Respiratory cell-type affinity and absolute *CFTR* expression in the primate airway upon nebulization of KB407

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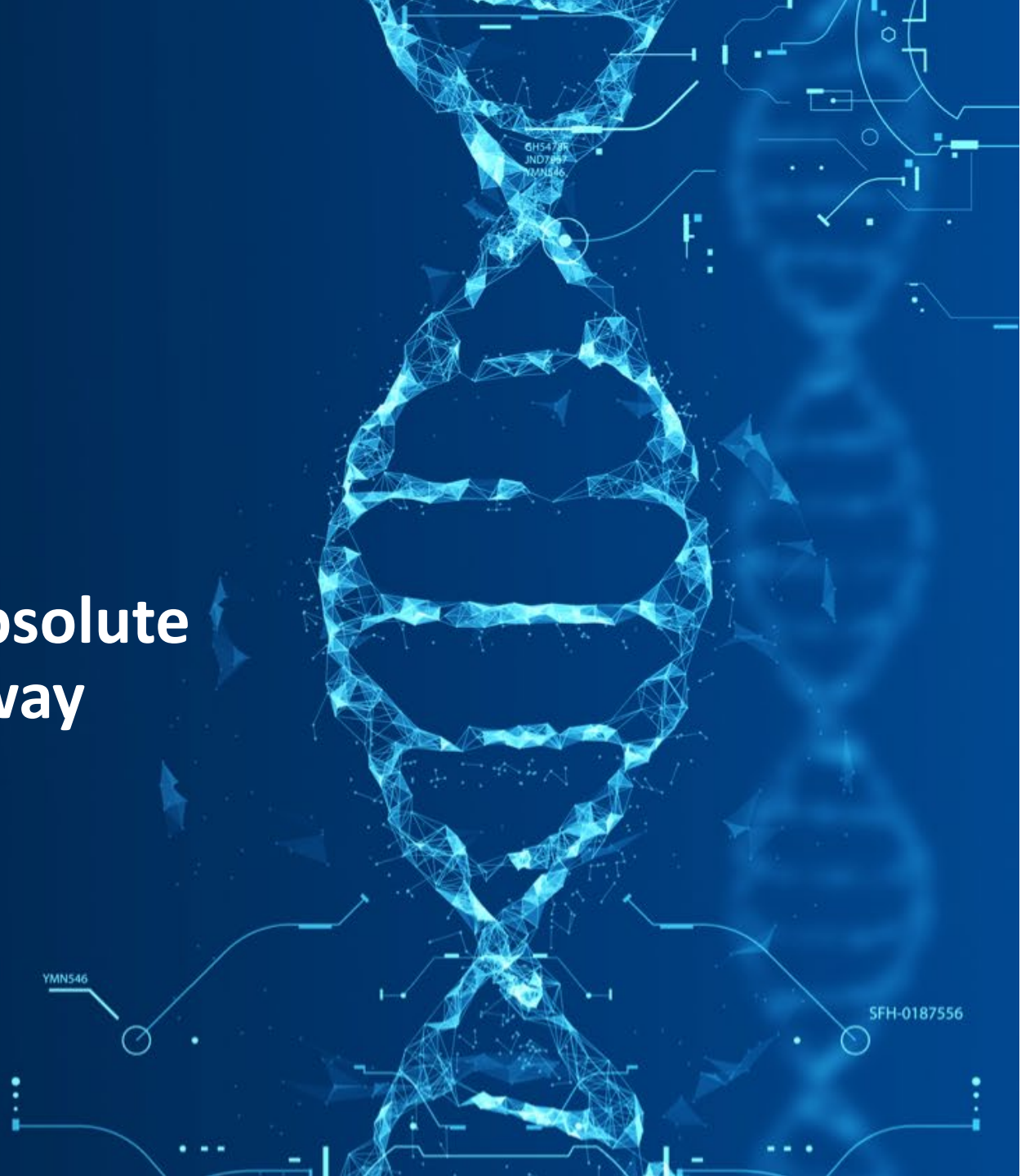


Disclosures

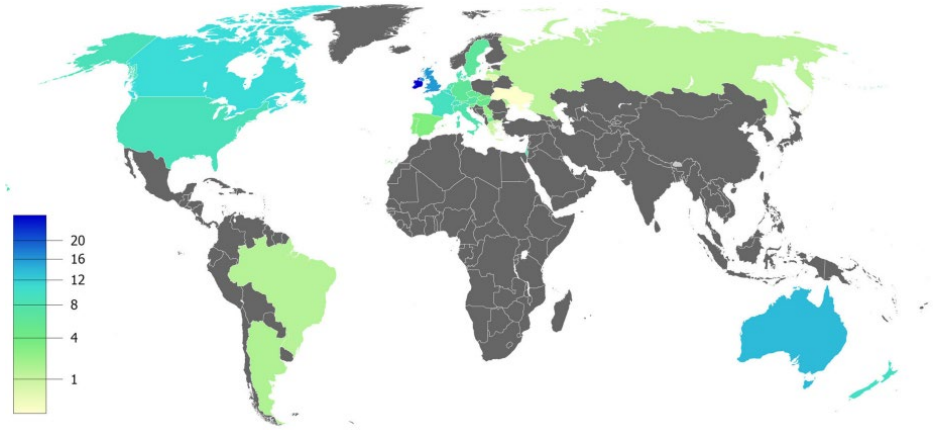
T. Parry, S. Artusi, J. Guzman-Lepe, M. Duermeyer and S. Krishnan are employees of, and have equity interest in, Krystal Biotech, Inc.

Cystic Fibrosis: Significant Unmet Need Despite Recent Approvals

Approximately 10% of CF patients have mutations that are not amenable to current small molecule approaches

Cystic Fibrosis

- Known as a life-threatening inherited disease, with an incidence of ~1/2,500 live births, affecting ~80,000 people worldwide[1]
- It is autosomal recessive, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), leading to reduced and/or loss of CFTR function[2-4]
- Progressive lung disease is the primary cause of morbidity and mortality where the loss of CFTR-mediated chloride and bicarbonate transport leads to airway mucus obstruction, recurrent bacterial infection, and inflammation[5]



Estimated prevalence of cystic fibrosis per 100,000 habitants[6]

Unmet need remains significant despite recent approvals

- Small molecule correctors work by improving the functions of mutated CFTR; however, they only restore ~50% of protein function in patients with certain amenable mutations
- These therapies are ineffective in the ~10% patients with mutations that do not produce any CFTR protein (null mutations)
- Suboptimal efficacy or tolerability issues remain even in those responsive to therapies

CF Prevalence & Incidence[1,6,7]

~80,000 patients with CF worldwide

~30,000 patients in US CF registry

~1,000 new cases of CF diagnosed each year in the US

1. Middleton PG et al., *NEJM* 2019;381(19): 1809-1919; 2. O'Sullivan BP et al., *Lancet* 2009;373:1891-904; 3. Elborn JS et al., *Lancet* 2016; 388:2519-31; 4. Sanders DB et al., *Pediatr Clin North Am* 2016;63:567-84; 5. Stoltz DA et al., *NEJM* 2015, 372 (4): 351-362; 6. Lopes-Pacheco M, Front. *Pharmacol.* 2016; 7:275; 7. US Cystic Fibrosis Foundation.
CF, cystic fibrosis.

