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Bioanalytical methods to assess pharmacokinetics and pharmacodynamics of BMN 349, a small molecule therapeutic for alpha-1 antitrypsin deficiency

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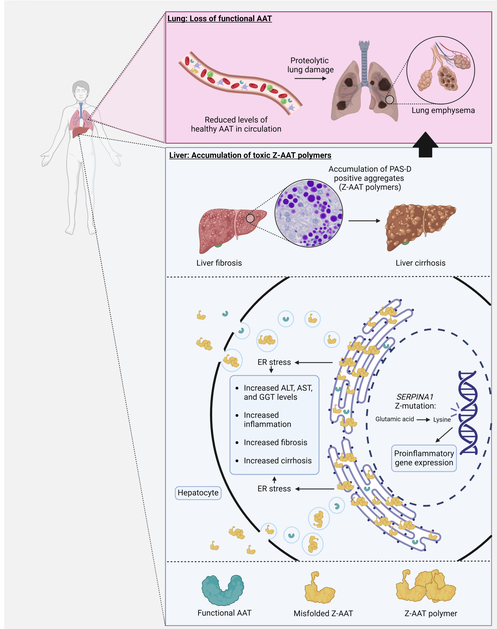
Background

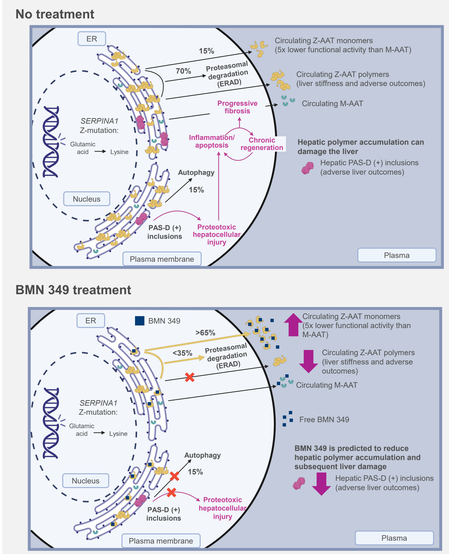
- Alpha-1 antitrypsin deficiency (AATD) is caused by point mutations in the *SERPINA1* gene encoding alpha-1 antitrypsin (AAT), an important protease inhibitor[1]
- The most severe AATD-causing mutation (E342K) produces a "Z" variant of AAT (Z-AAT) that is prone to polymerizing in hepatocytes[2-4]
- Polymerization leads to low levels of functional AAT in the blood due to hepatic accumulation of toxic Z-AAT polymers, which can lead to hepatitis and cirrhosis[1,3,4] (**Figure 1**)
  - Reduced circulating AAT can also damage alveolar tissue, which increases the risk of AATD-related lung disease[5]
- BMN 349, an investigational treatment for AATD, is a small molecule chaperone that binds to Z-AAT, preventing polymerization and increasing monomer AAT secretion from the liver into blood circulation (**Figure 2**)
  - Assessment of BMN 349 in a mouse model of AATD liver disease initiated the development of the bioanalytical plan described here[6]

Figure 1. Mechanism of AATD



AAT, alpha-1 antitrypsin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ER, endoplasmic reticulum; GGT, γ-glutamyl transferase; PAS-D, Periodic Acid Schiff-diastase; Z-AAT, mutated "Z" form of AAT.

Figure 2. Predicted effects of BMN 349 treatment in individuals with a Z-AAT allele



