Nonclinical pharmacology of KB408, an HSV-1-based vector designed for the treatment of alpha-1 antitrypsin deficiency, in the *SERPINA1* knockout mouse model

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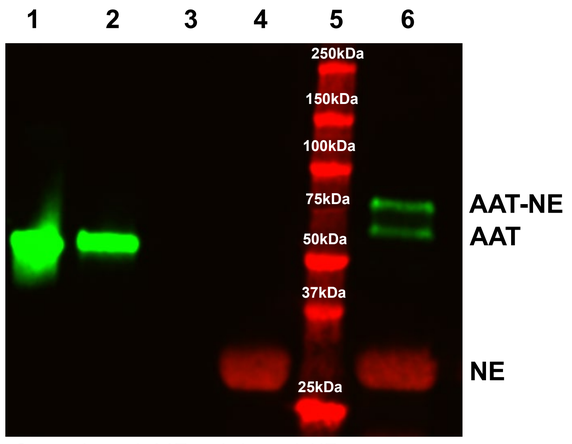
Background

- Alpha-1 antitrypsin deficiency (AATD) is a rare autosomal co-dominant inherited genetic disorder resulting from mutations in the *SERPINA1* gene encoding alpha-1 antitrypsin (AAT), a secreted α1-glycoprotein whose principal substrate is neutrophil elastase (NE) in the lungs[1-3].
- The primary function of AAT is to irreversibly bind and inhibit NE, thus protecting the lungs from unregulated NE protease activity which can result in parenchymal damage and loss of respiratory function[4].
- Despite causing both severe lung and liver pathology, lung disease is of the greatest clinical importance for most AATD patients due to progressive pulmonary impairment leading to eventual respiratory failure[4-6].
- Augmentation therapy, consisting of weekly intravenous infusions of plasma-derived AAT, remains the only FDA-approved therapy for AATD. However, its clinical efficacy in preventing progressive lung dysfunction is debated, and novel treatments targeting AATD pulmonary disease are needed.
- To this end, KB408, a replication-defective herpes simplex virus type 1 (HSV-1)-based gene therapy vector encoding full-length human AAT, was engineered by Krystal Biotech, Inc. for the treatment of AATD-related lung disease.
- Preliminary data indicated that KB408 efficiently transduced multiple mammalian cell types in culture, including clinically relevant primary human small airway epithelia cells (SAECs), resulting in secretion of full-length human AAT (Artusi *et al,* ESGCT 2021).

KB408-Derived Human AAT is Functionally Active *In Vitro*



Green channel: AAT; red channel: NE

Lane 1: Commercial native human AAT
Lane 2: KB408 supernatant
Lane 3: Mock supernatant
Lane 4: Commercial native human NE
Lane 5: Marker
Lane 6: KB408 supernatant + NE

Figure 1. *In vitro* binding of KB408-derived human AAT (hAAT) and its substrate native human NE (hNE) demonstrates the functional activity of the transgene. Supernatants from transduced HEK293 cells were collected at 24 hours post infection (h.p.i.) and incubated with commercially-available recombinant hNE prior to western blot (WB). Commercial native hAAT (lane 1) and hNE (lane 4) were loaded to verify unbound protein size (52 kDa and 25 kDa, respectively) and neat supernatants from transduced cells were loaded to verify proper secretion of KB408-derived hAAT (lane 2). WB showed that the combination of KB408-derived hAAT and recombinant hNE resulted in a shift of the AAT band from ~52 kDa to ~78 kDa (lane 6), suggesting that hAAT is able to irreversibly bind hNE and is therefore biologically active.

Wild-type (WT) Mice Express AAT Upon KB408 Inhalation

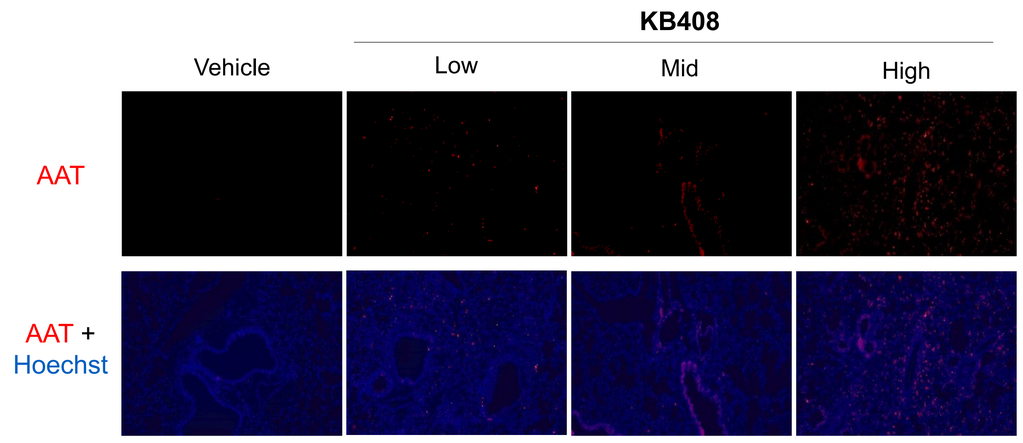


Figure 2. Representative immunofluorescence (IF) images of dose-dependent human AAT protein expression in the lungs of WT immunocompetent mice. KB408 at a low- (4.13 x 10^7 PFU), mid- (1.65 x 10^8 PFU), and high- (6.6 x 10^8 PFU) dose or vehicle was administered by intratracheal (IT) instillation to WT mice on days 1 and 3 and tissue was examined on day 4. Hoechst was used to stain nuclei.