KB407: A Differentiated Vector

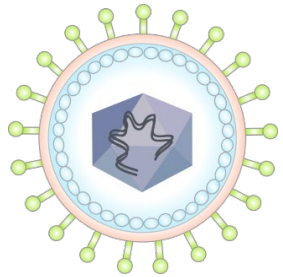
An investigational inhaled gene therapy designed with the ability to redose

Herpes Simplex Virus Type 1 (HSV-1) as a Gene Delivery Platform

	HSV-1
In vivo dosing	**Yes**
Potential baseline neutralizing immunity	**No**
Repeat-dose capabilities	**Yes**
Carrying capacity	**≥30 kb**
Integrates payload into host cell DNA	**No**
Efficiency of delivering genetic cargo	**High**
Regulatory precedent	**Yes**

- HSV-1 is a well characterized virus, highly prevalent in the human population, with some estimates suggesting at least 67% of the US population ≥12 years have been exposed to HSV-1[1]
- HSV-1 vectors efficiently transduce cells; their genomes remain episomal without integrating into host DNA[2,3], thus avoiding risks of insertional mutagenesis
- Additional benefit of the HSV-1 vectors include large payload capacities exceeding 30 kb and its natural property to resist immune clearance[4-6]

KB407



- Based on Krystal's differentiated HSV-1 vector platform that has been clinically validated in a Phase 3 study in dystrophic epidermolysis bullosa (NCT04491604)
- Engineered to be replication defective with reduced cytotoxicity
- Encodes two full-length copies of human *CFTR*
- Duration of nebulization <30 minutes
- Episomal delivery of *CFTR* transgene does not disrupt host cell DNA
- Ability to redose and/or adjust dose over time as lung cells turnover

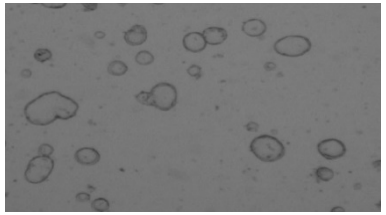
1. Xu F, et al. *J Infect Dis*. 2002;185(8):1019–24; 2. Heldwein EE, Krummenacher C. *Cell Mol Life Sci*. 2008;65(11):1653-68; 3. Goins WF, et al., Engineering HSV-1 Vectors for Gene Therapy, in Herpes Simplex Virus: Methods and Protocols, J.R. Diefenbach and C. Fraefel, Editors. 2014, Springer New York:New York, NY. p. 63-79; 4. Tognarelli EI, et al. *Front Cell Infect Microbiol*. 2019;9:127; 5. Yang L, et al. *Front Immunol*. 2019;10:2196; 6. Oldham ML, et al. *Nature*. 2016;529(7585):537-40.
HSV-1, herpes simplex virus type 1

KB407 Corrected CFTR Defect in 3D Patient-Derived Intestinal Organoids

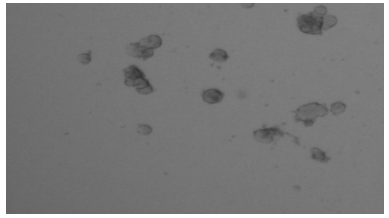
Restoration of normal cystic organoid morphology occurs irrespective of underlying CFTR mutation

