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BCX4430 — A broad-spectrum antiviral adenosine nucleoside analog under development for the treatment of Ebola virus disease

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Summary The adenosine nucleoside analog BCX4430 is a direct-acting antiviral drug under investigation for the treatment of serious and life-threatening infections from highly pathogenic viruses, such as the Ebola virus. Cellular kinases phosphorylate BCX4430 to a triphosphate that mimics ATP; viral RNA polymerases incorporate the drug's monophosphate nucleotide into the growing RNA chain, causing premature chain termination. BCX4430 is active in vitro against many RNA viral pathogens, including the filoviruses and emerging infectious agents such as MERS-CoV and SARS-CoV. In vivo, BCX4430 is active after intramuscular, intraperitoneal, and oral administration in a variety of experimental infections. In nonclinical studies involving lethal infections with Ebola virus, Marburg virus, Rift Valley fever virus, and

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Yellow Fever virus, BCX4430 has demonstrated pronounced efficacy. In experiments conducted in several models, both a reduction in the viral load and an improvement in survival were found to be related to the dose of BCX4430. A Phase 1 clinical trial of intramuscular administration of BCX4430 in healthy subjects is currently ongoing.

Background

Nucleoside analog drugs have been highly successful in the treatment of a range of serious or life-threatening viral infections. Nucleoside analogs target fundamental reproductive mechanisms of the viral genome. For example, acyclovir, which was introduced in the 1980s to treat herpesvirus encephalitis [1,2], targets DNA polymerase; zidovudine, which was introduced in the 1990s to treat infections of human immunodeficiency virus [3], targets reverse transcriptase; and sofosbuvir, which was recently approved for the treatment of hepatitis C, targets RNA polymerase [4].

A viral-encoded RNA polymerase is essential for replication of RNA viruses in host cells and catalyzes the synthesis of multiple copies of complementary RNA from the template viral RNA. Each base-paired nucleotide is incorporated through specific binding of the triphosphate nucleotide to the enzyme and the RNA template, followed by cleavage of the pyrophosphate and covalent bonding of the 5′-monophosphate nucleotide to the 3′ position in the growing RNA strand. Therefore, desirable characteristics for antiviral nucleosides include efficient uptake into cells and efficient conversion of the nucleoside to the triphosphate nucleotide by mammalian kinases.

In fresh hepatocytes from mice, rats, non-human primates and humans in vitro (Fig. 1, left panel) and after an IM injection in rats in vivo (Fig. 1, right panel), BCX4430 is efficiently taken up into cells and converted to BCX4430 triphosphate (TP) [5]. In vivo conversion of BCX4430 to its TP nucleotide is particularly efficient; for example, the intracellular BCX4430-TP levels are higher than plasma levels of the parent compound within 30 min of intramuscular administration and continue to exceed the plasma drug levels by 10- to 100-fold for at least 24 h after a single dose.

As the molecular goal of antiviral therapy with RNA polymerase inhibitors is to interrupt viral RNA synthesis, a further desirable characteristic of a candidate nucleoside drug is structural mimicry of the corresponding natural nucleotide. This mimicry allows the drug nucleotide triphosphate to be recognized by the viral RNA polymerase and to be substituted for the corresponding natural nucleotide. Artificial nucleotides that are similar to either a natural purine (adenosine and guanosine) or a natural pyrimidine (uracil or cytosine) nucleoside may have structural modifications to either the base or ribose sugar moieties or both. BCX4430 (Fig. 2) is an adenosine analog, with substitution of carbon for nitrogen at position 7 on the base and nitrogen for oxygen at position 1 on the ribose ring.

Steric and electrostatic interactions and substitutions on the ribose ring are known to alter the conformation of the sugar. These conformational changes can in turn affect nucleotide incorporation and chain extension by polymerases. A structural change from a furanose in adenosine to an azasugar ring in BCX4430 alters the electrostatic interaction of the ring, and the viral RNA-dependent RNA polymerase is unable to add more than one or two further nucleotides, as demonstrated in vitro using a hepatitis C RNA polymerase assay (Fig. 3) [5].

In studies of ^{3}H-BCX4430 and ^{3}H-adenosine, the HuH-7 human hepatocellular carcinoma cell line was incapable of incorporating labeled BCX4430 into either RNA or DNA, despite avid incorporation of the labeled natural nucleoside [5]. The specificity of BCX4430 inhibitory activity, and likely that of other antiviral nucleoside drugs, for the viral RNA polymerase compared to the mammalian RNA and DNA polymerases is possibly due to the superior error—correction capabilities of the mammalian enzymes; however, other factors cannot be excluded.

