#4468

BMN 349, a small molecule for alpha-1 antitrypsin-associated liver disease, stabilizes human Z-AAT and reduces severity of liver disease in the PiZ mouse model

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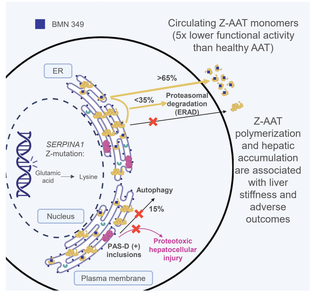
Background

- Alpha-1 antitrypsin deficiency (AATD) is caused by point mutations in the *SERPINA1* gene encoding antitrypsin (AAT), a protease inhibitor[1]
- The AATD-causing "Z" mutation (E342K) results in an unstable Z form of AAT (Z-AAT), which polymerizes and accumulates in hepatocytes, activating necrotic processes that lead to fibrosis and liver disease[1-3]
- There are currently no approved treatments for AATD-related liver disease other than liver transplant
- BMN 349 is a small chaperone molecule that binds and stabilizes Z-AAT monomers, which may enhance secretion of Z-AAT into plasma, thereby reducing hepatic polymerization and accumulation (**Figure 1**)

Figure 1. BMN 349 binds Z-AAT to prevent polymerization



AAT, alpha-1 antitrypsin; ER, endoplasmic reticulum; ERAD, ER-associated degradation; PAS-D (+), Periodic Acid Schiff-Diastase positive inclusion; Z-AAT, mutated form of AAT.

Objective

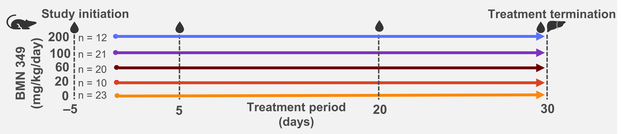
- Here, we describe the results from a study that used a mouse model of AATD liver disease to investigate the impact of BMN 349 treatment on levels of total plasma Z-AAT, plasma and liver Z-AAT polymers, and overall liver health

Methods

Study design and tissue collection

- Female mice expressing human Z-AAT (PiZ mice)[4] were dosed twice daily orally with BMN 349 at 0, 20, 60, 100, or 200 mg/kg/day for 30 days (**Figure 2**)
- Whole blood samples were collected 5 days prior to BMN 349 dose initiation and on days 5, 20, and 30
- Liver samples were collected following the last BMN 349 dose on day 30

Figure 2. Study design and assessments



Mice were 12 weeks of age at study initiation. AAT, alpha-1 antitrypsin; Z-AAT, mutated form of AAT.

Assessment of Z-AAT levels and polymer burden

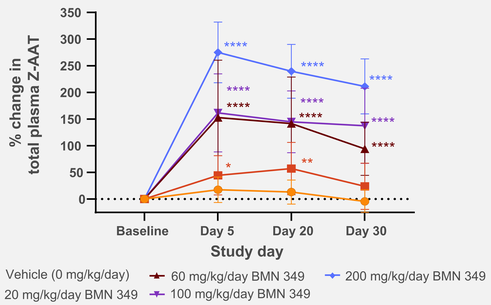
- Plasma total and polymer Z-AAT levels and liver Z-AAT polymer levels were measured by an enzyme-linked immunosorbent assay (ELISA)
- Liver Z-AAT polymer burden was visualized in tissue sections using Periodic Acid Schiff-Diastase (PAS-D) staining
- Plasma pharmacodynamic biomarkers of hepatocyte endoplasmic reticulum stress and biosynthetic function were assessed using liquid chromatography tandem mass spectrometry-based proteomics

Results

BMN 349 dose-dependently increased total plasma Z-AAT levels during the treatment period

- Total plasma Z-AAT levels rapidly increased at doses of ≥60 mg/kg/day BMN 349 (**Figure 3**)

Figure 3. Total plasma Z-AAT levels via ELISA



Data are mean ± SD percent change from baseline. Significance (vs vehicle) was determined with a mixed-effects analysis using Dunnett's multiple comparisons test. *, *P* <0.05; **, *P* <0.01; ****, *P* <0.0001.
AAT, alpha-1 antitrypsin; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; Z-AAT, mutated "Z" form of AAT.

Plasma and liver Z-AAT polymers were dose-dependently reduced by BMN 349 treatment

- Plasma polymers were reduced at all dose levels and nearly eliminated at 200 mg/kg/day BMN 349 (**Figure 4A**)
- Polymer accumulation in the liver was prevented by doses ≥100 mg/kg/day BMN 349 (**Figure 4B**)
- Liver histopathology also shows that 200 mg/kg/day BMN 349 was associated with fewer and smaller PAS-D (+) globules (**Figure 4C**)

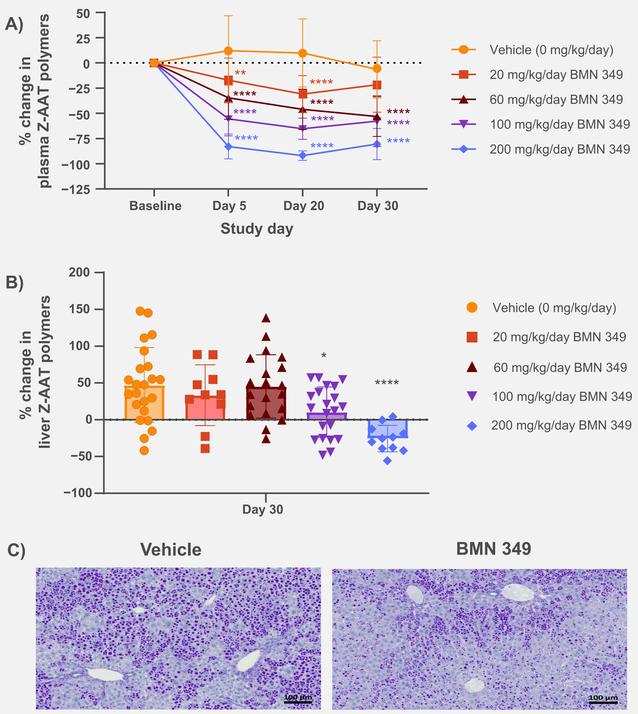
BMN 349 treatment improved liver health over time