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

Objective

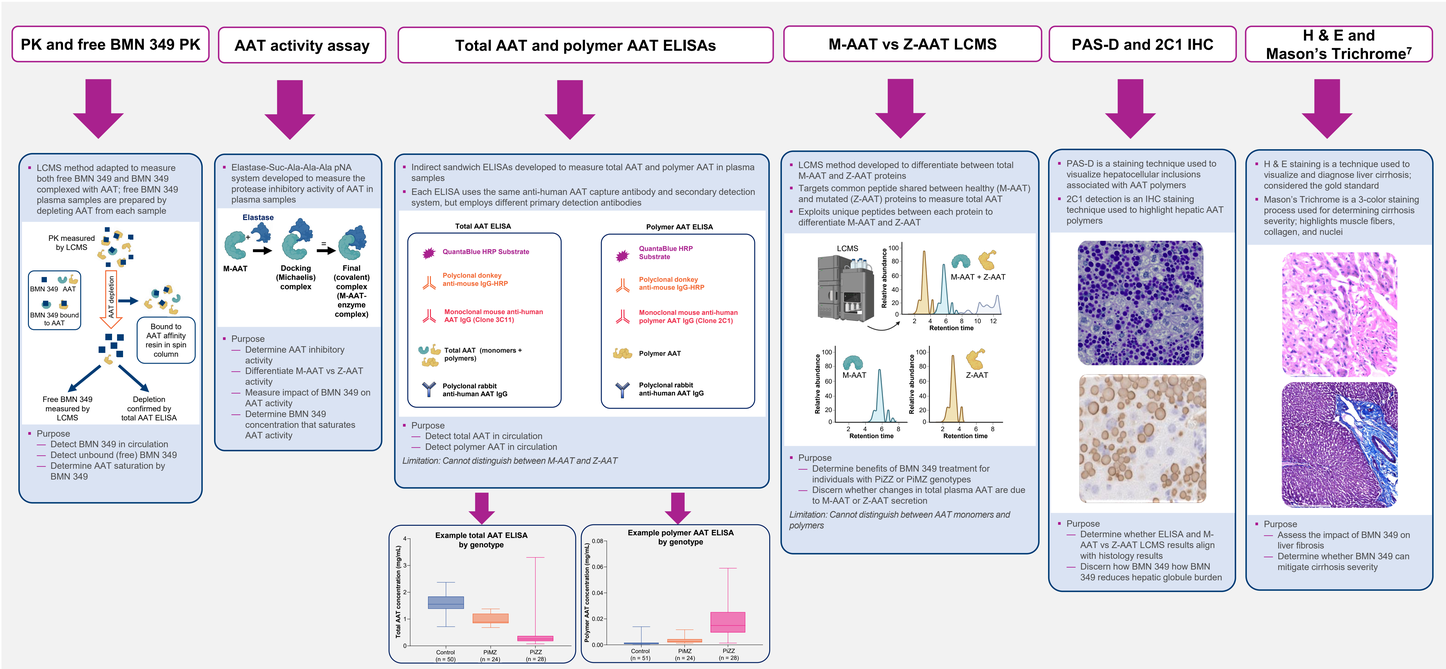
- Here, we present the bioanalytical plan for clinical assessment of BMN 349, including assays developed to measure pharmacokinetics of BMN 349 and its impact on AAT secretion and activity in individuals with AATD

Methods

BMN 349 assay plan

- A suite of bioanalytical methods was developed to provide a comprehensive approach for measuring BMN 349 activity and efficacy in clinical trial participants with AATD (**Figure 3, Table 1**)
- Assay method characterizations in human plasma samples show individuals with the PiZZ genotype have lower total and higher polymer AAT levels than healthy individuals or individuals with the PiMZ genotype (**shown in the total AAT and polymer ELISA panels**)

Figure 3. Planned assessments of BMN 349 activity and efficacy



Plasma samples for preliminary ELISAs were provided by Dr. Mark Brantly. Liver disease status was reported by the individual in a questionnaire. Box edges are the 25th and 75th percentiles, solid line is the median, and whiskers are the minimum and maximum values.
AAT, alpha-1 antitrypsin; AATD, alpha-1 antitrypsin deficiency; Ala, alanine; ELISA, enzyme-linked immunosorbent assay; H & E, hematoxylin and eosin; HRP, horseradish peroxidase; IgG, immunoglobulin G; IHC, immunohistochemistry; LCMS, liquid chromatography mass spectrometry; M-AAT, healthy form of AAT; PAS-D, Periodic Acid Schiff-Diastase; PK, pharmacokinetics; pNA, p-nitroanilide; Suc, succinyl; Z-AAT, mutated "Z" form of AAT.

Table 1. Validated assay parameters

Parameter	LCMS PK	AAT activity assay	Total AAT ELISA	Polymer AAT ELISA
Range	1.00–2000 ng/mL	4.17–14.9 mg/dL	3.1–200 µg/mL	1.08–69 µg/mL
Sensitivity	1.0 ng/mL	4.17 mg/dL	3.1 µg/mL	1.08 µg/mL
Precision	≤7.38%	≤7.0%	≤24.9%	≤12.8%
Accuracy	−8.75% to −6.0%	−0.2% to 6.5%	−0.4% to 9.7%	1.9% to 13.9%

Other parameters, such as parallelism, stability, specificity, robustness, and selectivity in normal, hemolyzed, and lipemic samples were all assessed using acceptance criteria set for each unique assay.
AAT, alpha-1 antitrypsin; ELISA, enzyme-linked immunosorbent assay; LCMS, liquid chromatography mass spectrometry; PK, pharmacokinetics.

Disclosures

CC, AG, SN, AD, JVV, SS, and KL are current or former employees and stockholders of BioMarin Pharmaceutical Inc.

Conclusion

- A suite of bioanalytical methods are being developed and validated to measure pharmacokinetics and pharmacodynamics of BMN 349 and its effect on AAT and Z-AAT in human plasma
- These assays will provide a holistic evaluation of BMN 349 during clinical development to determine a safe and efficacious dose for treating individuals with AATD

References

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