Antiviral effect of BCX4430 in cell culture

Virus proliferation in cell culture is inhibited by incubation with BCX4430. BCX4430 displays a broad spectrum of activity against a wide variety of RNA viral pathogens. Many of these viruses, including MERS-CoV, EBOV, MARV, and YFV, cause serious

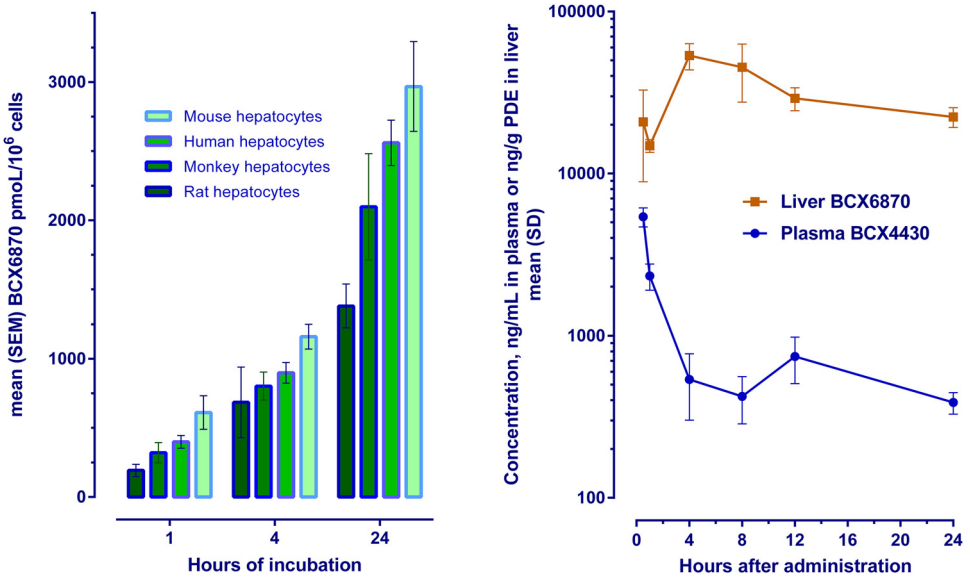

Figure 1 Anabolism of BCX4430 to BCX4430-TP (BCX6870) in hepatocytes in vitro (left panel) and levels of BCX4430 in the plasma and BCX4430-TP in the liver of rats after an IM injection of BCX4430 (right panel). Redrawn from [5]. Cells were incubated with 10 μM BCX4430 for the indicated times, and the levels of BCX4430-TP were quantitated by HPLC; the plasma samples were obtained and the livers were harvested from rats following administration of a 30 mg/kg BCX4430 dose by intramuscular injection, and the plasma BCX4430 and the liver BCX4430-TP were quantitated by HPLC/tandem MS.

or fatal infections and currently have either no approved treatments or treatments with limited efficacy. The antiviral activity of BCX4430 has been demonstrated for viruses from diverse taxa, including positive-sense *Flaviviridae* and *Coronaviridae* RNA viruses and negative-sense *Filoviridae*, *Arenaviridae*, *Bunyaviridae*, *Orthomyxoviridae*, *Picornaviridae*, and *Paramyxoviridae* RNA viruses [5]. The antiviral activity of BCX4430 against the filoviruses has also been evaluated in HeLa cell line assays by quantitation of intracellular viral RNA for MARV and EBOV and extracellular RNA for EBOV in the supernatant [5].

As reported in vitro, the EC_{50} values for BCX4430 vary across a wide range from ~3 to ~68 μM [5]; however, many cell lines used for antiviral drug sensitivity assays are inefficient at anabolizing BCX4430 to BCX4430-TP (Fig. 4). Examples of results of susceptibility assays with a very inefficient cell line, Vero, and the Jordan strain of MERS-CoV and 17D strain of the Yellow Fever virus are shown in Fig. 5; the EC_{50} estimates are in the double-digit micromolar range. Apparently, the observed high EC_{50} values for BCX4430 may be misleading, and in vivo studies are required to investigate the antiviral activity of this compound.

