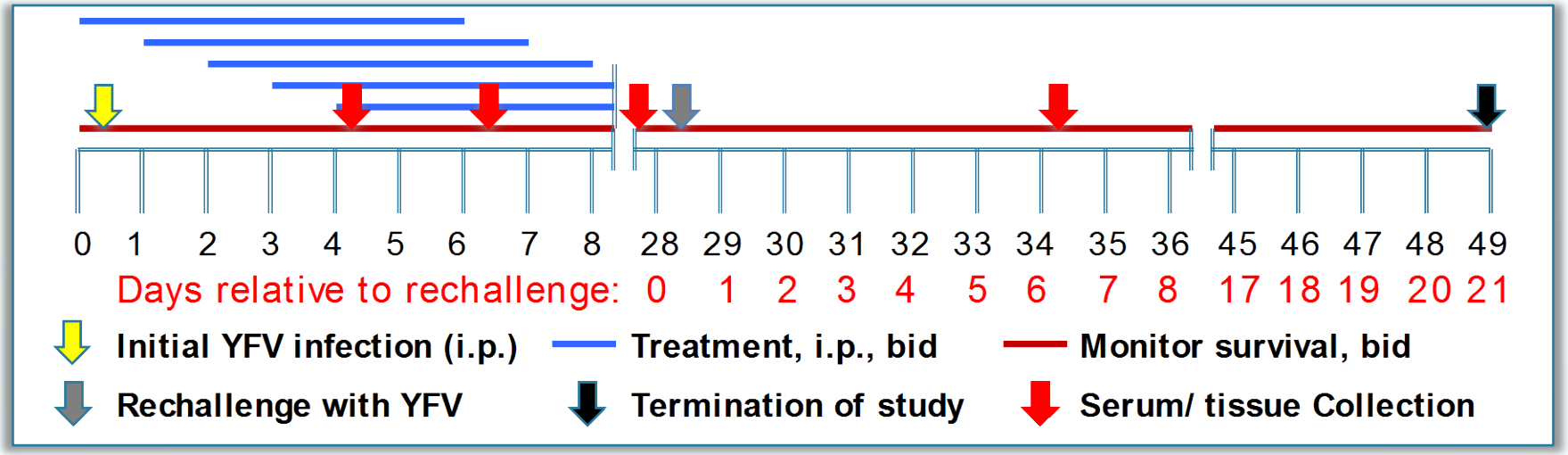


Data combined from 2 studies

Therapeutic Efficacy- Key Findings

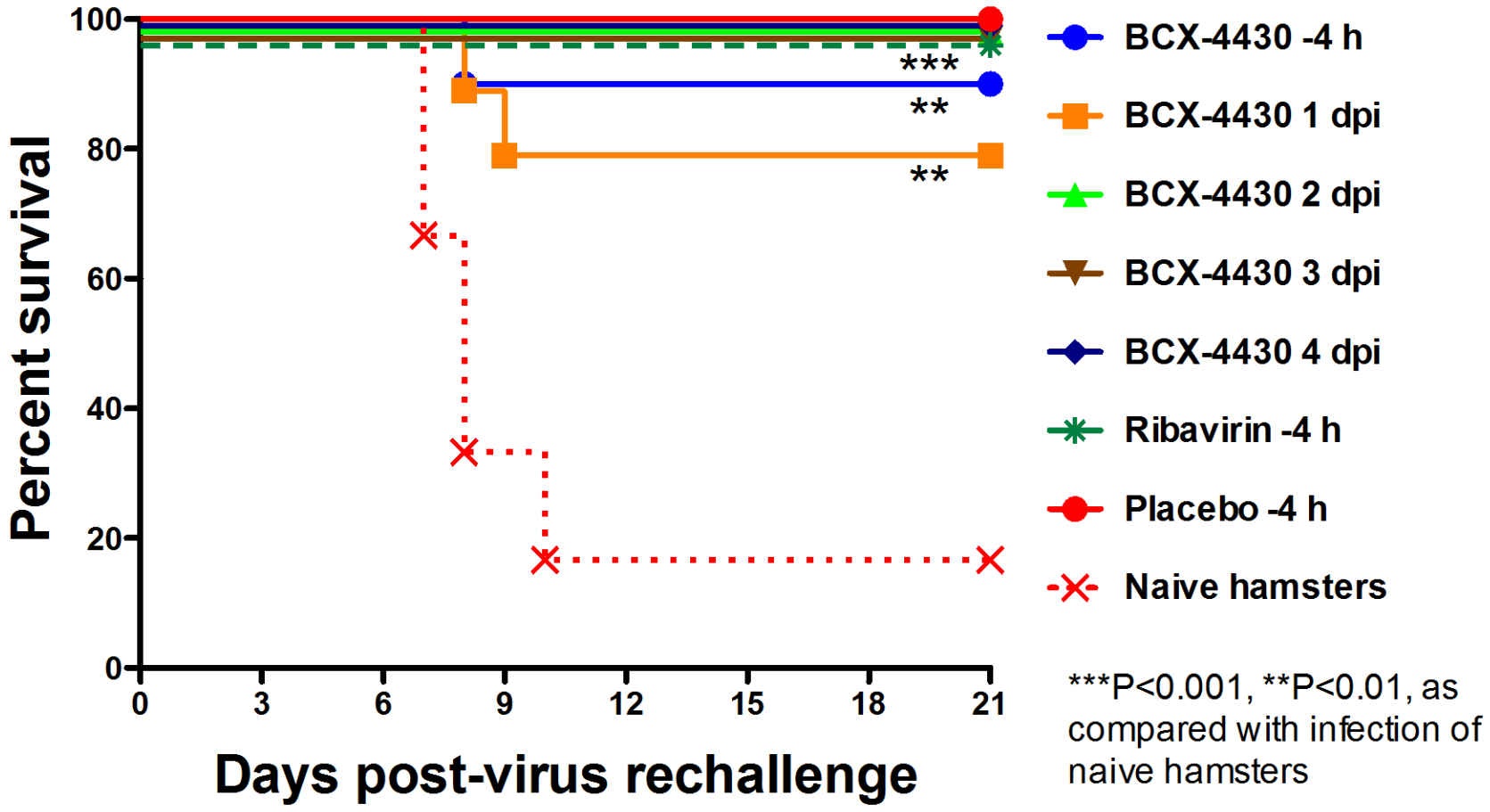
- BCX4430 treatment significantly improved survival and weight change when administered up to 4 days after virus challenge, despite minimal effect on ALT and serum virus titer
- Treatment beginning on 4 dpi coincides with peak viremia and liver titers
- Two separate studies confirmed the efficacy of treatment initiated 3 and 4 dpi