KB408 Locally Delivers Human *SERPINA1* to *SERPINA1* Knockout (KO) Mice

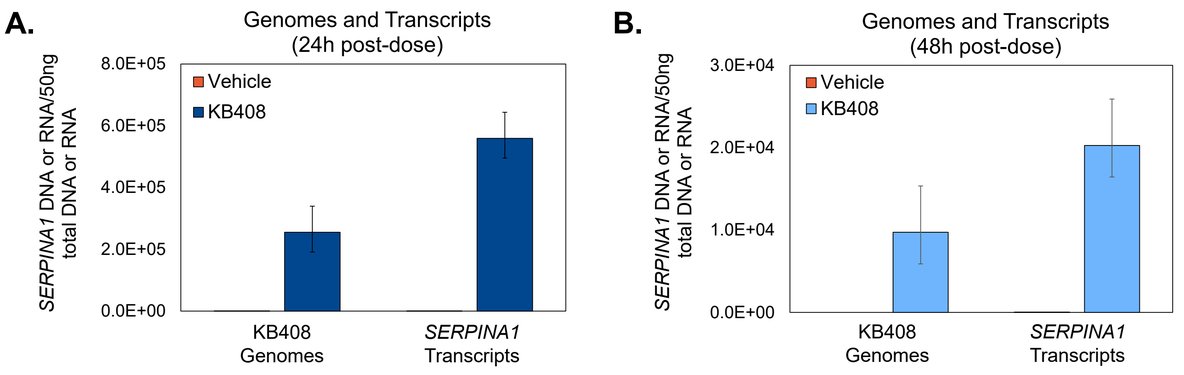


Figure 3. KB408 locally delivered human *SERPINA1* without systemic vector exposure. Following IT administration of KB408 (3.3 x 10^8 PFU) to *SERPINA1* KO mice, lung tissue homogenates were analyzed for KB408 genomes and human *SERPINA1* transcripts at **(A)** 24-hours post-dose and **(B)** 48-hours post-dose. Blood, brain, heart, spleen, liver, kidney, ovary, bone marrow, and lymph node samples were all below the limit of detection for vector genome copies. Vector transduction and *SERPINA1* gene expression were determined by qPCR and qRT-PCR, respectively. Data presented as the average ± SD.

KB408 Effectively Targets the Respiratory Tract of *SERPINA1* KO Mice and Promotes Expression of Human AAT in Lung Tissue and Fluid Without Toxicity

