Evaluation of KB409 and KB410, two HSV-1-based gene therapy vectors for the treatment of primary ciliary dyskinesia

Bruce Nmezi, Ph.D.[1]; Sara Artusi, Ph.D.[1]; Mary Jane Duermeyer, B.S.[1]; Jorge Guzman Lepe, M.D.[1]; Aiyana-Mei Tom, M.S.[1]; Trevor J. Parry, Ph.D.[1]; Suma M. Krishnan, M.S.[1]

[1]Krystal Biotech, Inc., Pittsburgh, Pennsylvania

Krystal®
ASGCT 2026 Annual Meeting
Poster #1423

Primary Ciliary Dyskinesia (PCD)

- PCD is a rare, genetically heterogeneous motile ciliopathy caused by pathogenic variants in one of at least 50 genes encoding structural and regulatory components of the ciliary axoneme.[1,2]
- PCD affects ~1 in 7,554 individuals globally and causes diverse clinical phenotypes including recurrent infections and bronchiectasis, and can ultimately progress to respiratory failure.[3,4]
- Dynein axonemal intermediate chain 1 (*DNAI1*) and dynein axonemal heavy chain 5 (*DNAH5*) encode key structural elements of the ciliary outer dynein arm and are among the most frequently reported genes with pathogenic variants in patients with PCD.[5]
- Due to the lack of approved therapies that address the underlying causes of PCD, treatment is restricted to symptomatic relief.
- Krystal Biotech, Inc. is currently developing KB409 and KB410, replication-defective, non-integrating herpes simplex virus type 1 (HSV-1)-based vectors encoding human DNAI1 and DNAH5, respectively, to be delivered via non-invasive inhalation for the treatment of PCD.

KB409 Transduction Leads to Dose-Dependent Full-Length DNAI1 Expression in Human Bronchial Epithelial Cells (HBECs) Without Cytotoxicity

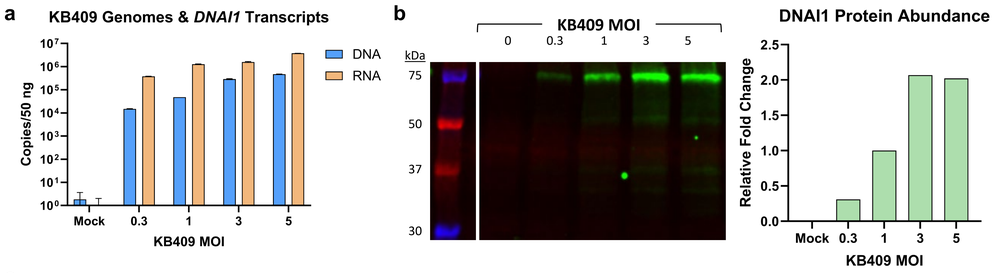

Figure 1. (**a**) Dose-dependent KB409 genomes via quantitative PCR (qPCR) and *DNAI1* transcripts via quantitative reverse-transcription PCR (qRT-PCR) in clinically relevant primary HBECs following transduction at different multiplicities of infection (MOIs). (**b**, left) Production of full-length DNAI1 protein analyzed by western blot and (**b**, right) protein quantification confirmed dose-dependent protein expression following transduction at different MOIs. Values normalized to MOI 1 for quantification. (**c**) Cell viability at 48 hours post-transduction, measured via flow cytometry, compared to mock-transduced cells or a vector control (same vector backbone as KB409 but lacking *DNAI1*). Mock viability is set to 100%.

KB410 Transduction Leads to Dose-Dependent Full-Length DNAH5 Expression in HBECs Without Cytotoxicity