Virus Rechallenge Study



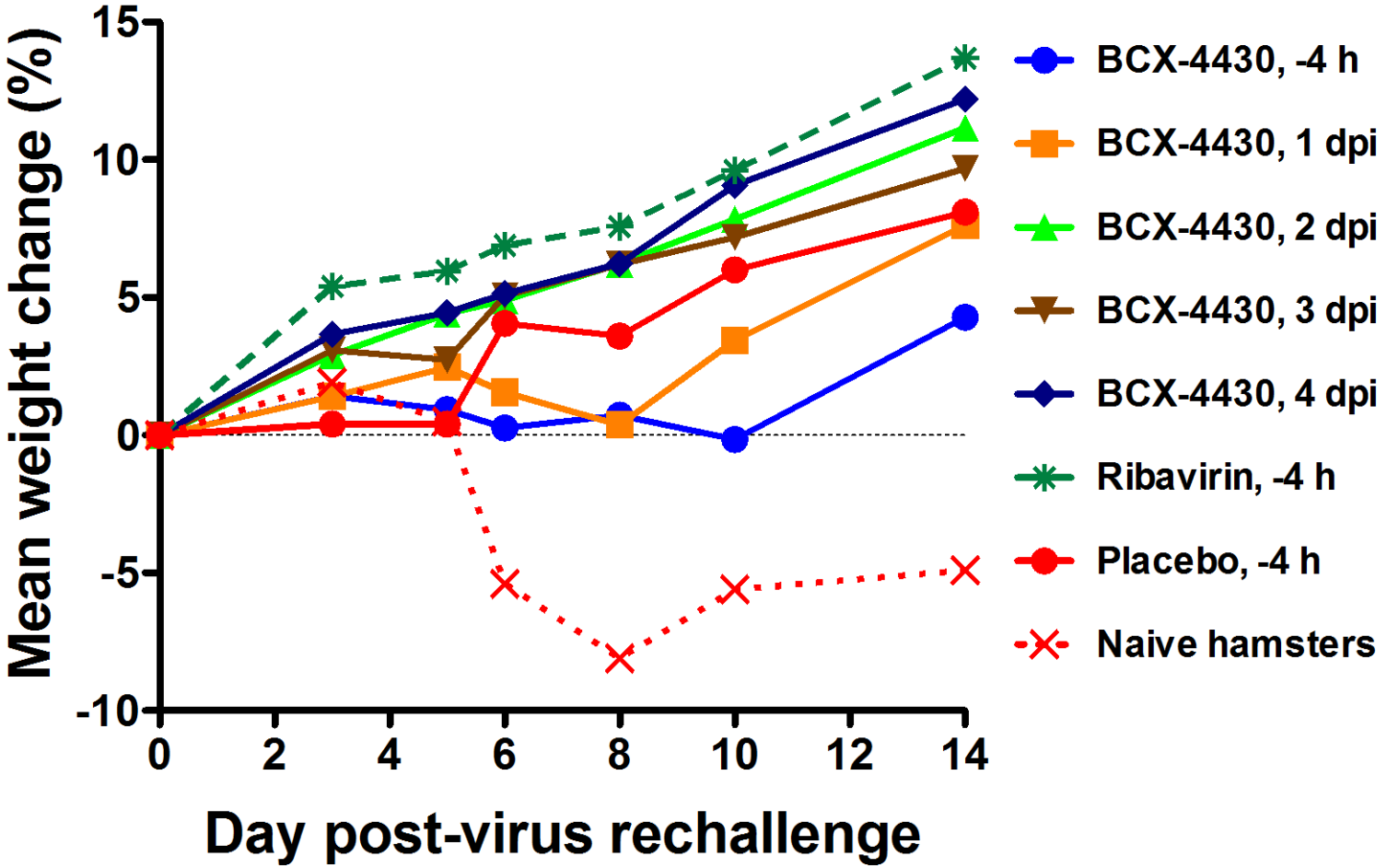
- 2˚ challenge of animals from therapeutic study compared with challenge of naïve indv.
- Disease parameters: survival, **Δ** weight, ALT (day 6), viremia (day 4), and nAb titer (day 0)

