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

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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

<table>
<thead>
<tr>
<th>Study</th>
<th>Concentration</th>
<th>Result</th>
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<tbody>
<tr>
<td>AMES</td>
<td>5 mg/plate</td>
<td>Negative</td>
</tr>
<tr>
<td>hERG</td>
<td>30 µM</td>
<td>Negative</td>
</tr>
<tr>
<td>Mammalian DNA incorporation</td>
<td>30 µM</td>
<td>Negative</td>
</tr>
<tr>
<td>Mammalian RNA incorporation</td>
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### Broad-Spectrum Activity of BCX4430

<table>
<thead>
<tr>
<th>Family</th>
<th>Virus</th>
<th>EC$_{50}$(µg/mL)</th>
<th>EC$_{90}$(µg/mL)</th>
<th>In Vivo PoP</th>
<th>Model</th>
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<tr>
<td><strong>Flaviviridae</strong></td>
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<tr>
<td></td>
<td>Yellow Fever</td>
<td>8.3</td>
<td>9.33</td>
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<td>Hamster</td>
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<tr>
<td></td>
<td>Dengue 2</td>
<td>13</td>
<td>13.05</td>
<td>Yes</td>
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<td>West Nile</td>
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<td>7</td>
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<tr>
<td></td>
<td>JEV (SA-14)</td>
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<td>Rift Valley Fever</td>
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<td>Maporal (Hantavirus) (HV97021050)</td>
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<td>VEE (TC83)</td>
<td>72</td>
<td>60</td>
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<td>Rhinovirus 2</td>
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<td></td>
<td>Adenovirus</td>
<td>60</td>
<td>25</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

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:
  \[
  \text{EC50: 8.3 } \mu\text{g/ml; EC90: 9.3 } \mu\text{g/ml; CC50: 320 } \mu\text{g/ml}
  \]
- Potential RNA polymerase inhibitor- results pending
- *In vivo* testing warranted
Hamster Model of Yellow Fever

• Adapted Jimenez strain, i.p.
• Serum AST > ALT
• 80% mortality around 6-9 dpi
• Renal dysfunction
• Several measurable blood parameters of disease

• Liver disease w/ microvesicular steatosis
• Viremia, 2-5 d

• Some hemorrhagic manifestations: petechiae, nosebleed

• Liver disease w/ microvesicular steatosis
• Adapted Jimenez strain, i.p.
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

![Graph showing mean weight change over days post-treatment initiation for different dose groups of BCX-4430 and placebo treatment.]

- BCX-4430 150 mg/kg/d
- BCX-4430 200 mg/kg/d
- BCX-4430 250 mg/kg/d
- BCX-4430 300 mg/kg/d
- Placebo treatment

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

**Percent survival**

**Day of death**

- BCX-4430, 125 mg/kg
- BCX-4430, 40 mg/kg
- BCX-4430, 12.5 mg/kg
- BCX-4430, 4 mg/kg
- BCX-4430, 1.25 mg/kg
- Ribavirin, 50 mg/kg
- Saline Placebo

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

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- NIH Project Officer: Heather Greenstone
- NIH Contract: HHSN272201000039I/A21, Division of Microbiology and Infectious Disease, NIAID, NIH
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, $\Delta$ weight (d 3 to 6), ALT (d 6)
BCX-4430 Significantly Improves Survival

The graph shows the percent survival over time for different treatment groups. BCX-4430 at 125 mg/kg, BCX-4430 at 40 mg/kg, Ribavirin at 50 mg/kg, and Saline Placebo are compared. The graph indicates that BCX-4430 at 125 mg/kg and BCX-4430 at 40 mg/kg significantly improve survival compared to Ribavirin and Saline Placebo, as indicated by the survival curves extending further to the right on the x-axis. The p-value for all comparisons is <0.001, as compared with placebo.
Treatment Significantly Improves Weight Change and Serum ALT

***P<0.001, as compared with placebo