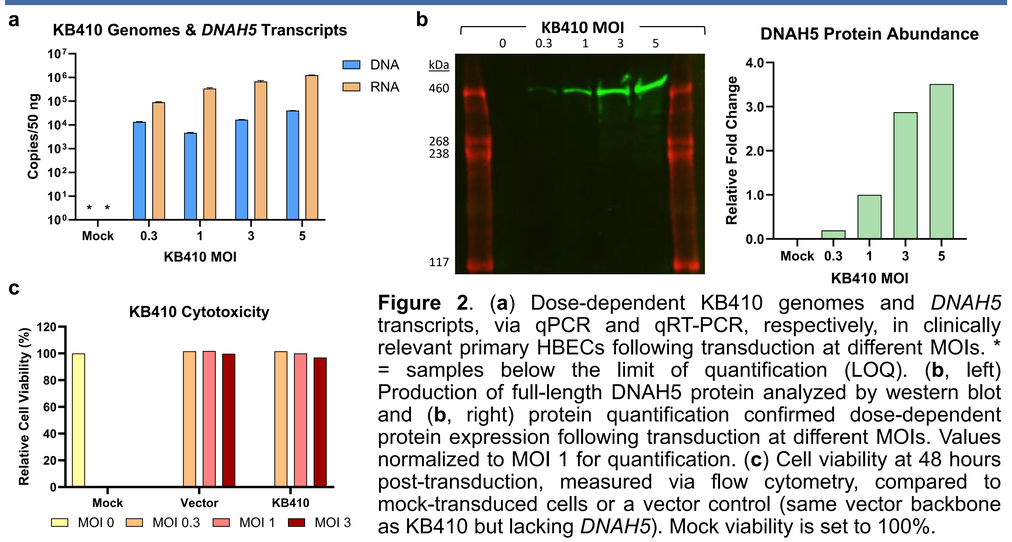

Figure 2. (**a**) Dose-dependent KB410 genomes and *DNAH5* transcripts, via qPCR and qRT-PCR, respectively, in clinically relevant primary HBECs following transduction at different MOIs. * = samples below the limit of quantification (LOQ). (**b**, left) Production of full-length DNAH5 protein analyzed by western blot and (**b**, right) protein quantification confirmed dose-dependent protein expression following transduction at different MOIs. Values normalized to MOI 1 for quantification. (**c**) Cell viability at 48 hours post-transduction, measured via flow cytometry, compared to mock-transduced cells or a vector control (same vector backbone as KB410 but lacking *DNAH5*). Mock viability is set to 100%.

Dose-Ranging Intratracheal (IT) Administration of KB409 Results in Rapid Transduction and Localized Expression of DNAI1 in Wild-Type (WT) Mice with Minimal Toxicity

a

Treatment (50 µL/ mouse)	Dose/Mouse
Vehicle Control	PBS/ 5% Glycerol
Dose 1	6.25 × 10^6 PFU
Dose 2	2.50 × 10^7 PFU
Dose 3	1.00 × 10^8 PFU
Dose 4	4.00 × 10^8 PFU

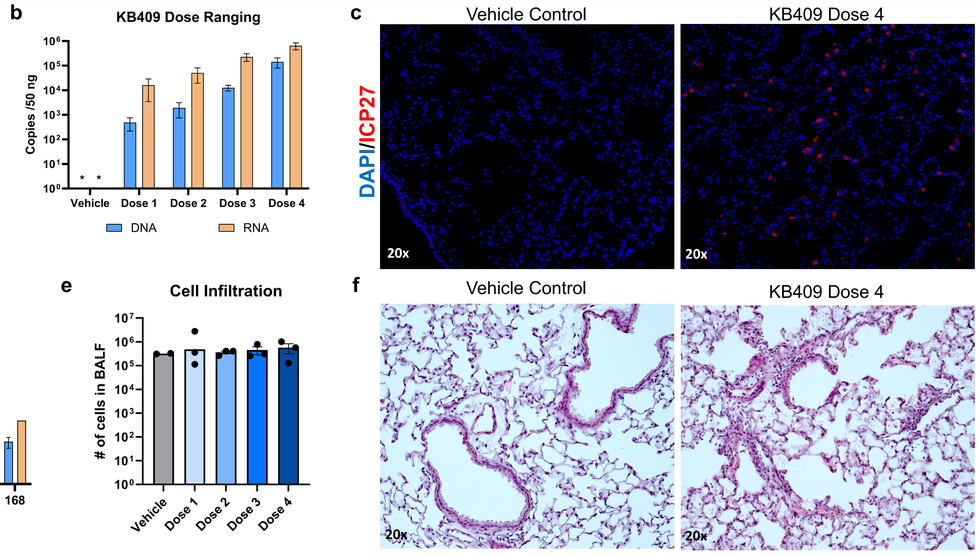

Figure 3. (**a**) A range of KB409 doses were tested via (IT) administration to WT mice. (**b**) KB409 genomes and *DNAI1* transcripts from a range of IT KB409 doses measured via qPCR and qRT-PCR, respectively. * = samples below the LOQ. (**c**) Immunofluorescence (IF) staining revealed localization of KB409-expressed HSV-1 protein ICP27 (red) to the lung parenchyma of IT-treated mice. DAPI nuclei staining is shown in blue. (**d**) Transduction of KB409 genomes and expression of *DNAI1* transcripts measured via qPCR and qRT-PCR, respectively, over 7 days post-IT dosing with 2 × 10^8 plaque forming units (PFU) KB409. (**e**) Cell infiltration post-IT dosing measured via analysis of bronchoalveolar lavage fluid (BALF). (**f**) Representative images of hematoxylin and eosin (H&E)-stained lung sections from IT KB409-treated mice. No significant accumulation of KB409 was observed in blood and non-targeted tissues. PBS, phosphate-buffered saline.