Reference Images

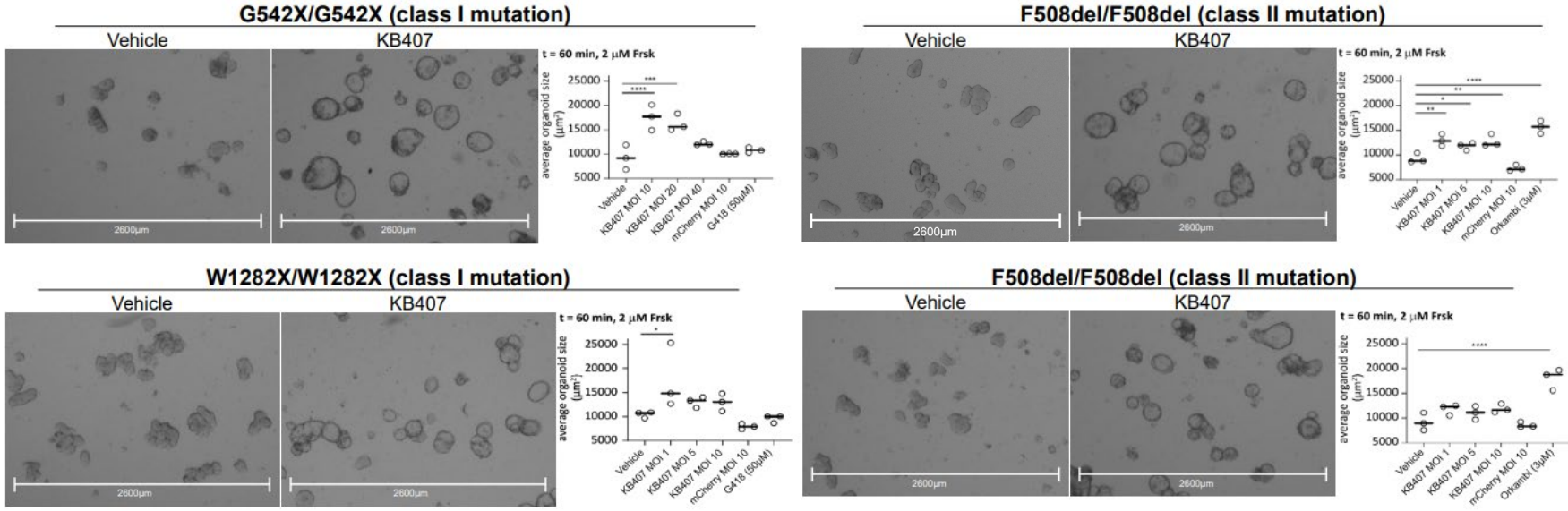
Healthy PDOs



CF PDOs



***Ex Vivo* KB407 Dose-Ranging and Pharmacodynamics in 3D Organotypic Cultures**



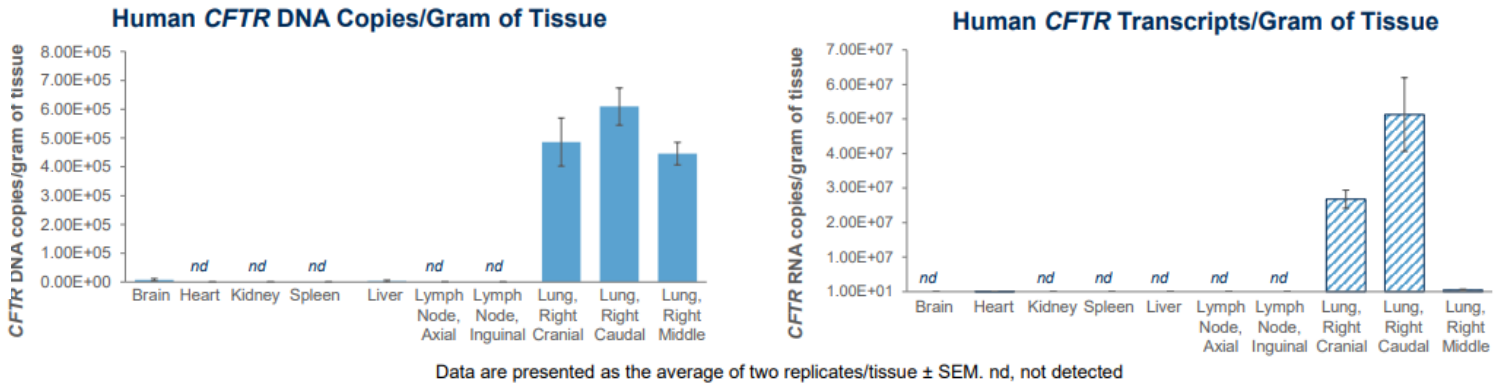
- In healthy patient derived organoids (PDOs, top left), CFTR protein functions properly and enables water transport across the membrane leading to plump, round appearing PDOs
- In PDOs derived from CF patients (bottom left, center, and right), CFTR does not work properly and water is not transported causing PDOs to appear shrunken or shriveled
- Transduction by KB407 leads to a striking restoration of normal cystic organoid morphology even at the lowest MOI tested within 24 hours of transduction, irrespective of the underlying CFTR mutation
- KB407 also found to transduce primary CF patient derived small airway epithelial cells in a dose-dependent manner; the vector efficiently produces functional, full-length CFTR protein that properly traffics to the cell membrane

Freedman C, et al. Poster at the ASGCT 2020 Annual Meeting. Virtual. May 12-15, 2020.
CF, cystic fibrosis; MOI, multiplicity of infection; PDOs, patient derived organoids.

Nebulized KB407 in Nonhuman Primates (NHPs)

Repeat doses of KB407 well tolerated and broadly distributed throughout lung tissue in NHPs

KB407 biodistribution & 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

- No abnormal cage-side of clinical observations
- No changes in food consumption, bodyweight, or behavior during dosing period
- KB407 was distributed throughout airways, including the bronchioles and alveoli, with little-to-no vector detected in all other tissues
- All blood samples below the limit of detection for vector at all timepoints

Parry T, et al. Poster #541 at the *2021 North American Cystic Fibrosis Conference (NACFC)*. Virtual. November 1-5, 2021.
NHPs, nonhuman primates.

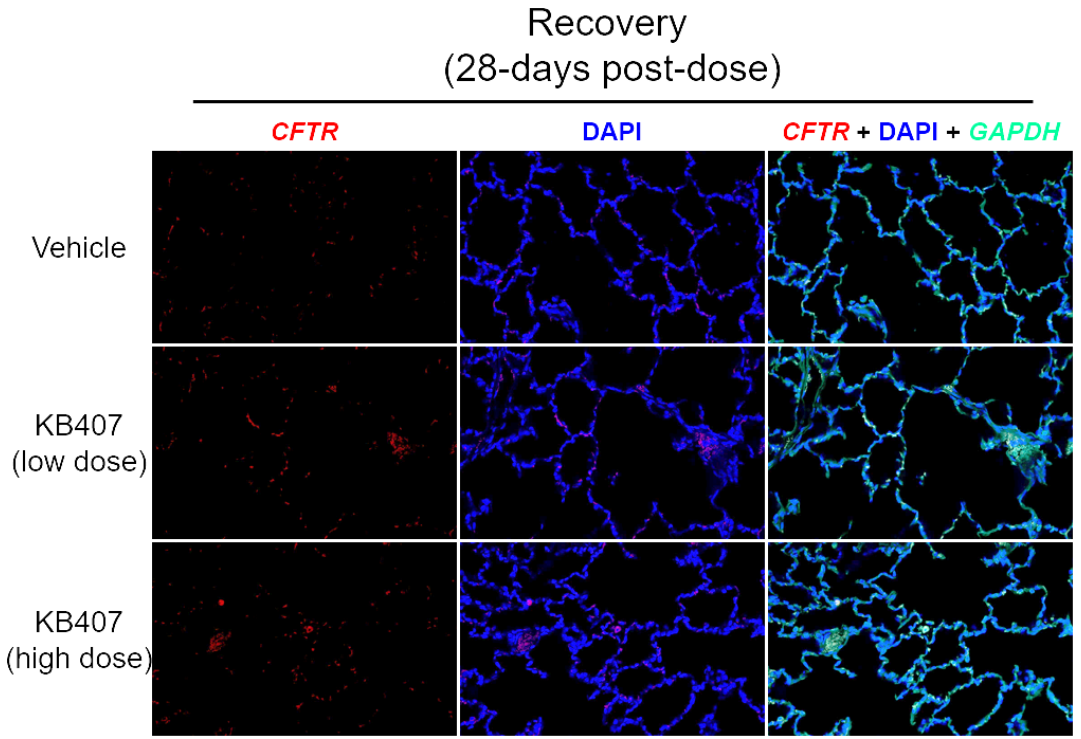
KB407 Repeat Dose (Weekly) GLP Toxicology Study in NHPs

Study Design

Group	n	Duration of Exposure (minutes)	Avg. Dose Deposited in Lungs (PFU/administration)	Dosing Days	Necropsy Days
Air	6	90	-	1, 8, 15	16
Vehicle	10	90	-	1, 8, 15	16, 43
Low Dose KB407	10	18	1.81×10^8 (male)	1, 8, 15	16, 43
			2.33×10^8 (female)		
High Dose KB407	10	90	1.43×10^9 (male)	1, 8, 15	16, 43
			2.11×10^9 (female)		

