Krystal

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Murine Toxicology Study of Repeat-Dose Inhaled KB408, an HSV-1-Based Vector for the Treatment of Alpha-1 Antitrypsin Deficiency

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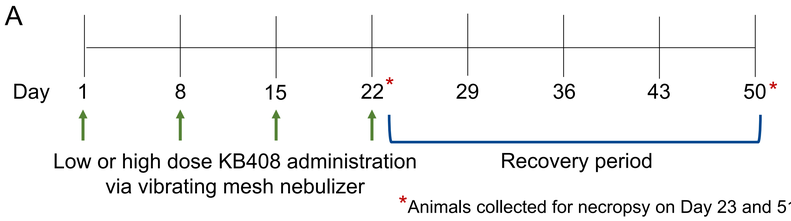
Introduction

- Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder resulting from mutations in the *SERPINA1* gene encoding alpha-1 antitrypsin (AAT), a secreted alpha-1 glycoprotein whose primary function is to inhibit neutrophil elastase (NE) in the lungs. Unregulated NE protease activity can result in progressive pulmonary impairment and respiratory failure.
- KB408 is a replication-defective herpes simplex virus type 1 (HSV-1)-based gene therapy vector encoding full-length human AAT developed for the treatment of AATD-related lung disease.
- In previous studies, KB408 efficiently transduced human small airway epithelial cells *in vitro,* and inhalation of KB408 resulted in local effector delivery to the respiratory tract of *SERPINA1* knockout mice, restoring AAT in lung tissue and epithelial lining fluid (Artusi, *ESGCT* 2023).
- An IND-enabling good laboratory practice (GLP) toxicology study of KB408 was initiated to support preliminary dose selection for an ongoing Phase 1 clinical trial (NCT06049082).

Study Design

A



	Air Control		Vehicle Control		Low Dose KB408		High Dose KB408	
No. of animals	M:15	F:15	M:15	F:15	M:18	F:18	M:18	F:18

B

Tissues for Biodistribution	Toxicology Endpoints
• Brain	• Body weight (%)
• Lymph nodes	• Food consumption
• Heart	• Clinical observations
• Lungs	• Pulmonary function
• Spleen	• Ophthalmology
• Bone marrow	• Electrocardiography (ECG)
• Kidney	• Hematology & serum chemistry
• Liver	• Organ weights
• Blood	• Gross pathology
• Reproductive tissues	• Histopathology

Figure 1. A GLP toxicology study was conducted to evaluate the biodistribution and potential toxicity of KB408 after 4 weeks of weekly nose-only inhalation delivery to healthy immunocompetent BALB/c mice, as well as to determine the reversibility of any effects after a 4-week recovery period. **A**. Study design. **B**. Tissues collected for biodistribution analysis via qPCR (left) and the toxicology endpoints assessed during the study (right). F, female, M, male.

Validation of Vibrating Mesh Nebulizer

A

Target Concentration

Testing Method	Performance Criteria	Result
Gravimetric (GFA)	2.40±1.00 mg/L	2.527 mg/L
Chemical (BioSampler)	4×10^7 to 7×10^8 genomes/L	3×10^8 genomes/L

B

Aerosol Concentration Repeatability

Testing Method	Repeatability Number	Concentration Results	Performance Criteria, RSD
Gravimetric (GFA)	1	1.220 mg/L	
	2	1.043 mg/L	
	3	0.950 mg/L	
	Average	1.071 mg/L	
	SD	0.137 mg/L	
	%RSD	12.8	<30%
Chemical (BioSampler)	1	3×10^8 genomes/L	
	2	3×10^8 genomes/L	
	3	3×10^8 genomes/L	
	Average	3×10^8 genomes/L	
	SD	3×10^7 genomes/L	
	%RSD	9.7	<30%