Dose-Ranging IT Administration of KB410 Results in Localized Expression of DNAH5 in WT Mice with Minimal Toxicity

a

Treatment (50 µL/ mouse)	Dose/Mouse
Vehicle Control	PBS/ 5% Glycerol
Low Dose	2.80 × 10^7 PFU
Mid Dose	1.13 × 10^8 PFU
High Dose	4.50 × 10^8 PFU

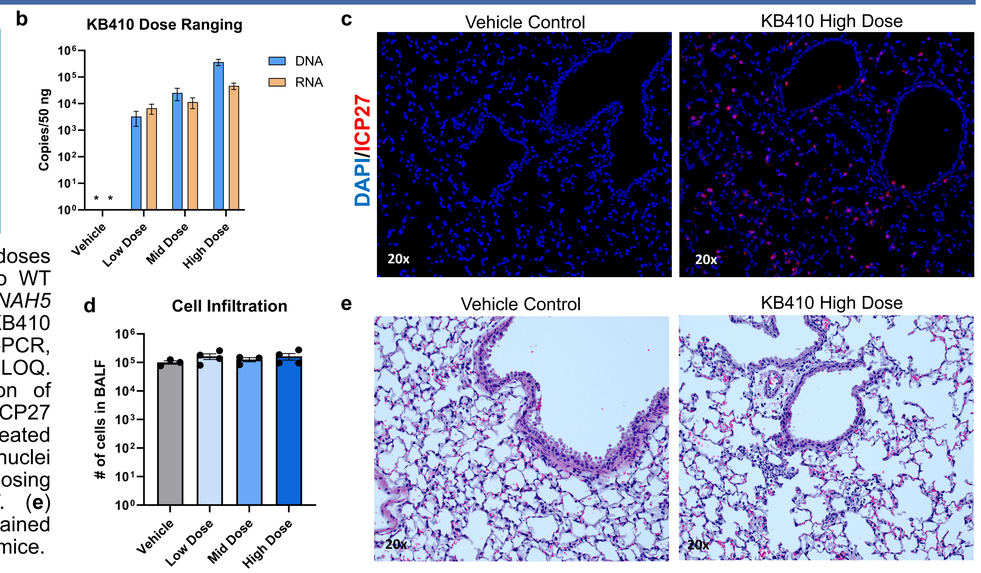

Figure 4. (**a**) A range of KB410 doses were tested via IT administration to WT mice. (**b**) KB410 genomes and *DNAH5* transcripts from a range of IT KB410 doses measured via qPCR and qRT-PCR, respectively. * = samples below the LOQ. (**c**) IF staining revealed localization of KB410-expressed HSV-1 protein ICP27 (red) to the lung parenchyma of IT-treated mice; DAPI was used to stain nuclei (blue). (**d**) Cell infiltration post-IT dosing measured via analysis of BALF. (**e**) Representative images of H&E-stained lung sections from IT KB410-treated mice.

Conclusions

KB409 and KB410 are able to transduce and express full-length DNAI1 and DNAH5, respectively, with minimal toxicity both in cell culture and in murine lungs, supporting continued development of both vectors as inhaled therapeutics for patients with PCD.

Acknowledgements, Disclosures, and References

These studies were funded by Krystal Biotech, Inc. (Krystal). All animal studies were performed in an AAALAC accredited facility and protocols were IACUC approved prior to initiation. Krystal would like to thank Hilltop Laboratory Animals, Inc. for their contributions to the work presented here. All authors are current or former employees of Krystal. We would like to acknowledge Dr. Jada George (Krystal) for drafting this poster. **References**: 1. Horani A, *et al*. Paediatr Respir Rev. 2016 Mar;18:18-24. 2. Despotes KA, *et* al. Cells. 2024 Jun 4;13(11):974. 3. Hannah WB, *et al*. Lancet Respir Med. 2022 May;10(5):459-468. 4. Shapiro AJ, *et al*. Pediatr Pulmonol. 2016 Feb;51(2):115-32 .5. Djakow J, *et al*., Pediatr Pulmonol. 2012 Sep;47(9):864-75.