Findings: NOAEL was determined to be the high dose

- No toxicity based on mortality, cage side/clinical observations, body weights, and clinical and anatomic pathology
- No changes in tidal volume, respiratory frequency, or minute volume at any dose level
- Mild mononuclear or mixed cell infiltrates in lungs and minimal to mild neutrophilic infiltration in nasal turbinates
- Effects were considered non-adverse due to the mild severity, lack of impact on health, and reversible on recovery



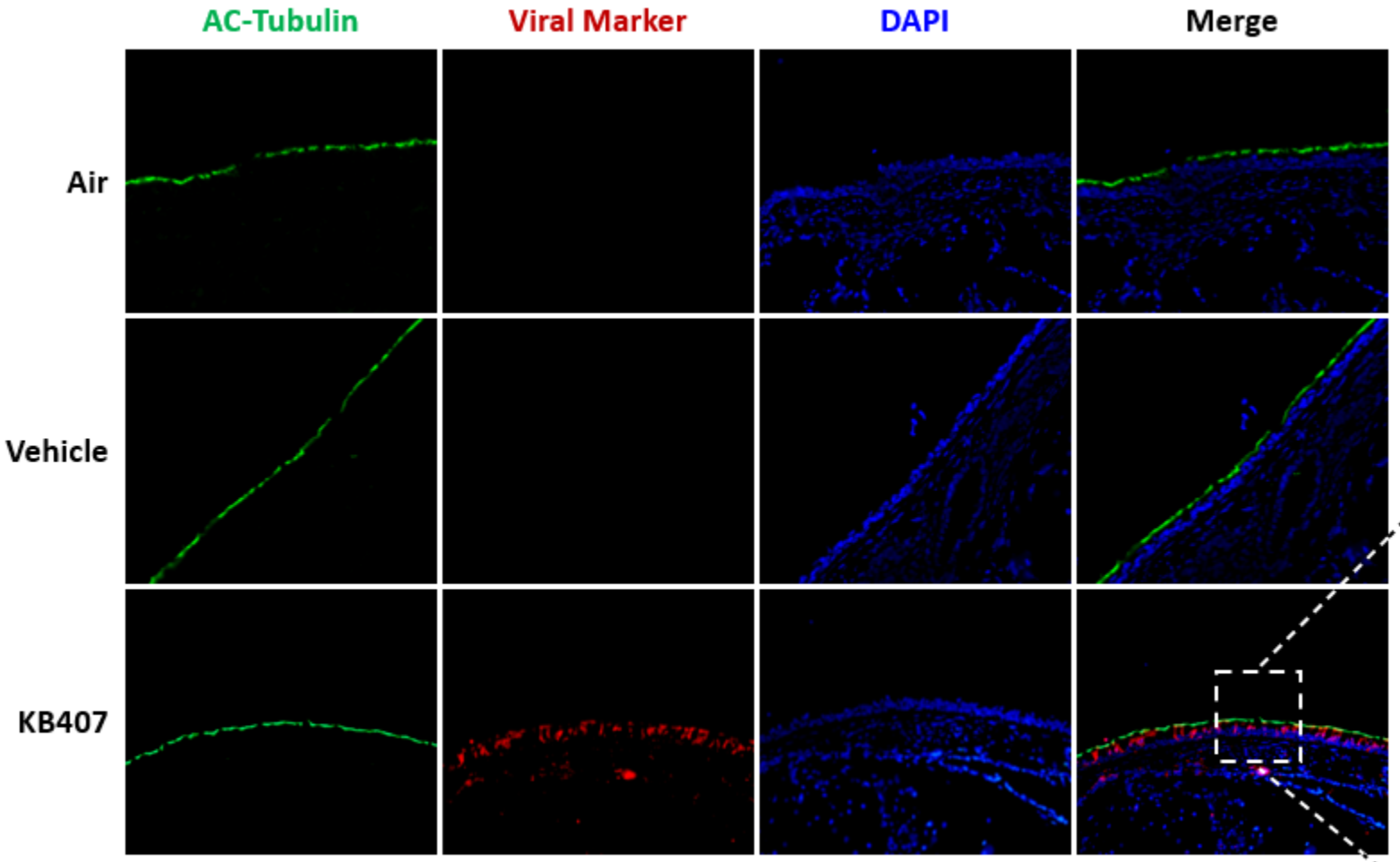
Fluorescent in situ hybridization

- Lung samples harvested 28 days after the last dose demonstrate persistence of the vector and CFTR expression

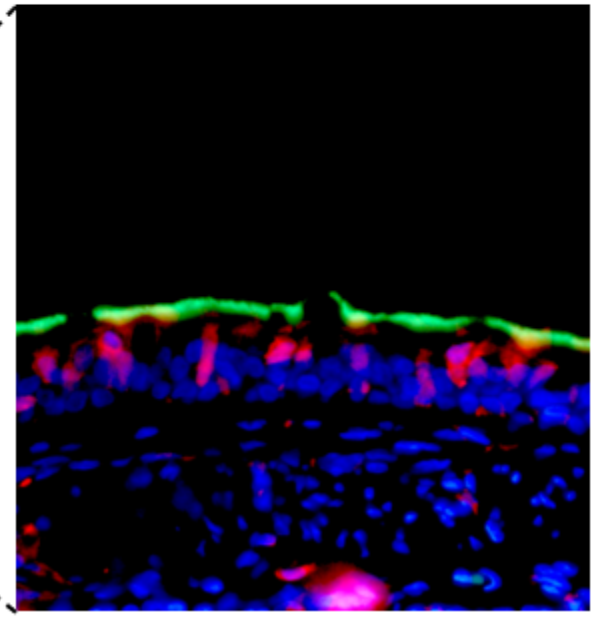
Parry T, et al. Poster #541 at the *2021 North American Cystic Fibrosis Conference (NACFC)*. Virtual. November 1-5, 2021.
GLP, good laboratory practice; NHPs, nonhuman primates; NOAEL, no observed adverse effect level.