- BMN 349 dosed at ≥100 mg/kg/day significantly decreased proteins associated with hepatic endoplasmic reticulum stress and unfolded protein response pathways, as well as normalized markers of biosynthetic liver function in PiZ mice (**Figure 5**)

Conclusions

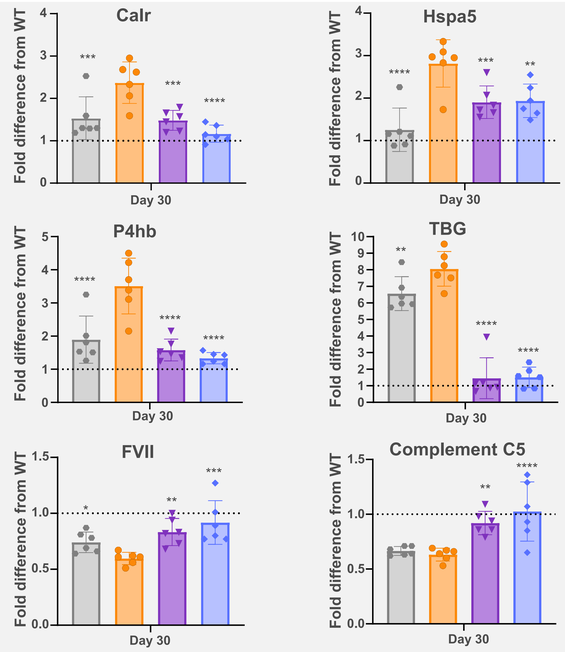
- BMN 349 stabilizes monomeric human Z-AAT, blocking formation of hepatic polymers to enable normal secretion to the plasma in PiZ mice
- BMN 349 treatment reduced pro-necro-inflammatory processes in the liver and improved overall biosynthetic liver function, suggesting BMN 349 may slow or prevent AATD liver disease progression

Figure 4. Z-AAT polymer levels in A) plasma and B) liver via ELISA, and C) liver Z-AAT polymer PAS-D (+) staining



A and B) Data are mean ± SD percent change from baseline. Significance (vs vehicle) was determined using a one-way ANOVA **A)** Dunnett's or **B)** Šídák's multiple comparisons test. **C)** PAS-D (+) globules are indicators of Z-AAT polymers (magenta). Liver histopathology images are from PiZ mice that received vehicle (left) or 200 mg/kg/day BMN 349 (right) for 30 days.
*, *P* <0.05; **, *P* <0.01; ***, *P* <0.0001; ****, *P* <0.0001.
AAT, alpha-1 antitrypsin; ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; PAS-D (+), Periodic Acid-Schiff with diastase positive; PiZ, transgenic mouse model with E342K mutation; SD, standard deviation; Z-AAT, mutated "Z" form of AAT.

Figure 5. Plasma pharmacodynamic biomarker levels via LC-MS/MS



Data are mean ± SD, measured in plasma samples. Proteomics assessments were only carried out in mice that received BMN 349 at 100 or 200 mg/kg/day. Values (n = 6/cohort) were normalized to those of control WT mice of the same age and reported as fold-change from WT. Proteins Calr, Hspa5, and P4hb are biomarkers for hepatic ER stress; TBG, FVII, and complement C5 are associated with hepatic biosynthetic function. Significance (vs vehicle) was determined using a one-way ANOVA, Fisher's uncorrected LSD test. *, *P* <0.05; **, *P* <0.01;***, *P* <0.001; ****, *P* <0.0001.
ANOVA, analysis of variance; Calr; calreticulin; C5, component 5; ER, endoplasmic reticulum; FVII, factor VII coagulation protein; Hspa5, heat shock protein 5; LC-MS/MS, liquid chromatography tandem mass spectrometry; LSD, least significant difference; P4hb, prolyl 4-hydroxylase subunit beta; SD, standard deviation; TBG, thyroxine binding globulin; WT, wild type.

References

1. Seixas S, et al. *Appl Clin Genet*. 2021;14:173-94. **2.** Lomas DA, et al. *Thorax*. 2004;59:529-35. **3.** Schneider CV, et al. *Gastroenterol*. 2020;159:534-48. **4.** Carlson JA, et al. *J Clin Invest*. 1989;83(4):1183-90.

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Disclosures

BH, **HW**, **DM**, **CC**, **AG**, **JV**, **BH**, **KL**, **HZ**, **LS**, **KE**, and **SB** are employees and stockholders of BioMarin Pharmaceutical Inc. **IT** and **SF** are former employees of BioMarin Pharmaceutical Inc. and are potential stockholders. **DL** is supported by the Medical Research Council (UK), the NIHR UCLH Biomedical Research Centre, and BioMarin Pharmaceutical Inc., and he is also an inventor on the patent PCT/GB2019/051761 that describes the development of BMN 349 used in this work. **RR** and **JI** declare no competing interests.

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