Significantly Improved Survival in Rechallenged Versus Naïve Animals

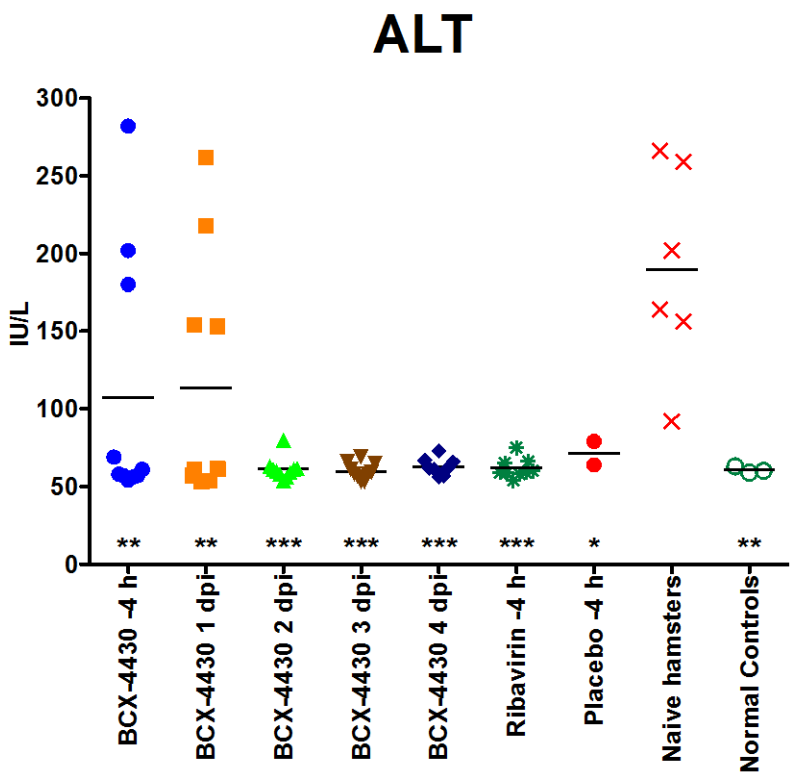


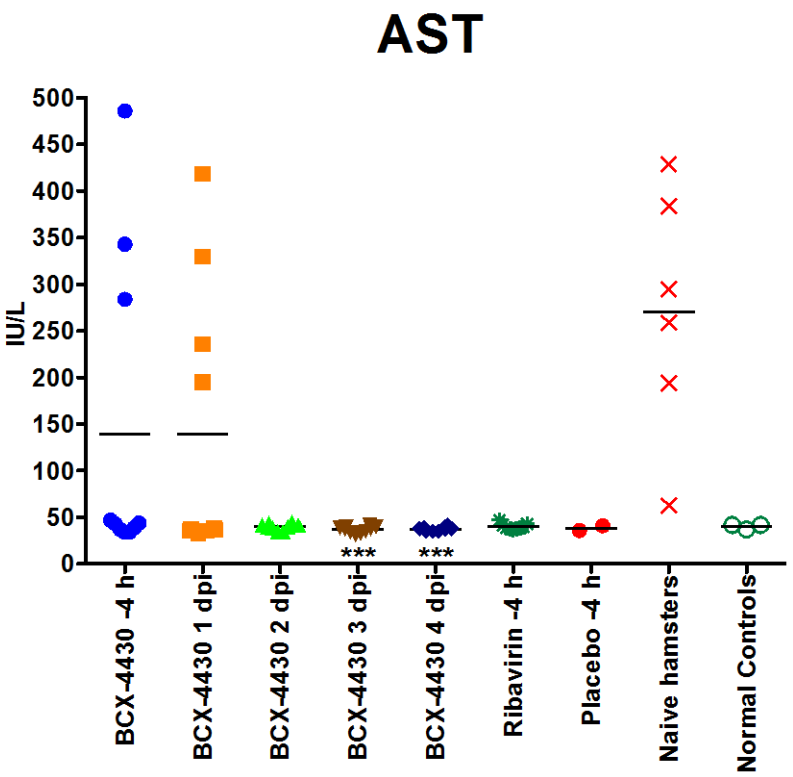
***P<0.001, **P<0.01, as compared with infection of naive hamsters

Weight Increases After Rechallenge vs. Naïve Animals



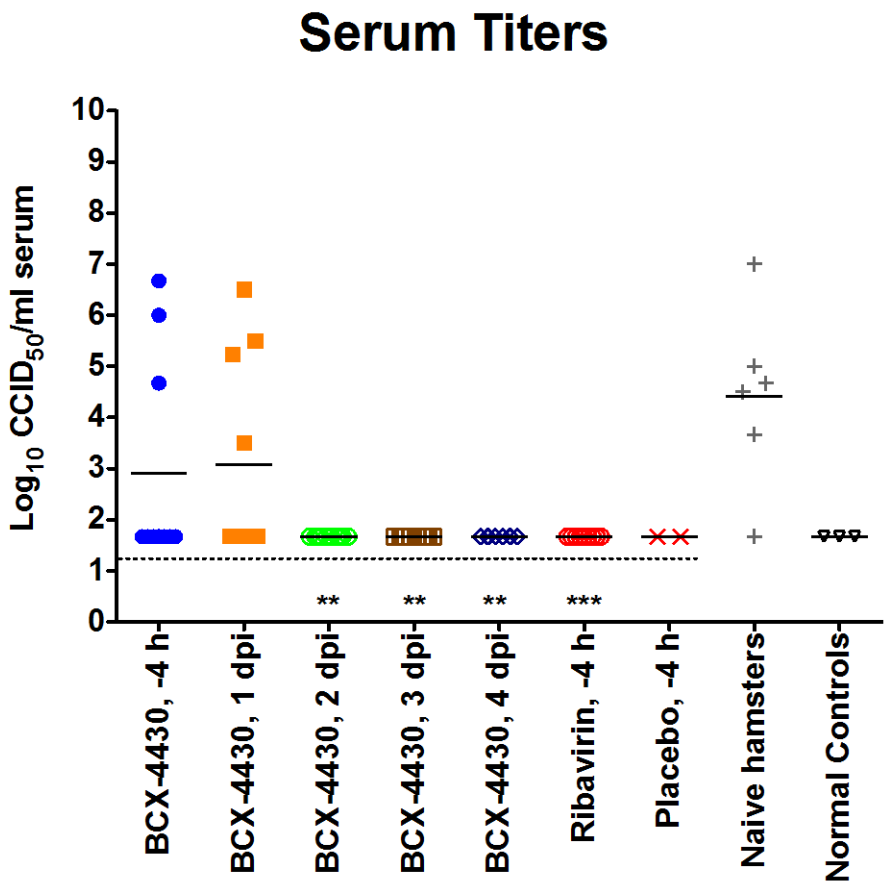
Serum ALT and AST were Significantly Improved after Rechallenge