KB407 Cell-Type Affinity in NHP Lungs

Ciliated cells (AC-Tubulin⁺), 24-hours after last dose administered (Day 16 of GLP toxicology study)



- A majority of KB407-positive respiratory epithelium was identified as ciliated cells, consistent with the observation that ciliated cells are the predominant cell type found in the conducting airways[1]

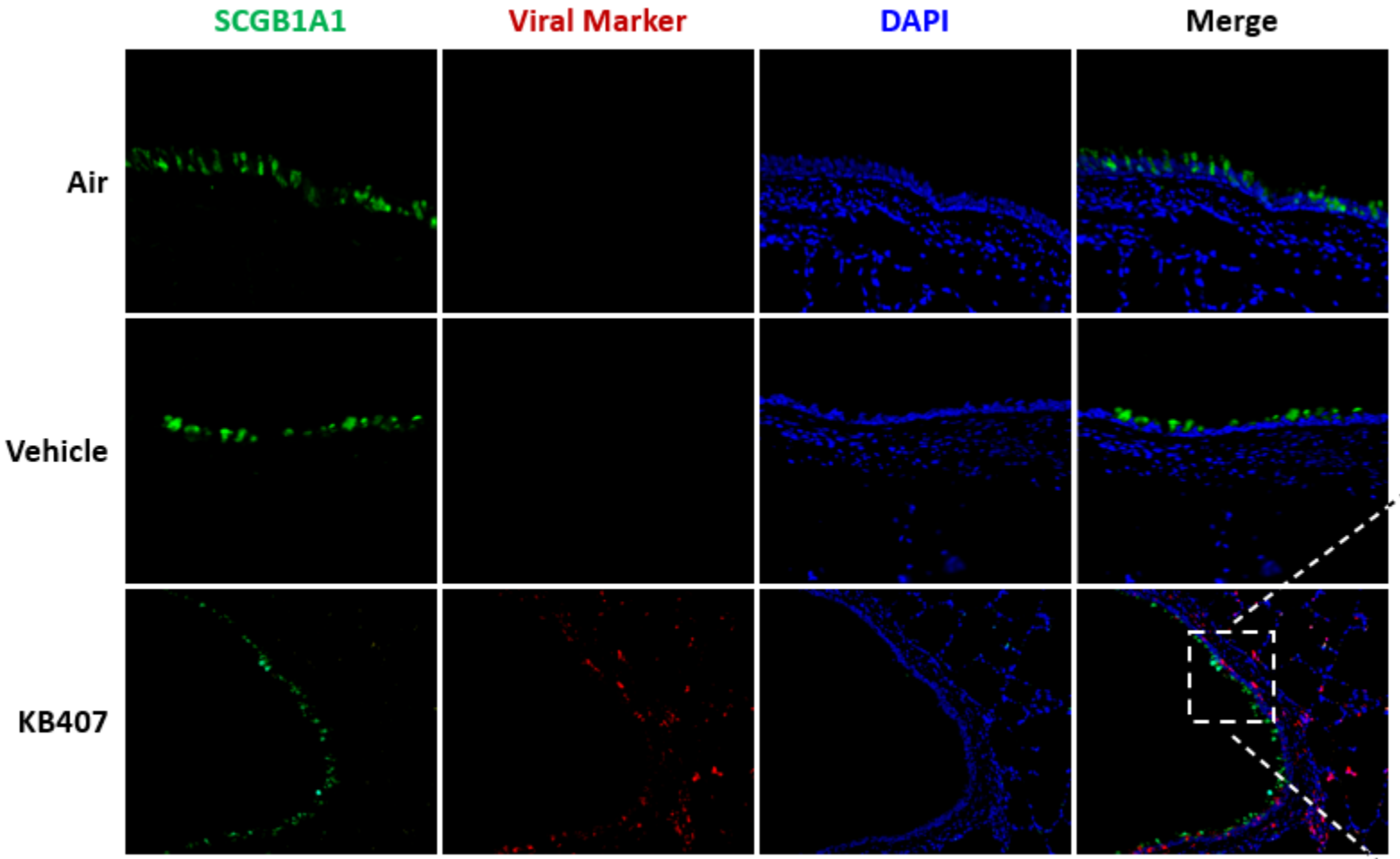


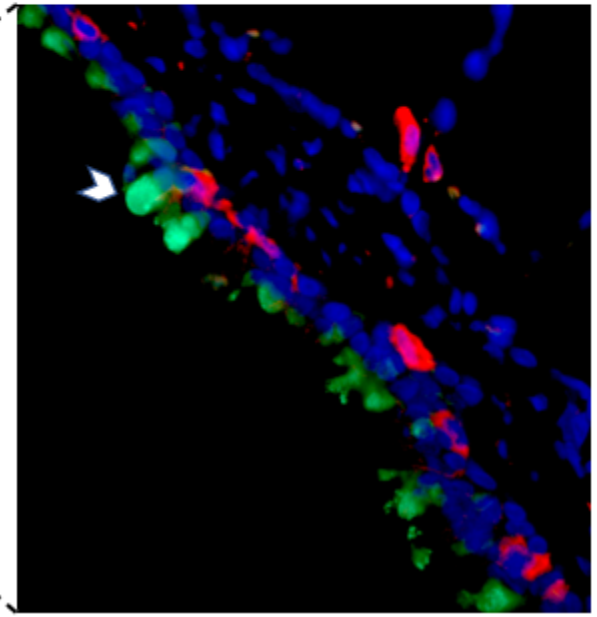
59.6% (298/500) of AC-Tubulin⁺ cells were KB407⁺ (10 fields of view)

1. Okuda, K. et al. *Am J Respir Crit Care Med.* 2021 203(10): 1275-1289.
GLP, good laboratory practice; NHP, nonhuman primate.

KB407 Cell-Type Affinity in NHP Lungs

Club cells (SCGB1A1⁺), 24-hours after last dose administered (Day 16 of GLP toxicology study)





