# Nonclinical Pharmacology of Nebulized KB407 for the Treatment of Cystic Fibrosis

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## Introduction

- Small-molecule modulators targeting specific classes of cystic fibrosis transmembrane conductance regulator (CFTR) protein dysfunction have been the focus of recent drug development, thereby targeting only a subset of the CF population[1,2]
- Regrettably, no mutation-agnostic corrective therapy is approved
  - To this end, KB407, a replication-defective gene therapy vector encoding full-length human CFTR, has been engineered for the treatment of cystic fibrosis (CF)
  - Previous *in vitro* and *ex vivo* studies demonstrated KB407 transduction efficiency and effector expression, localization, and activity in 2-D cell culture of primary CF patient small airway epithelial cells and in 3-D cell culture of CF patient-derived intestinal organoids (PDOs)[3]
- Here, KB407 was investigated to determine whether it is safe for, and amenable to, non-invasive inhaled administration upon single and repeat dosing, including in a good laboratory practice (GLP) toxicology study in non-human primates (NHPs)

## Materials and Methods

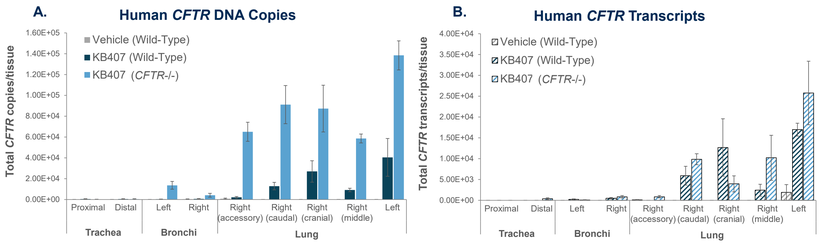
- Test Article - KB407, Krystal Biotech, Inc.'s proprietary replication-incompetent, non-integrating HSV-1-based vector encoding two copies of full-length human *CFTR*

## Results: *In vivo* Proof-of-Concept Study of KB407 Inhaled Administration

**Table 1. Experimental design for KB407 nebulization in immunocompetent mice**

| Treatment | Dose Duration (minutes) | Mouse strain | Number | Necropsy |
|---|---|---|---|---|
| Vehicle | 60 | C57BL/6 mice | 4 | 48 hours |
| KB407 | 60 | C57BL/6 mice | 4 | |
| KB407 | 60 | Cftr$^{tm\ 1Unc}$ Tg(FABPCFTR)1 Jaw/J mice (*CFTR-/-*) | 4 | |

**Figure 1. Transduction and human *CFTR* expression in the airways of wild-type and *CFTR-/-* mice following nebulization of KB407**

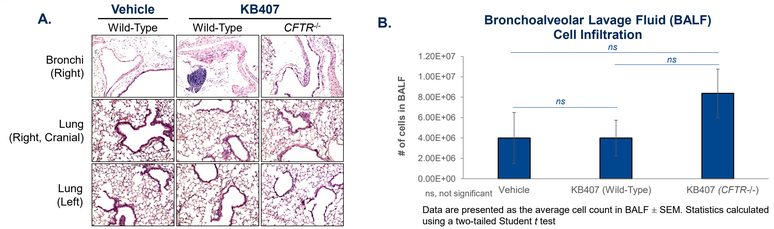



Data are presented as the average of two tissue samples (two replicates/tissue) ± standard error of the mean (SEM).

- KB407 can be effectively aerosolized and capably infects relevant airway epithelia after nebulization
  - KB407 was below the limit of detection in all blood samples tested

## Results: *In vivo* Proof-of-Concept Study of KB407 Inhaled Administration (Cont'd)

**Figure 2. Evaluation of airways following nebulization of KB407**




Data are presented as the average cell count in BALF ± SEM. Statistics calculated using a two-tailed Student *t* test

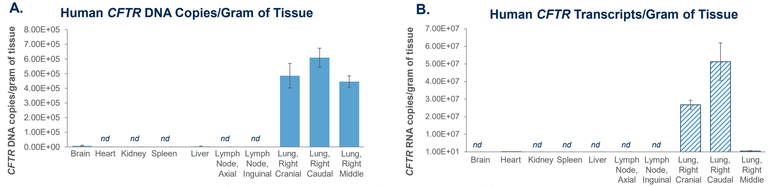
- **Figure 2A** shows representative hematoxylin and eosin–stained airway tissue samples from the indicated animals after nebulization of KB407 or negative control (vehicle)
- **Figure 2B** shows BALF harvested 48 hours post infection.
- No obvious signs of immune cell invasion, fibrosis, or necrosis were detected in any KB407-dosed tissues, as compared to vehicle- treated mice, indicating the safety of the vector after nebulization
- No significant differences in cell infiltration into the lungs were noted between vehicle and KB407 administration groups

## Results: *In vivo* Tolerability and Biodistribution of Repeat-Dose KB407 Inhaled Administration

**Table 2. Experimental design for KB407 repeat-dose nebulization: tolerability and biodistribution in an NHP**

| Time point | Treatment | Dose Duration, min | Study Timeline |
|---|---|---|---|
| Day 1 | Vehicle | 90 | Vehicle, KB407 (low dose), KB407 (high dose), Day 0, Day 19, Blood Collections, Tissue Harvest, Necropsy 48 hours after high-dose KB407 |
| Day 5 | KB407 | 54 | |
| Day 17 | KB407 | 90 | |