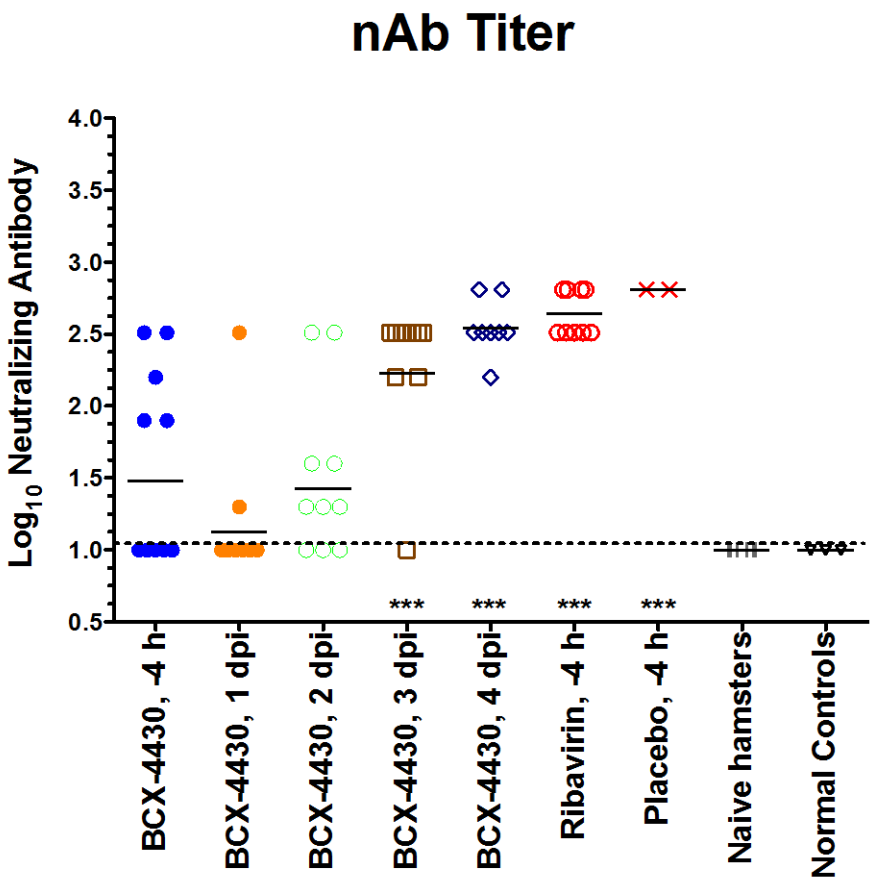




***P<0.001, **P<0.01, *P<0.05, as compared with placebo

Significantly Lower Virus Titers Correlate with Higher nAb Levels



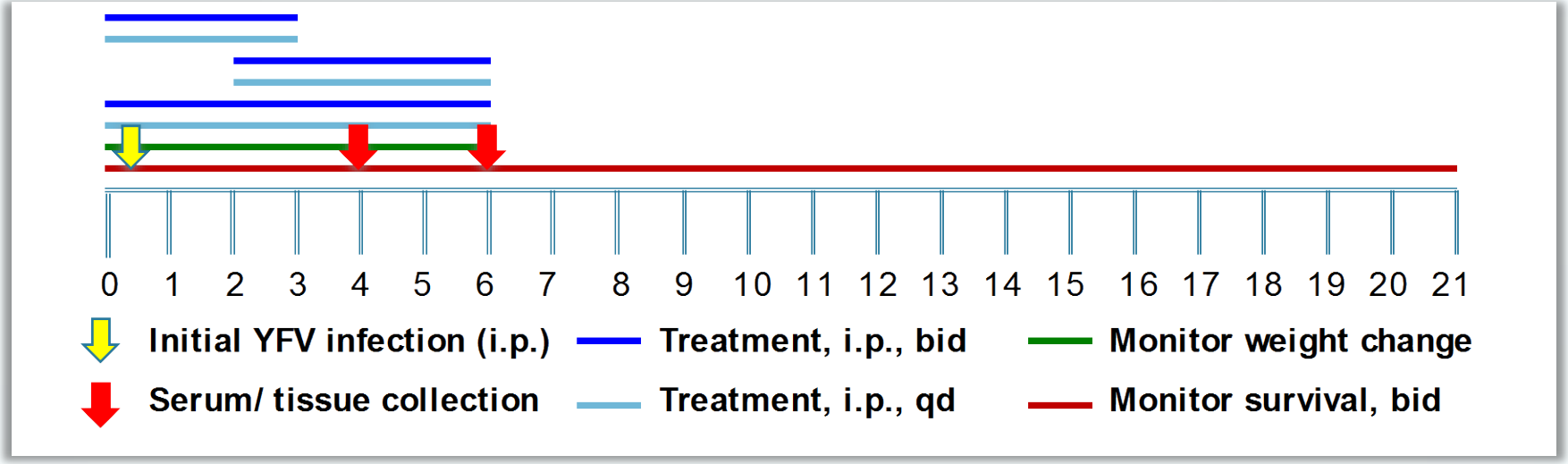


***P<0.001, **P<0.01, as compared with placebo

Key Findings

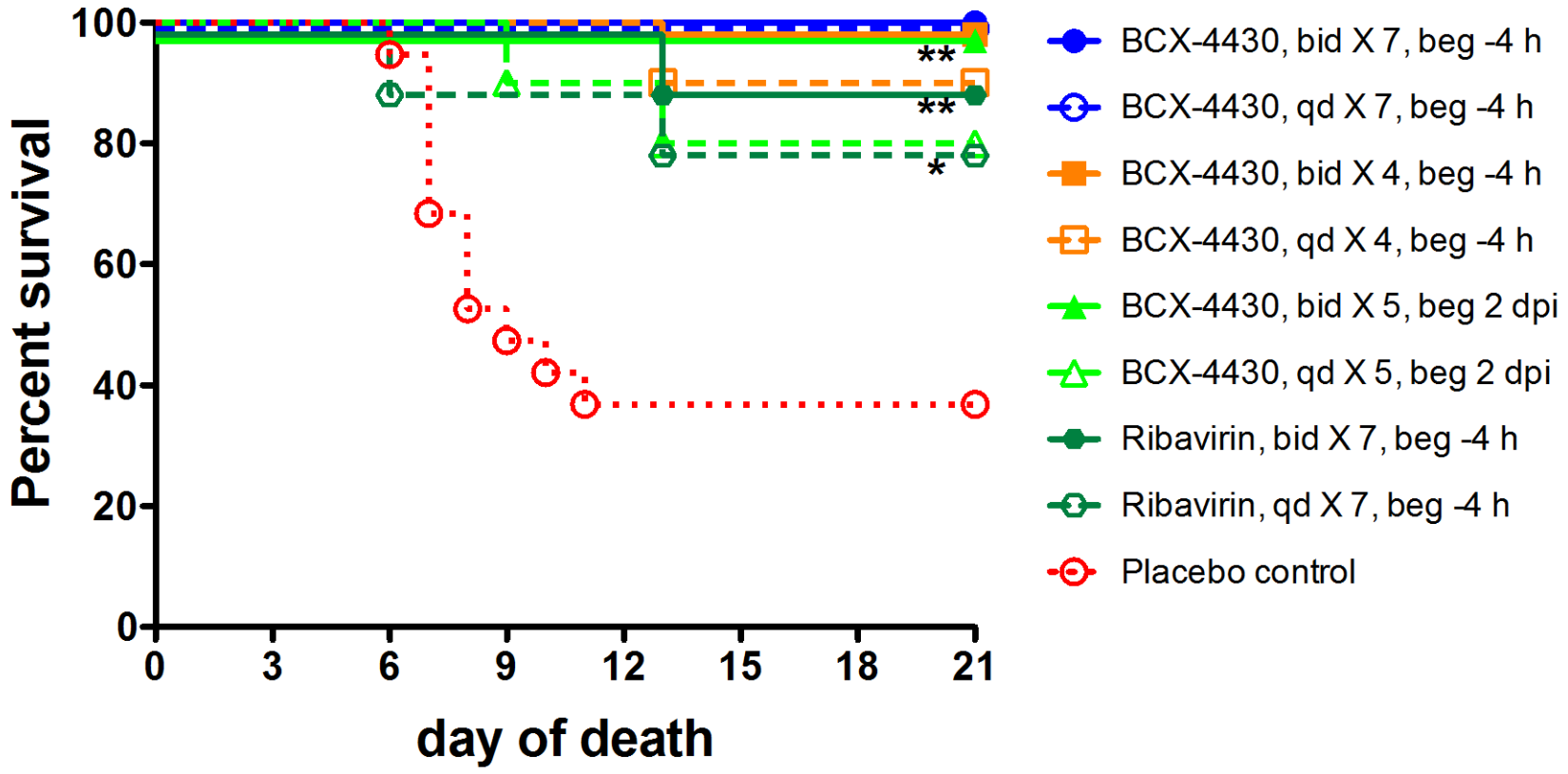
- BCX4430 treatment up to 24 h after infection results in efficient clearance of YFV
- Animals treated beginning 2 dpi or later demonstrated complete immune response and protection against other disease parameters
- Earlier treatment initiation (<2 dpi) resulted in a less effective protection to secondary virus challenge