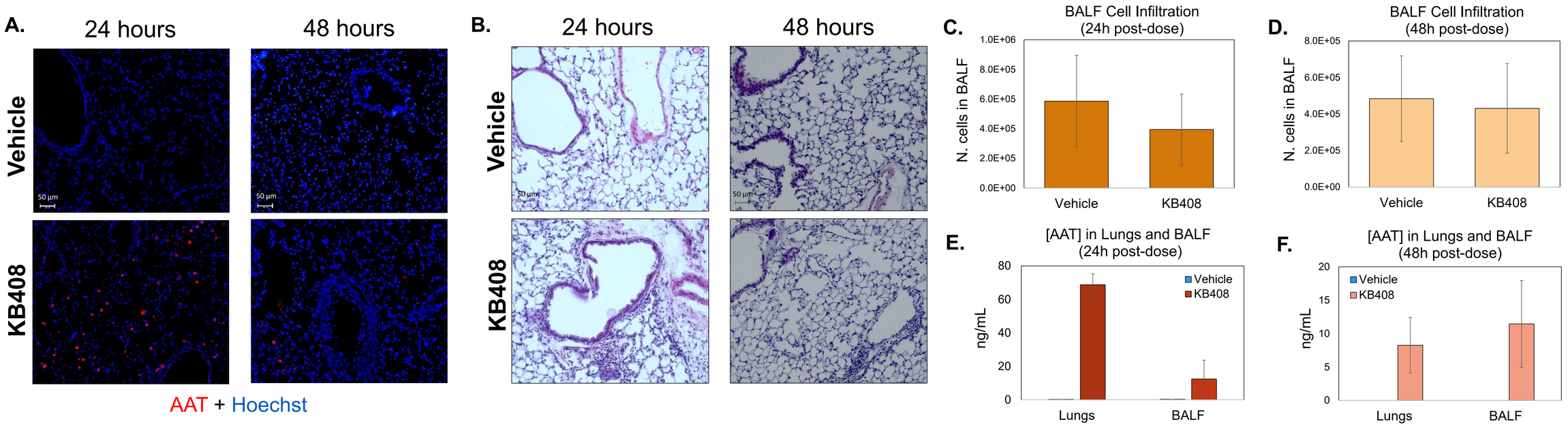


Figure 4. Human AAT protein in the lungs and bronchoalveolar lavage fluid (BALF) of *SERPINA1* KO mice 24- and 48-hours post-IT administration of KB408. (A) Representative IF images show AAT expression in *SERPINA1* KO mice lungs at 24- and 48-hours post-IT administration of KB408. Hoechst was used to stain nuclei. Magnification is 20X. **(B)** Representative hematoxylin and eosin (H&E) staining of *SERPINA1* KO lung tissue sections reveals no visible toxicity at 24- or 48-hours post-dose. Magnification is 20X. **(C,D)** Total cell counts in the BALF at **(C)** 24- and **(D)** 48-hours post-dose of KB408 or vehicle. **(E,F)** Human AAT protein detected in the lung homogenates and BALF of *SERPINA1* KO mice at **(E)** 24- and **(F)** 48-hours post-dose of KB408 or vehicle as determined through ELISA. KB408 dose used in these experiments was 3.3 x 10^8 PFU. Data presented as the average ± SD.

Comparison of AAT Levels in the Lungs and Relevant Fluids of *SERPINA1* KO Mice After KB408 Inhalation Vs. Intravenous (IV) AAT Protein Administration

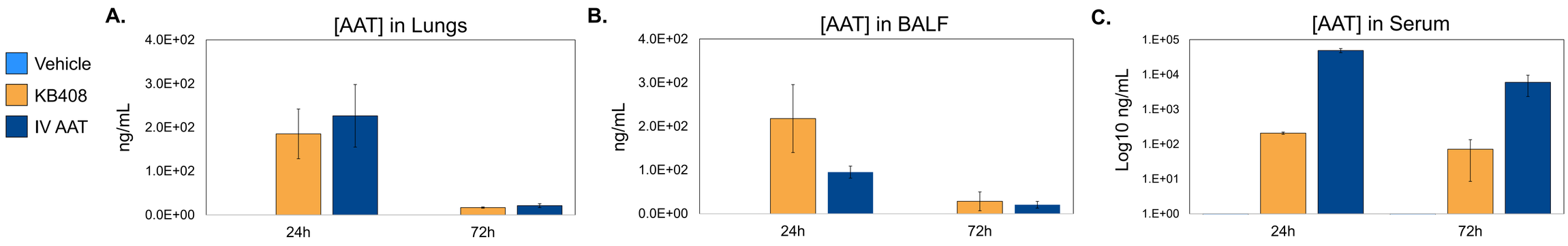


Figure 5. AAT detection in lung tissue and clinically relevant fluids of *SERPINA1* KO mice after IT administration of KB408 vs. IV injection of AAT protein. To compare between the current standard of care for AATD patients (augmentation therapy consisting of weekly IV infusions) and inhalation of KB408, *SERPINA1* KO mice were treated with a single dose of either vehicle control, KB408 via IT administration (5.8 x 10^8 PFU), or IV administration of commercially-available plasma-derived human AAT protein by tail vein injection (0.4 mg). The concentration of plasma-derived human AAT used in this study was selected based on the animal equivalent of augmentation therapy, though was slightly lower due to protein solubility and injection volume limits. AAT protein was measured by a human AAT-specific ELISA within the **(A)** lung tissue, **(B)** BALF, and **(C)** serum at 24- and 72-hours post-IT/IV administration. In lung tissue, the two approaches show comparable levels of AAT, but KB408 induced higher amounts of AAT accumulation on the lung surface as measured through BALF. IV led to higher serum levels than IT as expected due to the local delivery mechanism of KB408 inhalation. Data is presented as the average ± SD.

Conclusions

- Human AAT protein produced by KB408 transduction is biologically active and can irreversibly bind its substrate NE *in vitro*.
- KB408 effectively targets the respiratory tract when administered via inhalation in both healthy and *SERPINA1* KO immunocompetent mice, promoting secretion of human AAT into both the serum and lung lining fluid.
- Inhaled KB408 therapy resulted in higher concentrations of AAT on the lung surface as compared to a surrogate animal dose of IV augmentation therapy, with similar lung tissue exposure levels.
- KB408 is well tolerated in the airways of both healthy and *SERPINA1* KO mice with no significant toxicity.
- These observations support the development of KB408 as a novel gene therapy for the treatment of AATD-related lung disease.

Acknowledgements/Disclosures/References

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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; **6:** Black *et al.* Eur Respir J. 2008;31(5):998-1004.