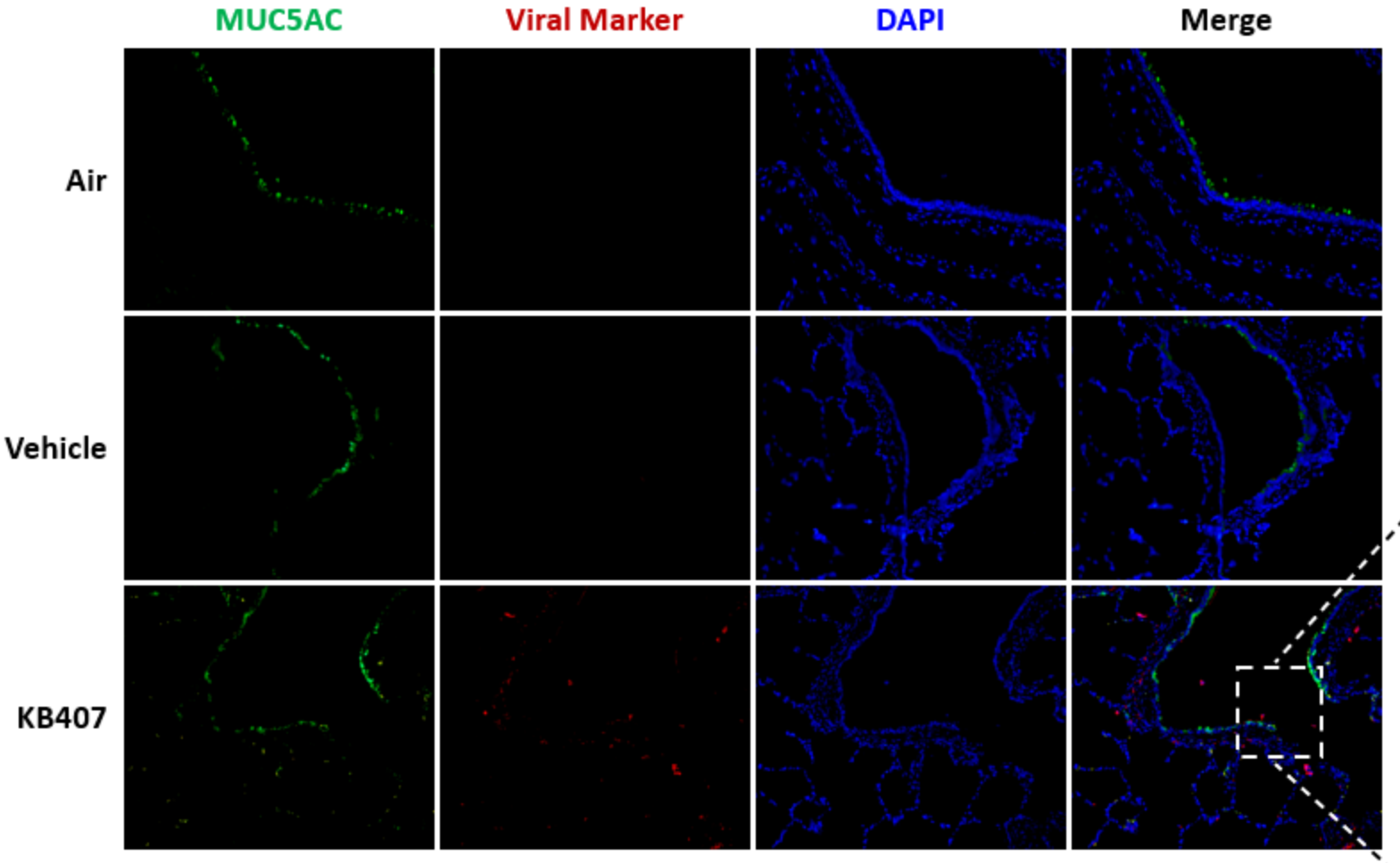
- KB407-positive club cells identified in the airways of NHPs after repeated nebulization

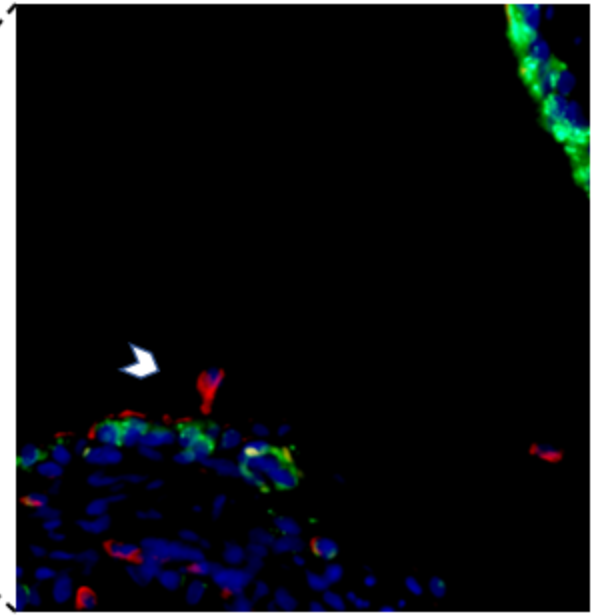
17.4% (38/218) of SCGB1A1⁺ cells were KB407⁺ (10 fields of view)

GLP, good laboratory practice; NHP, nonhuman primate.

KB407 Cell-Type Affinity in NHP Lungs

Goblet cells (MUC5AC$^+$), 24-hours after last dose administered (Day 16 of GLP toxicology study)





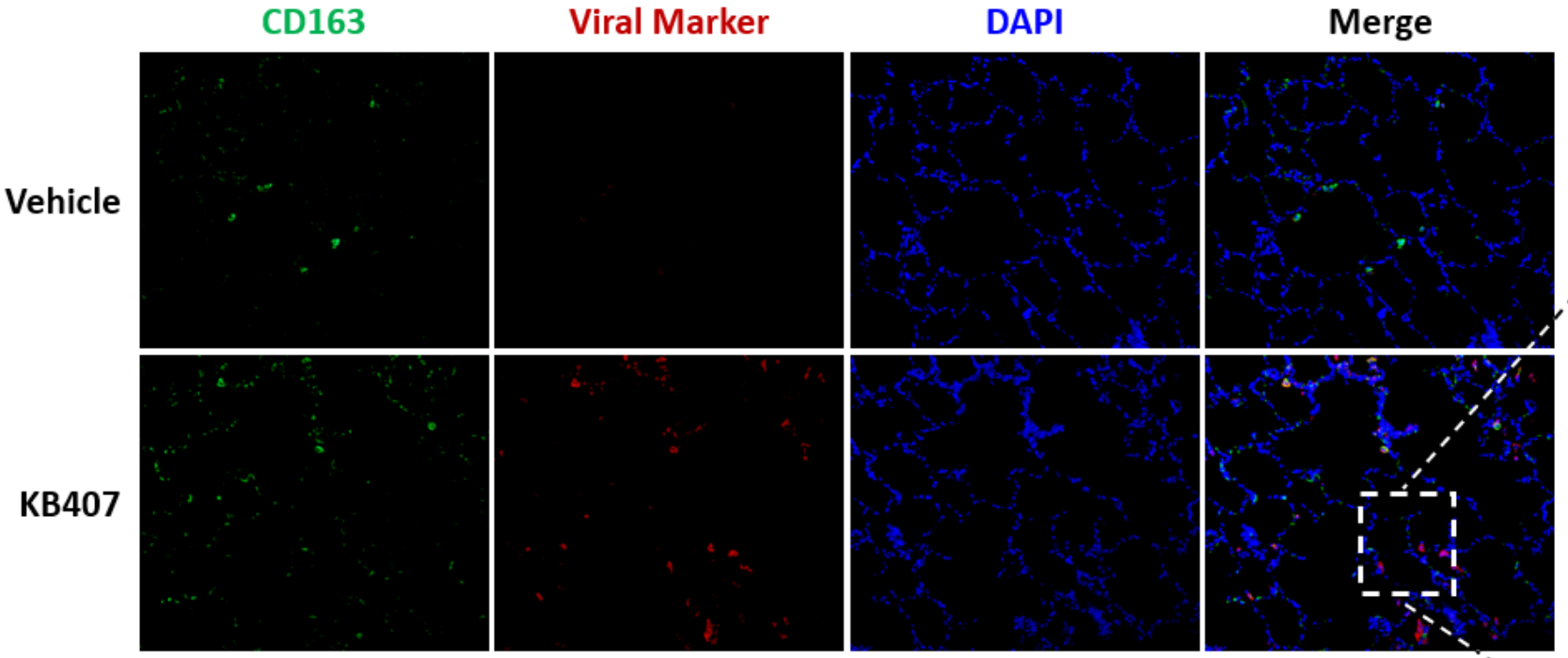
- KB407-positive goblet cells also observed in the airways of NHPs after repeated nebulization