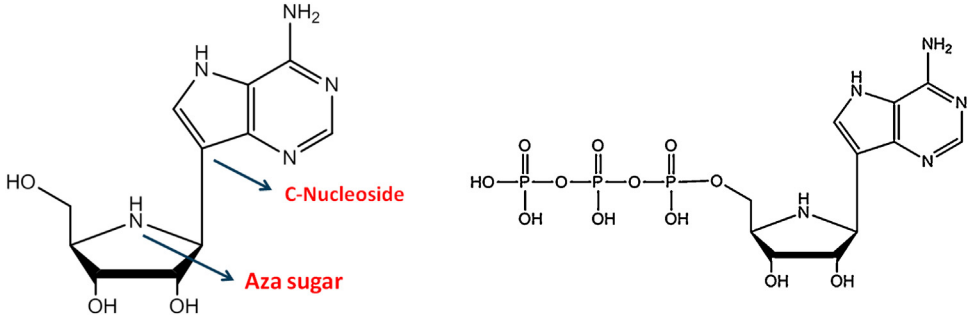

Figure 2 Structure of BCX4430 (left panel), an adenosine nucleoside analog broad-spectrum antiviral drug, and its active nucleotide form, BCX4430-triphosphate (also designated as BCX6870) (right panel).

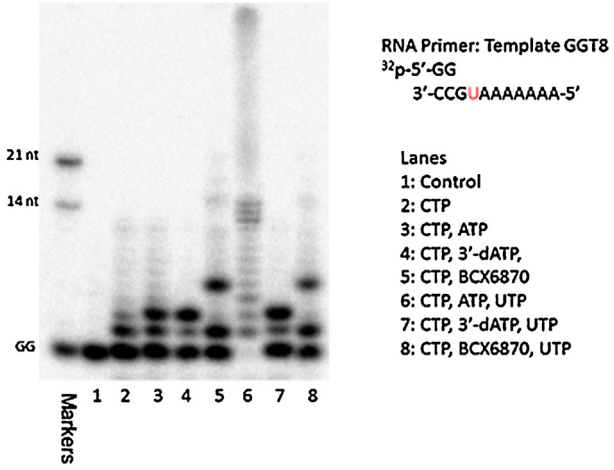

Figure 3 Non-obligate chain termination of hepatitis C through RNA-dependent, template-directed RNA synthesis by incubation with BCX4430 triphosphate (BCX6870) in vitro. Chain termination as a result of incorporation of BCX4430 is shown in lanes 5 and 8. Lanes 4 and 7 show 3′-deoxyATP, a known obligate chain terminator positive control. Lane 6 shows the pattern of RNA oligomers made with no inhibitors present or normal nucleotides present. Reproduced from [5].

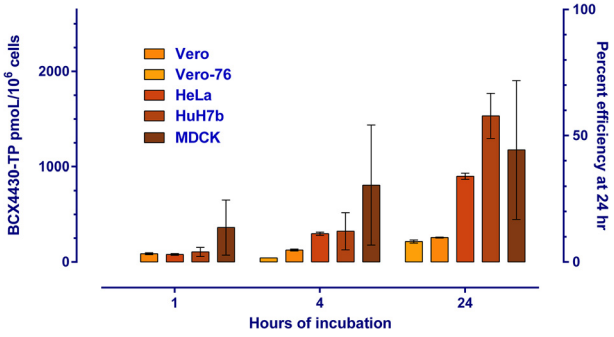

Figure 4 Variation in and low efficiency of conversion of BCX4430 to BCX4430-TP in cell lines commonly used for in vitro evaluation of antiviral activity of test agents; the data shown indicate mean and SD. Redrawn from [5]; right-hand scale indicates the percent of BCX4430-TP mean levels observed at 24 h in fresh human hepatocytes. The cell lines were incubated with 10 μM BCX4430 for the indicated times, and the levels of BCX4430-TP were quantitated by HPLC.

Efficacy of BCX4430 in nonclinical virus disease models

Experimental disease models of lethal virus infections in rodents or non-human primates are helpful for understanding the potential utility of candidate antiviral drugs. To date, BCX4430 has shown efficacy in a lethal rodent and an NHP model of filoviral diseases (Marburg virus disease [MVD] and Ebola virus disease [EVD]) [5], as well as in rodent models of Yellow Fever [6] and Rift Valley fever [5].

In a study in cynomolgus macaques infected with MARV [5], 0/6 controls survived compared to 17/18 animals treated with a 15 mg/kg BD dose of BCX4430 by IM injection ($p < 0.0001$), with the start of treatment delayed up to 48 h post inoculation. The maximum viral load, as measured by quantitation of viral RNA copies in the peripheral blood, was reduced by ~600-fold (geometric mean 0.008×10^9 compared to 4.79×10^9 copies/mL, $p < 0.0005$). Other disease markers, such as elevated bilirubin and AST from hepatitis and prolonged aPTT and PT from coagulopathy, were also markedly improved by BCX4430 ($p < 0.005$).