Reduced Treatment Freq./Duration



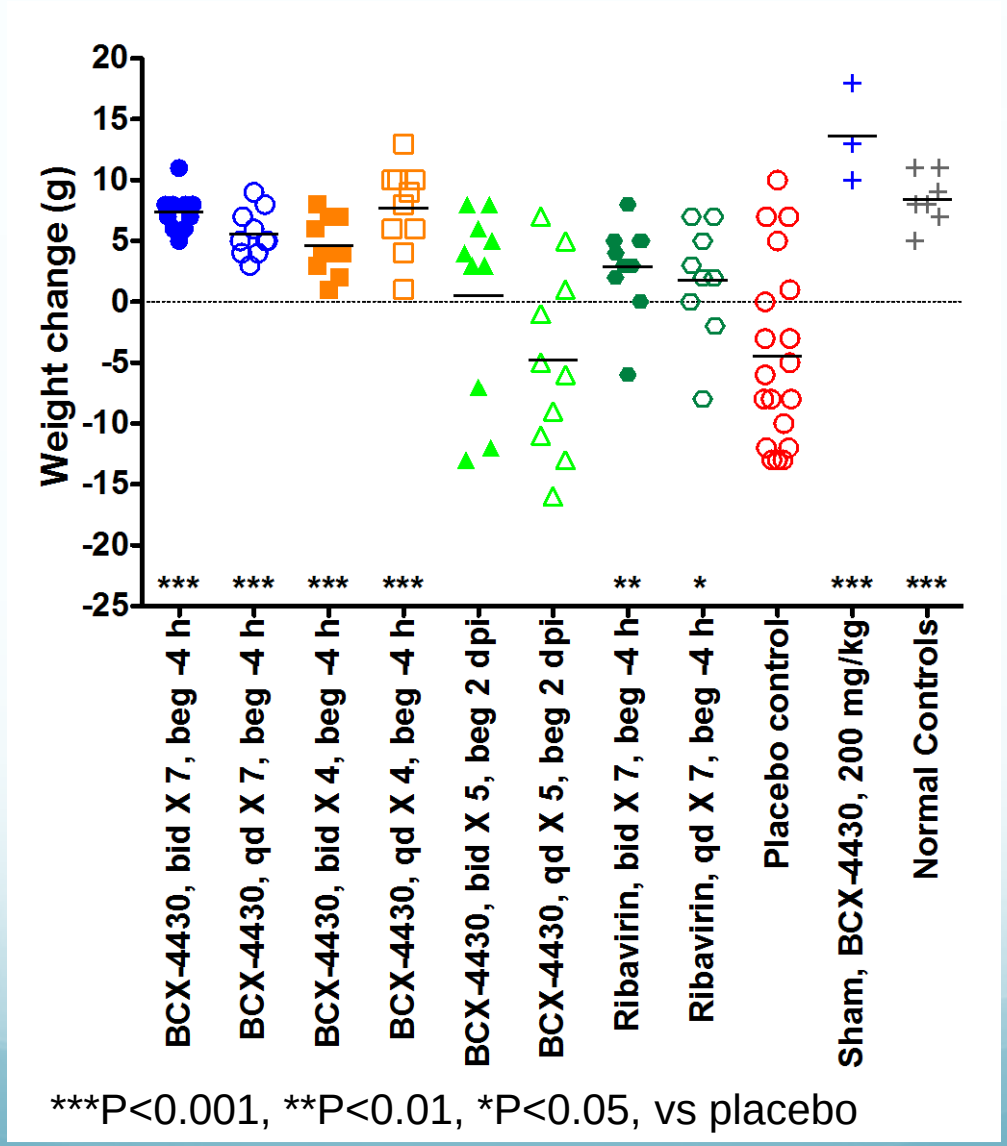
- 12 mg/kg/d of BCX4430 in 0.2 ml; Ribavirin control, 50 mg/kg/d
- Twice daily (bid) vs once daily (qd) treatment
- Treatment duration of 4 or 7 days, initiated -4 h
- Treatment duration of 5 days, initiated 2 dpi
- Disease parameters: Survival, weight change, serum ALT (6 dpi) and serum virus titer (4 dpi).

