Preclinical pharmacology of KB408, an HSV-1-based gene therapy vector, for the treatment of alpha-1 antitrypsin deficiency

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Introduction

- Alpha-1 antitrypsin deficiency (AATD) is a rare autosomal co-dominant inherited genetic disorder resulting from mutations in the gene (*SERPINA1*) encoding alpha-1 antitrypsin (AAT), a secreted α1-glycoprotein whose principal substrate is neutrophil elastase in the lungs[1-3].
- For the large majority of AATD patients, lung disease is of the greatest clinical importance[4], as it often results in life threatening progressive pulmonary impairment and severe respiratory insufficiency[5-6], underscoring the need for continued drug development efforts directed towards effective treatments targeting pulmonary AATD.
- To this end, Krystal Biotech, Inc. developed KB408, a replication-incompetent, non-integrating herpes simplex virus type 1 (HSV-1)-based gene therapy vector encoding two copies of full-length human *SERPINA1*, for the potential use in treating AATD lung disease.
- Objective: to determine (1) whether KB408 was capable of efficiently transducing clinically relevant primary human small airway epithelial cells (SAECs) in vitro and inducing production and secretion of full-length human AAT, and (2) if the vector was safe for, and amenable to, inhaled administration in immunocompetent animals, including in *SERPINA1* knockout mice.

Materials and Methods

- Vector efficiency and molecular correction were assessed for KB408 *in vitro* and *in vivo*.

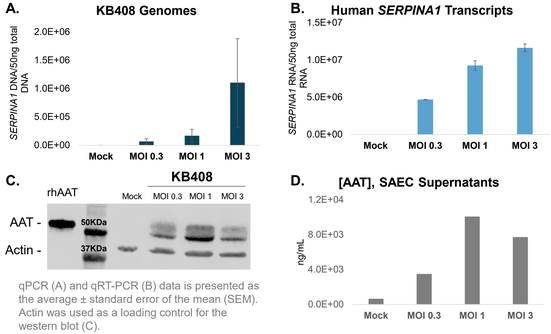
Table 1. Critical reagents

Material Description	Application	Source	Catalog No.
Recombinant human AAT (rhAAT)	Western Blot	Abcam	ab91136
AAT antibody	Western Blot	Abcam	ab9400
Actin	Western Blot	Lycor	926-42210
SERPINA1 knockout (KO) mice	In Vivo Dosing	The Jackson Laboratory	Strain: *Serpina1*^{em3Chmu}
AAT antibody	Immunofluorescence	ThermoFisher	PA5-16661
Anti-rabbit antibody	Immunofluorescence	Abcam	ab150080
AAT ELISA	ELISA	ALPCO	30-6752

Results: *In Vitro*

Figure 1. Clinically relevant primary airway cells efficiently targeted by KB408 to induce secretion of full-length AAT

- Vector transduction of primary human SAECs, assessed via qPCR analysis **(Figure 1A)**
- Dose-dependent human *SERPINA1* transcript expression in transduced SAECs, via qRT-PCR analysis **(Figure 1B)**
- Detection of intracellular full-length AAT protein production in transduced airway cells via western blot **(Figure 1C)**
- Quantification of AAT protein secretion into the cell culture supernatant via ELISA **(Figure 1D)**



qPCR (A) and qRT-PCR (B) data is presented as the average ± standard error of the mean (SEM). Actin was used as a loading control for the western blot (C).

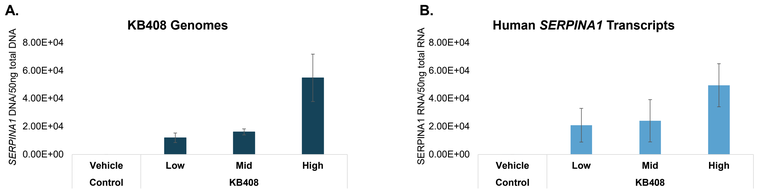
Results: *In Vivo* – Proof-of-Concept in Wild-Type Mice

Table 2. Experimental design for repeat-dosing of KB408 in healthy immunocompetent mice

Treatment	Animals	Dose	Route of Administration	Administration (Days)	Necropsy (Days)
Vehicle	C57BL/6 mice	-	Intratracheal Instillation	1 & 3	4
KB408	C57BL/6 mice	**Low-Dose** (4.125E7 PFU)			
KB408	C57BL/6 mice	**Mid-Dose** (1.65E8 PFU)			
KB408	C57BL/6 mice	**High-Dose** (6.6E8 PFU)			

PFU: plaque forming units

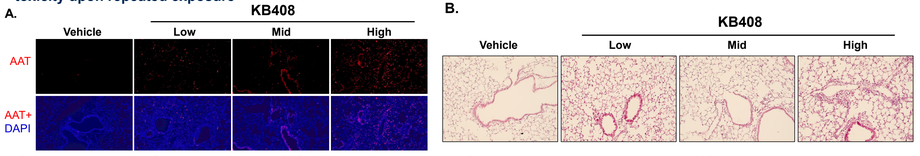
Figure 2. KB408 capably transduced the lungs of immunocompetent animals and expressed its cargo therein upon repeat dosing



qPCR (A) and qRT-PCR (B) data is presented as the average ± SEM.