**Figure 3. KB407 biodistribution and human *CFTR* expression in NHP tissues 48 hours following repeat dosing**




Data are presented as the average of two replicates/tissue ± SEM. nd, not detected

- KB407 capably infects airway tissue of NHPs and expresses its encoded human transgene
- All blood samples were below the limit of detection for the assay, suggesting that the vector was restricted to the airways without significant dissemination into the circulatory system
- No abnormal cage-side or clinical observations were noted, and no changes in food consumption or body weight were found during the dosing period, indicating that repeated dosing of KB407 was well tolerated

## Results: Repeat-dose GLP Toxicology Study

**Table 3. Experimental design for KB407 repeat-dose nebulization: NHP GLP toxicology study**

| Group | Dose Duration, min | Average Dose Deposited in Lungs (PFU/administration) | Dosing Days | Main Study | | | Recovery (4 Weeks) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | M | F | Necropsy Day | M | F | Necropsy Day |
| Air | 90 | - | 1, 8, 15 | 3 | 3 | 16 | - | - | 43 |
| Vehicle | 90 | - | 1, 8, 15 | 3 | 3 | | 2 | 2 | |
| KB407 (low) | 18 | 1.81E+8 (M)<br>2.33E+8 (F) | 1, 8, 15 | 3 | 3 | | 2 | 2 | |
| KB407 (high) | 90 | 1.43E+9 (M)<br>2.11E+9 (F) | 1, 8, 15 | 3 | 3 | | 2 | 2 | |

M, male; F, female.

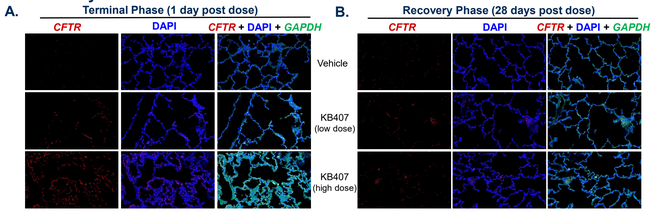
**Table 4. Findings from toxicity assessment of KB407 repeat dose**

| Treatment Group | Macroscopic[a] | | Microscopic | | Systemic[b] | | Pulmonary Function[c] | |
|---|---|---|---|---|---|---|---|---|
| | Main Study | Recovery | Main Study | Recovery | Main Study | Recovery | Main Study | Recovery |
| KB407 (low)<br>KB407 (high) | None | None | • Mild mononuclear or mixed cell infiltrates in lungs[d]<br>• Minimal to mild neutrophilic infiltration of lumen in nasal turbinates[e] | Compared to main study findings, infiltrates reduced in incidence and/or severity | None | None | None | None |

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight changes, and clinical and anatomic pathology.
[a]Macroscopic findings include identification of changes at necropsy inclusive of organ weight changes. [b]Systemic adverse findings inclusive of observations of mortality, body weight change, health/well-being. [c]Parameter measurements include breathing rate (breaths/min), tidal volume, minute volume (mL/min) obtained within 24 hours after the first dose, and after last dose (main study dosing period), and within 3 days of recovery necropsy (recovery study period); average of all measurements for the interval was calculated for each animal. [d]Both low- and high-dose groups; males and females. [e]Low dose, 3/3 females; high dose, 1/3 females; finding not observed in male animals.

- Effects for both doses were considered non-adverse due to the mild severity of findings and the lack of impact on the health and well-being of animals. Mild inflammation observed was determined to be reversible after cessation of dosing
- The no-observed-adverse-effect level ( NOAEL) was determined to be the high dose (1.43E+9 to 2.11E+9 deposited plaque-forming units ( PFU) in the lungs per administration)

**Figure 4. Persistence of KB407-encoded human *CFTR* transcripts in the lungs of dosed NHPs, as assessed by fluorescence in situ hybridization**




- KB407-encoded *CFTR* transcripts are present at both main study terminal phase and recovery phase in the lungs of dosed NHPs (probes specific for human *CFTR* or monkey glyceraldehyde-3-phosphate dehydrogenase(*GAPDH*))

## Conclusions

- **Inhalation of nebulized KB407 efficiently transduces airway epithelial tissue and directs localized expression of human CFTR in mice and NHPs**
- **No significant toxicity or systemic vector distribution was observed in immunocompetent animals upon single or repeated exposure to aerosolized KB407**
- **The NOAEL was determined to be the high dose of KB407 due to the lack of impact on the health and well-being of animals in the repeat-dose GLP toxicology study**
- **Taken together, these data provide strong support for the potential of inhaled administration of KB407 as a broadly applicable gene therapy for the treatment of CF**

## References

1. Clancy JP, Jain M. Personalized medicine in cystic fibrosis: dawning of a new era. *Am J Respir Crit Care Med*. 2012;186(7):593-597.
2. Clancy JP, Cotton CU, Donaldson SH, et al. CFTR modulator theratyping: current status, gaps and future directions. *J Cyst Fibros*. 2019;18(1):22-34.
3. Freedman C, Parry T, Agarwal P, et al. In vitro pharmacology of KB407, an HSV-1-based gene therapy vector, for the treatment of cystic fibrosis. ASGCT 23rd Annual Meeting; May 12-15, 2020.

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T. Parry, N. Reitze, S. Banerjee, N. Sarma, and S. Krishnan are current employees of Krystal Biotech, Inc.