Shorter, Less Frequent Dosing was Still Protective, Even Therapeutically



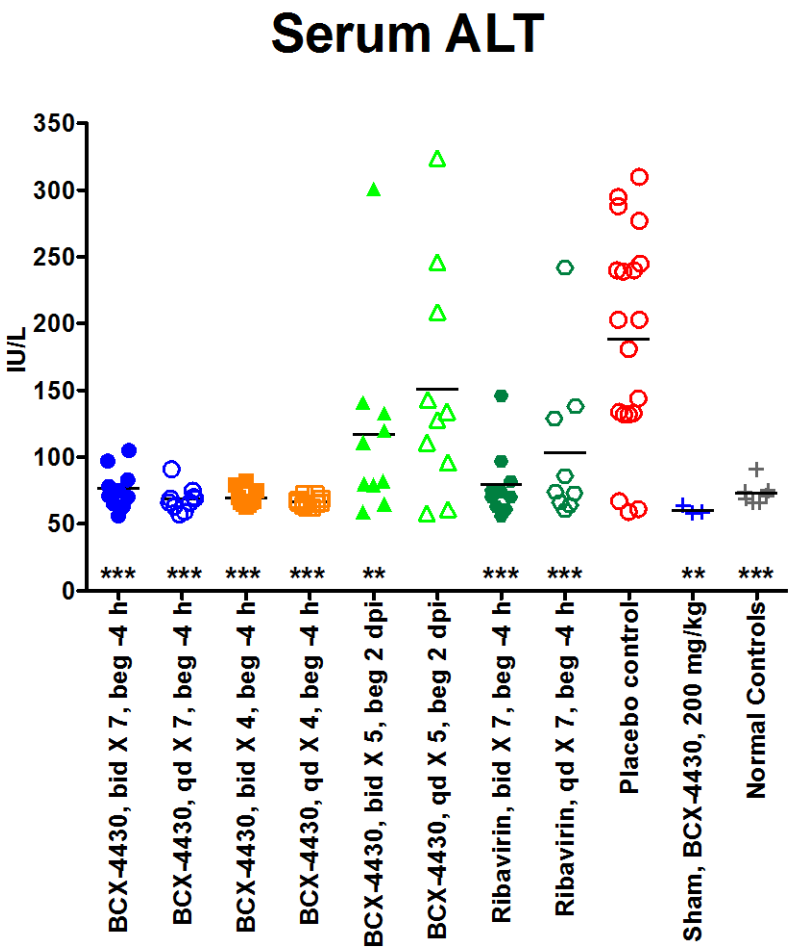
**P<0.01, *P<0.05, as compared with placebo

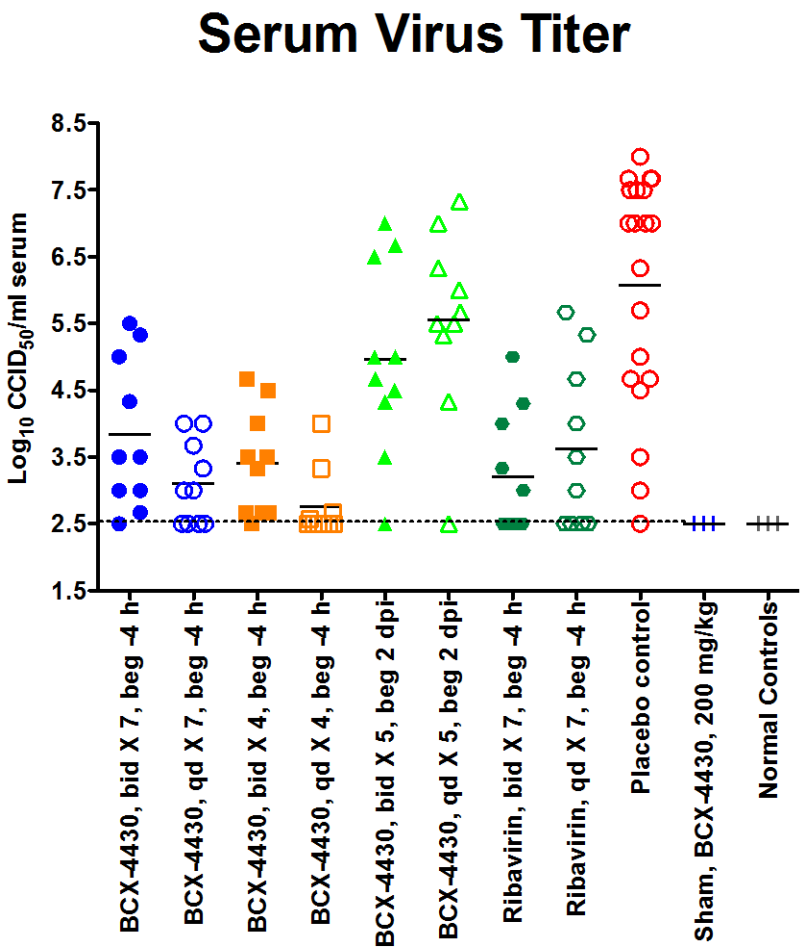
Altered Treatment Regimen Significantly Improves Weights



***P<0.001, **P<0.01, *P<0.05, vs placebo

Less Frequent, Shorter Treatment Regimen Improves ALT, Virus





***P<0.001, **P<0.01, as compared with placebo

Key Findings

- QD treatment is not significantly different than BID treatment
- A 4 day treatment regimen appears to be as effective as a 7 day regimen
- BCX-4430 (12 mg/kg) compared favorably with the positive control Ribavirin (50 mg/kg)
- A 5 day treatment initiated on 2 dpi was effective and resulted in significantly reduced mortality, regardless of treatment frequency

Summary of Findings- BCX-4430

- Tolerable in hamsters up to 200 mg/kg/day, i.p. for 7 days
- Anti-YFV activity at doses as low as 4 mg/kg/d
  - Tolerability index of ~50
- Improves survival from 10-30% in controls to 70-100%
- Reduces/prevents viremia and hepatic viral proliferation

Summary of Findings- BCX-4430

- Reduces/prevents transaminitis
- Demonstrates dose-response relationship
- Effective when administered bid X 7 days at a dose of 200 mg/kg/d when initiated up to 4 dpi
  - Coincides with onset of disease signs
- Permits induction of protective immunity