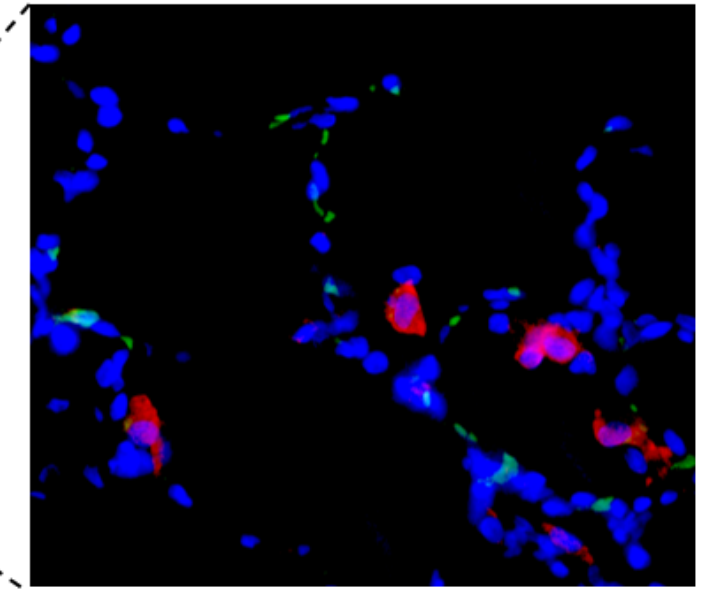
8.0% (8/100) of MUC5AC$^+$ cells were KB407$^+$ (10 fields of view)

GLP, good laboratory practice; NHP, nonhuman primate.

KB407 Cell-Type Affinity in NHP Lungs

Macrophages (CD163^{+}), 24-hours after last dose administered (Day 16 of GLP toxicology study)





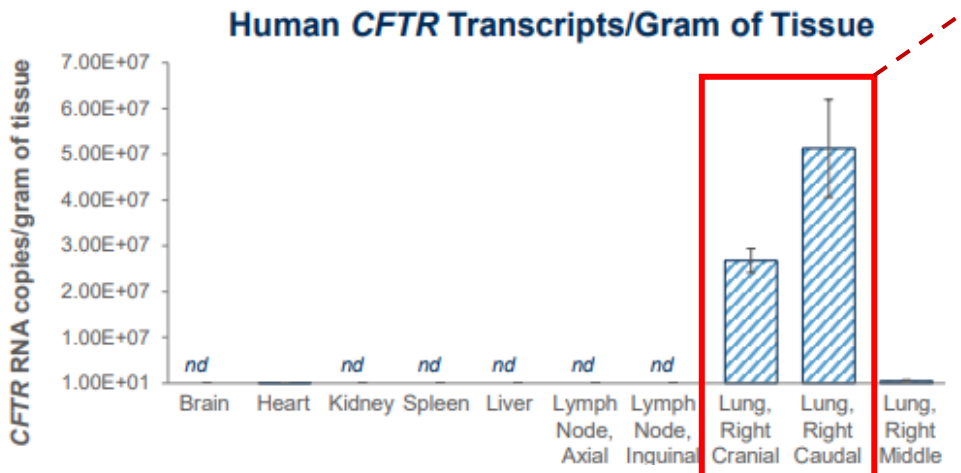
- Significant majority of KB407-positive cells are CD163-negative
- No significant colocalization of the viral and basal cell markers was detected, indicating that transduction was limited to air-exposed cells

20.6% (33/160) of KB407^{+} cells were CD163^{+} (10 fields of view)

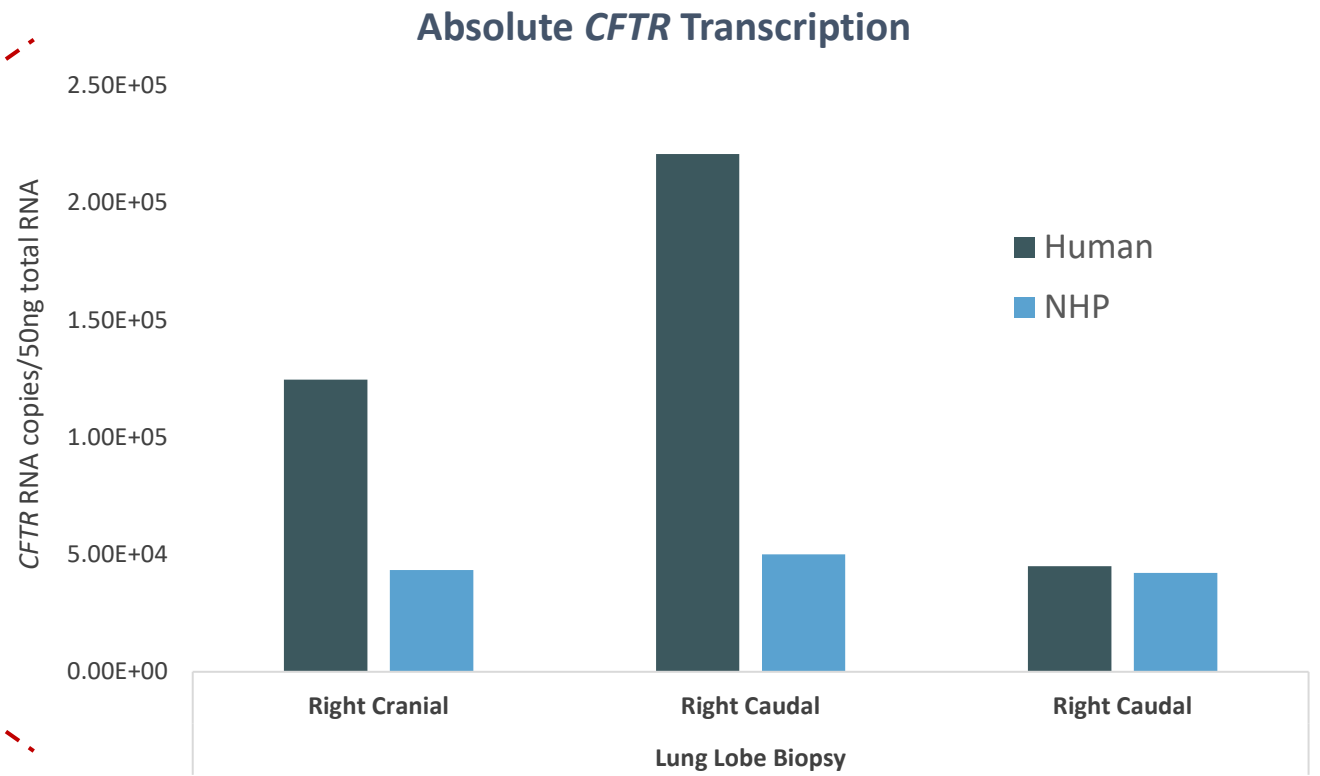
GLP, good laboratory practice; NHP, nonhuman primate.