A dose-range study of BCX4430 in a cynomolgus macaque EVD model evaluated doses ranging from 3.4 to 16 mg/kg BID IM starting 48 h after inoculation. No animals survived; however, in the 16 mg/kg BID dose group, the mean time to death was significantly prolonged compared to that of the control group (13.2 vs. 7.2days, $p = 0.005$), and the geometric mean viral RNA copies/mL in serum was significantly reduced on day 7 (0.049×10^9 vs.

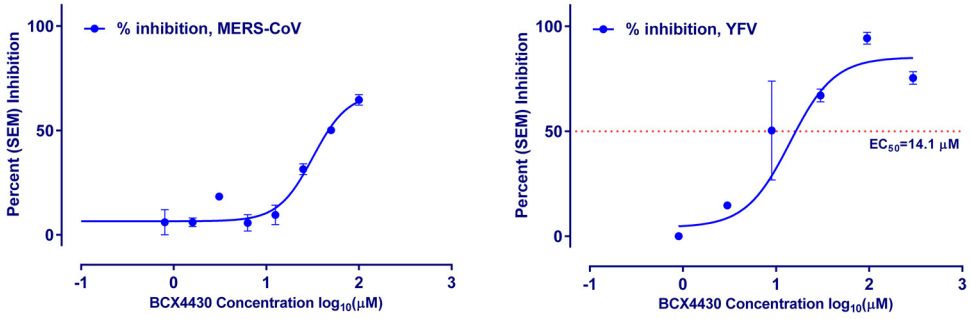

Figure 5 Concentration—response results for the inhibition of virus proliferation in cell culture assays using a Vero-E6 cell line infected with MERS-CoV (Jordan N3 strain) (left panel) and the Yellow Fever Virus (YFV, 17D strain) (right panel).
Redrawn from [5].

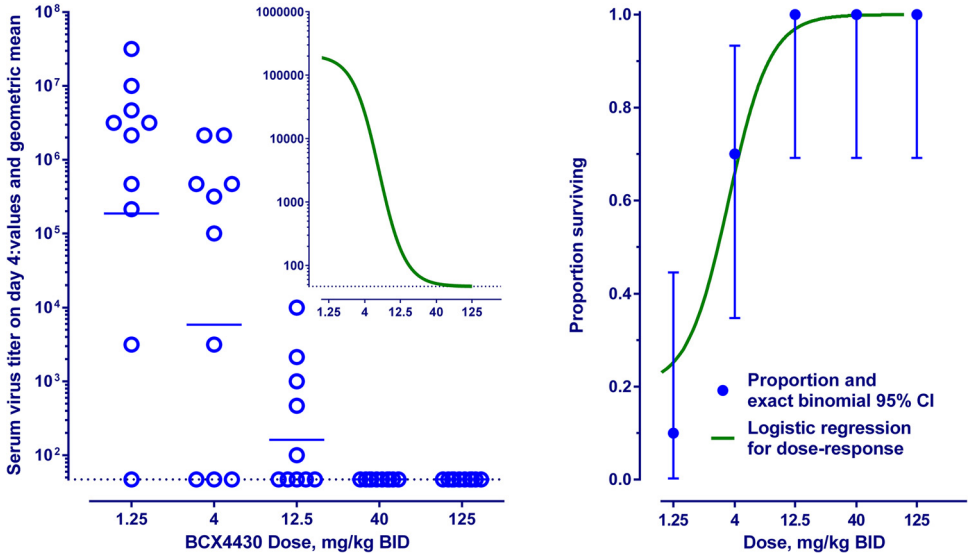

Figure 6 Relationship between the dose of BCX4430 to the antiviral effect (left panel) and survival (right panel) in Syrian golden hamsters inoculated with the Yellow Fever virus. The left panel open symbols shows data for each animal ($n = 10$ per dose group), and the horizontal bars shows the geometric mean for each dose group; the inset shows the best-fit nonlinear regression of BCX4430 dose with viral load (GraphPad Prism v6.07), indicating a typical and steep concentration—inhibition relationship. The right panel closed symbols show the proportion of animals surviving at each dose level in the same experiment, with error bars indicating the exact binomial confidence intervals. The line shows the best-fit logistic regression of dose with proportion surviving (XLSTAT Version 2015.5.01.23039).
Data from [6].

1.8×10^9 copies/mL for controls, a difference of 1.6 logs, $p = 0.028$).

In a subsequent study of BCX4430 in a rhesus macaque EVD model, all six animals administered a dose of 25 mg/kg IM BID of BCX4430 starting 30—60 min after inoculation survived compared to zero of six controls ($p < 0.001$). The viral load was reduced by 2.9 logs for this group on day 6, and the geometric mean viral RNA copies/mL in serum was 0.035×10^8 vs. 3.1×10^8 for controls ($p = 0.004$).