Acknowledgements

- Institute for Antiviral Research: Isaac Wong, Choi Jung, Joe Hagloch, Shelby Wilcox, and Makda Gebre
- BioCryst: Debra Kellogg, YeHong Luo, Cynthia Parker, Ramanda Upshaw, Pooran Chand, and Pravin Kotian
- NIH Project Officer: Heather Greenstone
- NIH Contract: HHSN272201000039I/A21, Division of Microbiology and Infectious Disease, NIAID, NIH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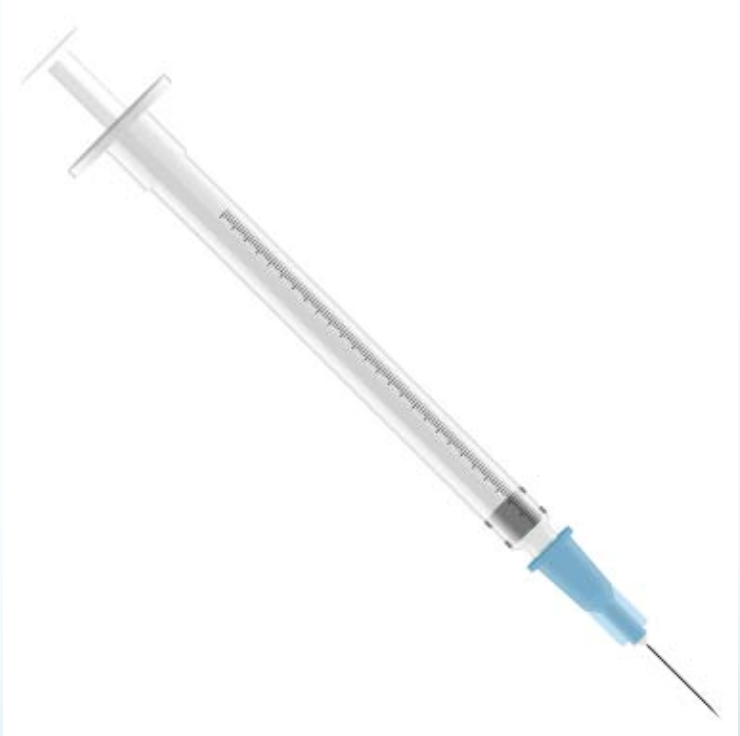
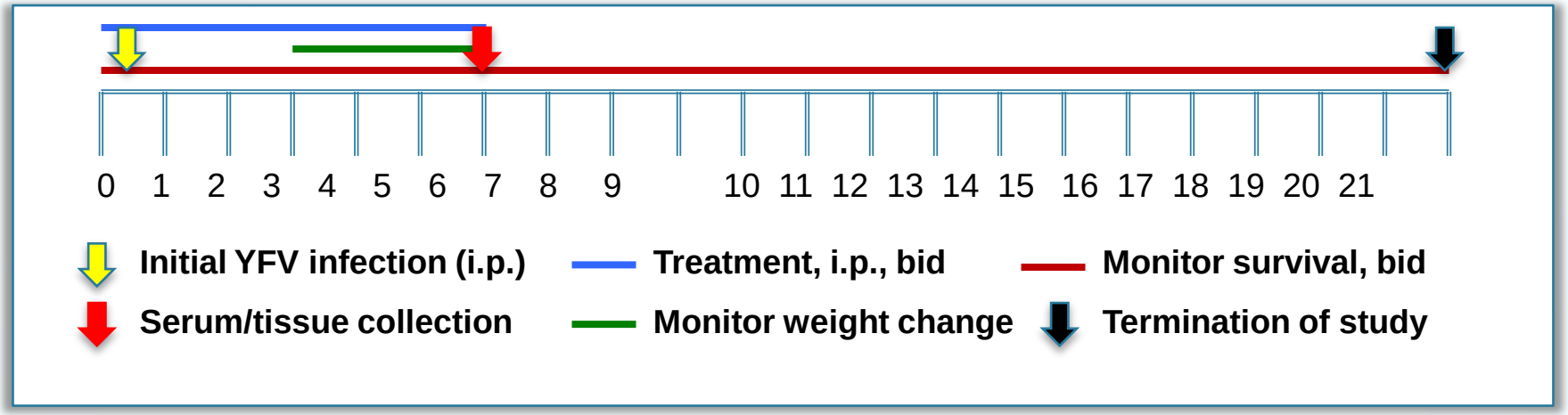
Characterizing the Activity of BCX4430

- Dose range finding study
- Post-virus challenge activity
- Rechallenge after treatment
- Frequency of dosing
- Treatment duration