- Detection of dose-dependent transduction **(Figure 2A)** and subsequence expression of the human transgene **(Figure 2B)** in lung tissue homogenates following intratracheal administration of KB408 by qPCR and qRT-PCR analyses, respectively

Figure 3. Dose-dependent human AAT protein expression was observed in the lungs of immunocompetent mice without visible toxicity upon repeated exposure



- Representative immunofluorescence images of KB408- or vehicle-treated mouse lungs. DAPI was used to stain nuclei **(Figure 3A)**
- Representative hematoxylin and eosin (H&E)-stained lung tissue sections harvested from KB408- or vehicle-treated lungs **(Figure 3B)**

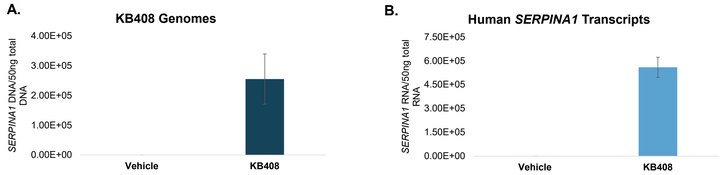
Results: *In Vivo* – *SERPINA1* Knock-Out Mice

Table 3. Experimental design for a single dose of KB408 in *SERPINA1* knock-out (KO) mice

Treatment	Animals	Dose	Route of Administration	Administration (Day)	Necropsy (Day)
Vehicle	*SERPINA1* KO (*Serpina1*^{em3Chmu})	-	Intratracheal Instillation	1	2
KB408	*SERPINA1* KO (*Serpina1*^{em3Chmu})	3.3E8 PFU			

Results: *In Vivo* – *SERPINA1* Knock-Out Mice

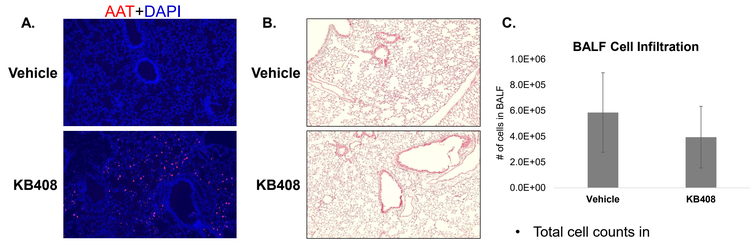
Figure 4. KB408 locally delivered human *SERPINA1* without systemic vector exposure upon inhalation



qPCR (A) and qRT-PCR (B) data is presented as the average ± SEM.

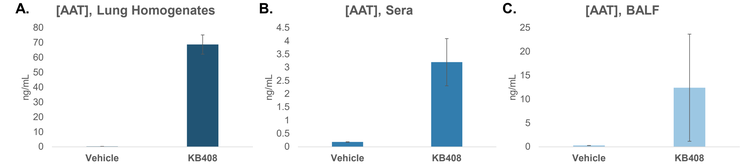
- Vector transduction **(Figure 4A)** and human *SERPINA1* transcript expression **(Figure 4B)** in lung tissue homogenates of *SERPINA1* KO mice following intratracheal administration of KB408.
- Whole blood, brain, heart, spleen, liver, kidney, ovary, bone marrow, and lymph node samples were all below the limit of detection for vector genome copies.

Figure 5. Human AAT protein expressed in the lungs following intratracheal administration of KB408 in *SERPINA1* knock-out mice without apparent vector- or effector-mediated toxicity



- Representative IF images DAPI was used to stain nuclei **(Figure 5A)**
- Representative H&E-stained lung tissue sections **(Figure 5B)**
- Total cell counts in bronchoalveolar lavage fluid (BALF). Data presented as the average ± SEM. **(Figure 5C)**

Figure 6. Human AAT protein detected in the lung tissue (A), sera (B), and BALF (C) of *SERPINA1* KO mice, suggesting protein expression in the lung epithelium and trafficking to both the lung surface and through the lung interstitium upon KB408 inhalation



Conclusions

- KB408 can efficiently transduce clinically relevant human SAECs, promoting intracellular expression and subsequent secretion of full-length human AAT.
- The vector effectively transduces the airways of immunocompetent animals and directs expression of human AAT after inhalation without significant toxicity or systemic vector biodistribution.
- Following intratracheal administration in *SERPINA1* knock-out mice, KB408 promoted robust expression of human AAT in lung homogenates, with protein also being detectable in the serum and BALFs, indicating trafficking of the effector through the pulmonary interstitium.
- Taken together, these data provide strong support for the potential of inhaled KB408 as a novel gene therapy candidate for the treatment of AATD lung disease.

References

1: De Serres *et al.* COPD. 2006 Aug;3(3):133-9; 2:Gooptu *et al.* Eur Respir J. 2009 Aug;34(2):475-88; 3: Greene *et al.* Nat Rev Dis Primers. 2016 Jul 28;2:16051; 4: Brode *et al.* CMAJ. 2012 Sep 4;184(12):1365-71; 5: Elliot *et al.* Am J Respir Cell Mol Biol. 1998 May;18(5):670-4.

Acknowledgements/Disclosures

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