BCX4430 showed potent in vivo antiviral activity in studies using a Yellow Fever model in Syrian golden hamsters [6]. The administration of BCX4430 at a dose of 12 mg/kg QD was highly effective in reducing mortality even when the initiation of treatment was delayed by as much as 4 days after inoculation. It is important to note that the in vitro susceptibility testing for YFV has shown a double-digit micromolar EC_{50} (Fig. 5, right panel), emphasizing that it would be unwise to reject the utility of BCX4430 for a particular virus based solely on in vitro susceptibility testing.

The slope of the relationship of the dose of BCX4430 to both antiviral effect and survival in non-clinical models of lethal viral infections is steep. An example is shown for experimental Yellow Fever in hamsters in Fig. 6. The estimated Hill slope for the log antiviral effect was 2.3, and the ED_{50} dose was estimated to be 6.3 mg/kg BID ($r = 0.75$) (Fig. 6, left panel). The ED_{50} dose for survival was estimated to be 2.75 mg/kg BID, and in the logistic regression best fit for the experimental data ($p < 0.001$, $r = 0.86$), doubling the dose from ~3 mg/kg BID to 6 mg/kg BID increased the odds of survival by approximately 5-fold. The dose—response for survival was also very steep in a mouse model of Marburg virus disease [5].

Clinical studies with BCX4430

A Phase 1 first-in-human study of intramuscular injection of BCX4430 (NCT02319772) in healthy subjects is ongoing; for single doses of 0.3 to10.0 mg/kg, plasma exposure to BCX4430 was dose-related and linear. The first multi-day cohort was evaluated at a dose of 2.5 mg/kg QD for 7 days, and the dose escalation is continuing. To date, BCX4430 injections have been safe and well tolerated. Additional studies of BCX4430 in healthy subjects are planned using the intravenous route of administration.

Integrated assessment of BCX4430 as a candidate antiviral drug for highly pathogenic emerging and neglected infectious diseases

The mechanism of action, in vitro antiviral activity, and nonclinical efficacy of BCX4430 discussed above represent a highly attractive profile for a broad-spectrum antiviral agent. The body of evidence for BCX4430 has attracted funding for continued development from US Government agencies; this program is now supported in part with US Federal contracts from NIH/NIAID[1] and HHS/BARDA.[2] The BCX4430 program has now advanced into early clinical development, together with late-stage nonclinical toxicology and drug supply scale-up activities.

The BCX4430 drug substance is a crystalline solid that has proven to be stable in ongoing studies for at least 2 years. As a traditional small molecule chemical entity, development of both the BCX4430 drug substance synthetic processes and the formulated drug product can be pursued using standard chemistry, manufacturing, and formulation research practices, with the expectation that large-scale synthesis of a high-quality finished product can be achieved. The pharmacokinetic profile of BCX4430 in non-clinical species [5] supports the evaluation of daily dosing, and its efficacy has been demonstrated in experimental viral infection models by both parenteral (IM and IP) and oral routes [5,6]. BCX4430 administration has been generally safe and well tolerated in an ongoing healthy human subjects safety study. The clinical pharmacology profile that emerges from these studies will help to refine and optimize therapeutic dosing schedules to complete the development of BCX4430 under the Animal Rule [7] and for use in clinical testing in relevant disease settings.

Recommendations for future research with BCX4430

The recent disastrous outbreak of Ebola virus disease in West African nations and the emergence of MERS-CoV infection in the Middle East underscore the importance and urgency of successful development of effective and safe antiviral drugs for treatment of these life-threatening illnesses. In addition to continued drug synthesis and formulation research to enable large-scale drug supply and inventory, the most important categories of research to complete for BCX4430 in the near term are in the fields of nonclinical pharmacology, nonclinical disease models, and clinical pharmacology:

- Dose—response studies in nonclinical infection models.
- Nonclinical pharmacology studies of plasma and tissue pharmacokinetics in healthy and experimentally infected animals.
- Optimized dose-schedule studies in nonclinical disease models.
- Delayed dosing studies in nonclinical disease models.
- Completion of Phase 1 studies in healthy subjects to define the maximum tolerated dose, and explore exposure via IM and IV routes of administration.
- Completion of nonclinical safety studies to support emergency usage readiness evaluation.
- Evaluation of the relationship of both dose and plasma drug exposure to virologic response in patients with relevant viral infections where there is an opportunity to do so.

Evidence from this research program would provide a sound basis for the selection of BCX4430 doses and regimens for use in an emergency response setting.

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Competing interests

RT, SD, AR, YE-K, YB, and WPS are employees and/or stockholders of BioCryst Pharmaceuticals Inc.

Ethical approval

Not required.

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