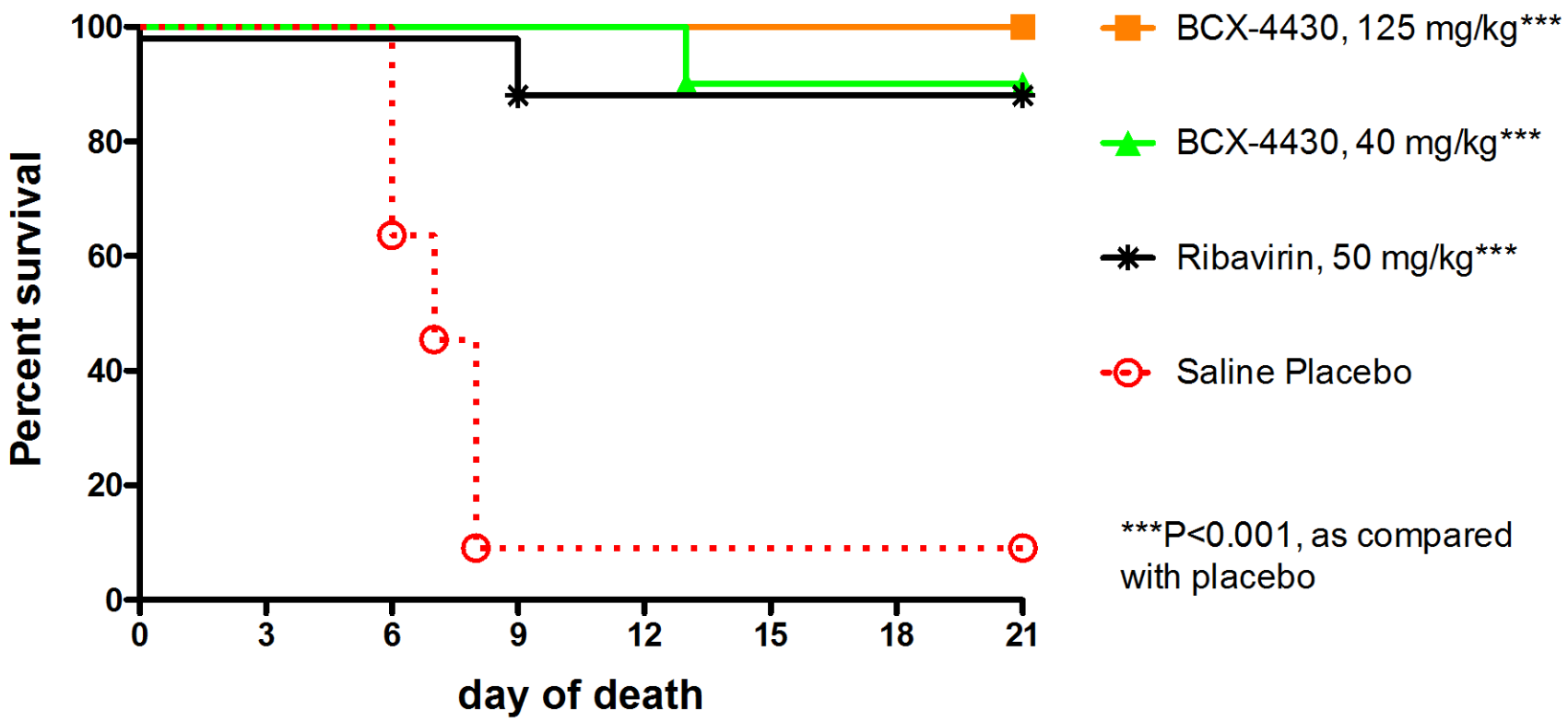


Dose Response- Study 1

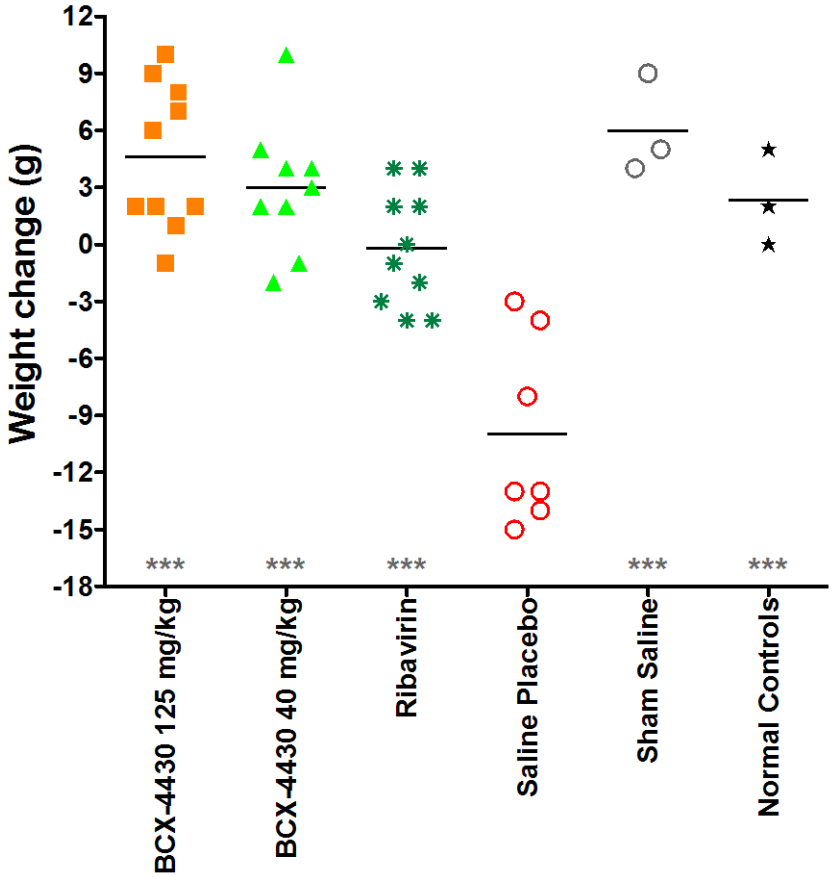


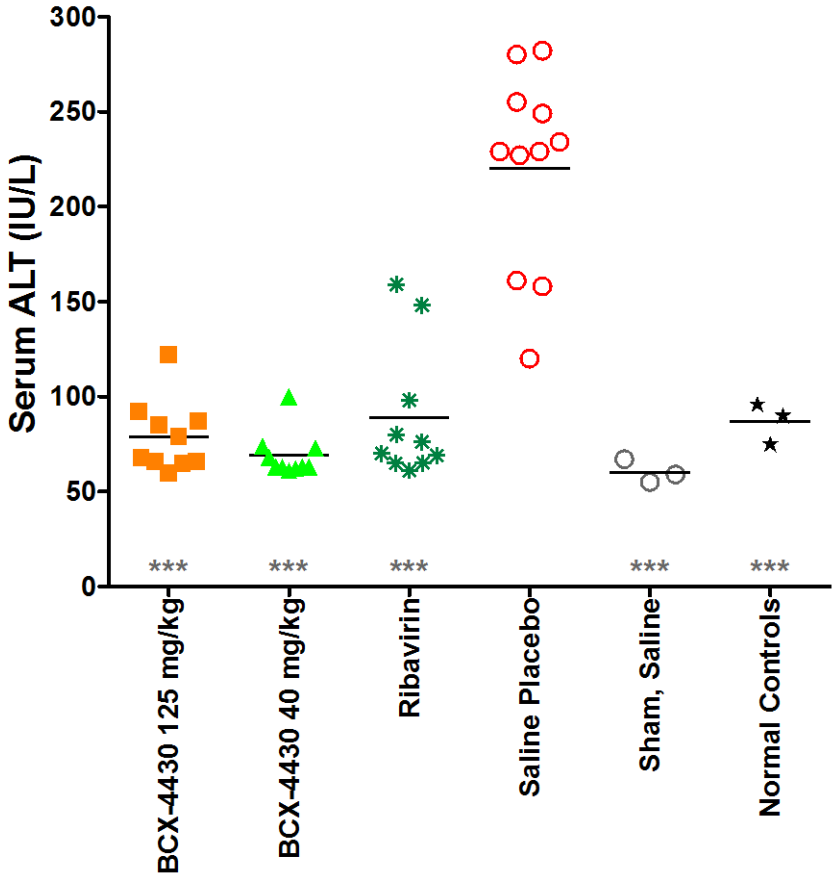
- 10 hamsters/group infected, 5/group tox
- BCX4430 doses of 40 and 125 mg/kg/d tested
- Administered i.p., bid X 7 days beginning -4 h
- Parameters: survival, Δ weight (d 3 to 6), ALT (d 6)

BCX-4430 Significantly Improves Survival



Treatment Significantly Improves Weight Change and Serum ALT





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