C

Particle Size Distribution

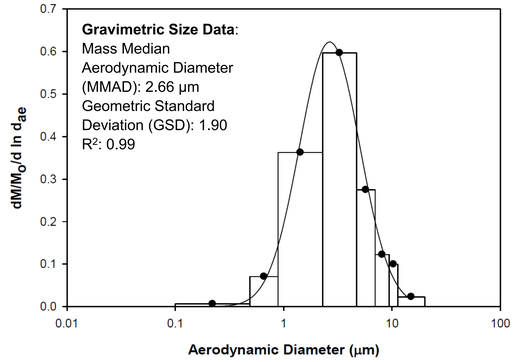


Figure 2. The ability of a vibrating mesh nebulizer to deliver a consistent dose of KB408 was evaluated as part of the GLP toxicology study. The system was validated through (**A**) total aerosol concentration, (**B**) aerosol concentration repeatability, and (**C**) particle size distribution. This same system is being used for clinical delivery of KB408. GFA, Glass Fiber. SD, standard deviation. RSD, relative standard deviation.

Assessment of Human *SERPINA1* Transcripts in Mouse Tissues Following KB408 Nebulization

Males	Left Lung	Right Cranial Lung	Right Caudal Lung	Axillary Lymph Nodes	Inguinal Lymph Nodes	Brain	Bone Marrow	Testis	Kidney	Spleen	Liver	Heart	Blood
KB408 Low Dose	3.02×10^3	3.03×10^3	3.11×10^3	5.86×10^1	6.44×10^1	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
KB408 High Dose	2.97×10^3	8.81×10^3	6.38×10^3	2.04×10^2	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

Females	Left Lung	Right Cranial Lung	Right Caudal Lung	Axillary Lymph Nodes	Inguinal Lymph Nodes	Brain	Bone Marrow	Ovary	Kidney	Spleen	Liver	Heart	Blood
KB408 Low Dose	1.79×10^3	2.81×10^3	1.52×10^3	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
KB408 High Dose	3.84×10^3	5.66×10^3	7.06×10^3	5.99×10^1	5.18×10^1	1.31×10^2	1.21×10^2	BQL	BQL	BQL	BQL	BQL	BQL

High Detection (>1,500 copies/mg) | **Low Detected (50 – 200 copies/mg)** | **Below Quantification Limit (BQL) (<50 copies/mg)**

Figure 3. KB408 exposure was highest in the lungs, with little-to-no detection in other tissues, as assessed by qPCR 24 hours after last dose administered. Results are the average of 6 animals per group. Transcripts were below the limit of detection for all control animals.

Toxicology Endpoints After Weekly KB408 Nebulization and Recovery Phase

Endpoint	Air Control	Vehicle Control	Low Dose KB408	High Dose KB408	Low Dose KB408 Recovery	High Dose KB408 Recovery
Body weight (%)						
Food consumption						
Clinical observations						
Pulmonary function, respiratory rate						
Pulmonary function, tidal volume						
Ophthalmology						
Electrocardiography						
Hematology & serum chemistry						
Organ weights						
Gross pathology						
Histopathology						

= no noteworthy findings | = mild findings | = moderate findings | = severe findings

Increased respiratory rate in low and high dose groups at Day 22 and in low dose group at Day 51.

Decreased tidal volume: body weight in high dose group after first dose (Day 1).

>10 fold increase in basophils at Day 23 and Day 51, 8- to 10-fold decrease in monocytes on Day 51 for low and high dose groups.

Mixed cell peribronchovascular infiltrates in low and high dose groups at Day 23, resolved by Day 51.

Figure 4. No KB408-related effects were identified on body weights, food consumption, clinical observations, ophthalmic examinations, heart function, and gross pathology, while minor, non-adverse KB408-related histopathology changes in the lungs and respiratory rate increases were observed one day post-final dose, resolving during recovery phase. This suggests a transient, non-adverse effect of KB408 on respiratory tissues.

Conclusions

The absence of adverse events and the limited biodistribution of KB408 after inhaled delivery to mice supports the safety profile of KB408. Combined with molecular studies from mice, these data support the initiation of a Phase 1 study for patients with AATD.

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