KB407 Expresses Human *CFTR* ≥ Endogenous *CFTR* in NHP Lungs

Absolute quantitation of exogenous human and endogenous NHP CFTR transcripts 48 hours post-KB407 nebulization



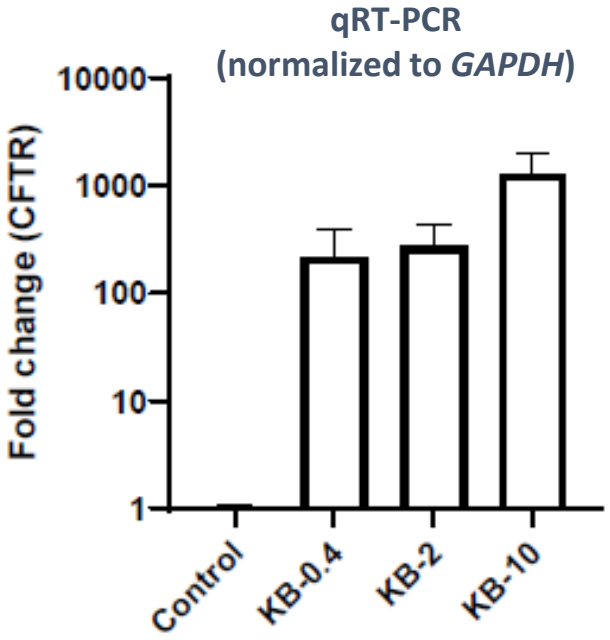
- Absolute quantification of exogenous human *CFTR* transcripts in multiple lung tissue biopsies were found to be 106%-440% of the endogenous levels observed in otherwise healthy primate airways



NHP, nonhuman primate.

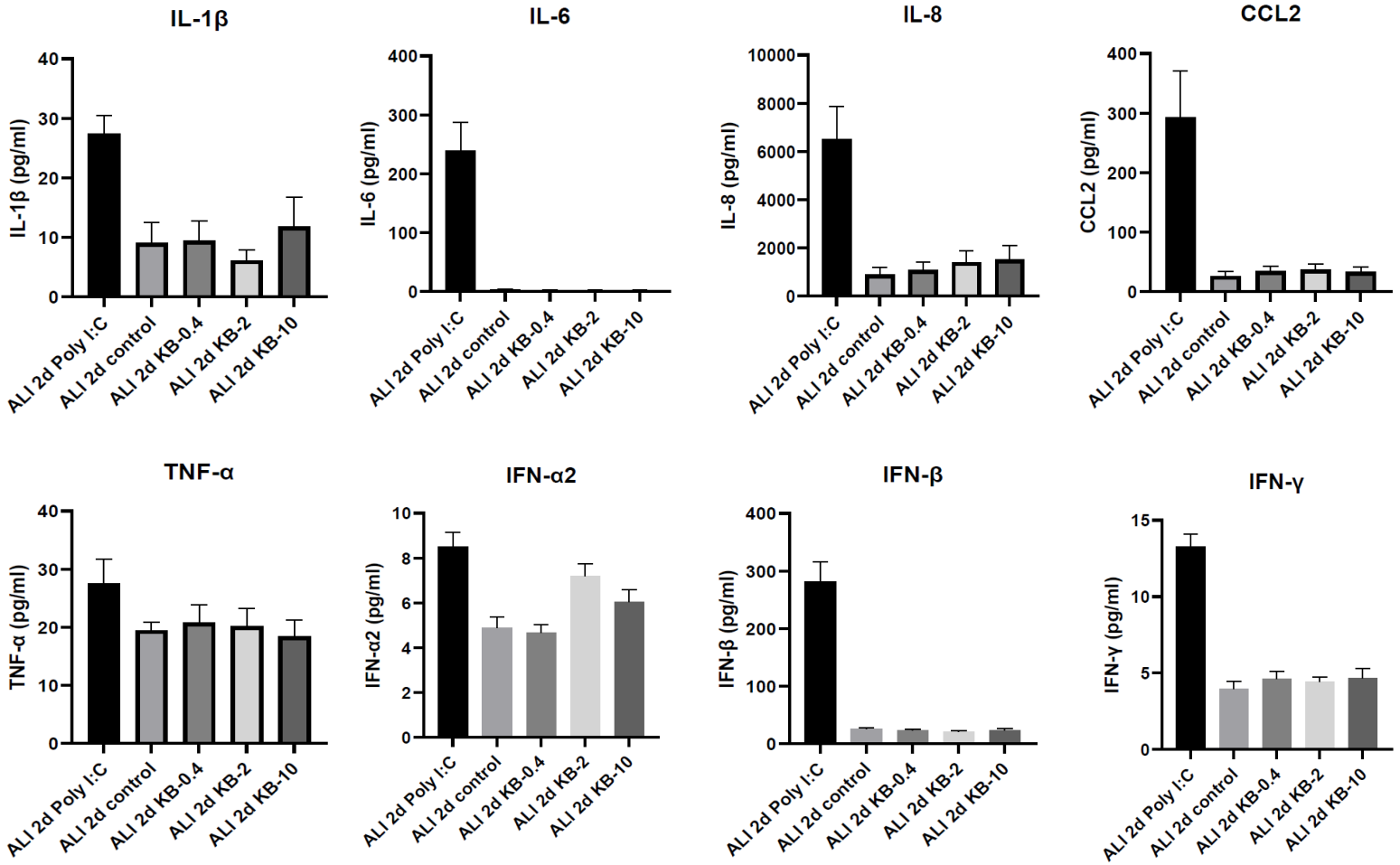
Assessment of Inflammatory Induction in Human CF Cells

No significant cytokine induction, even at MOI 10 and in presence of high levels of KB407-mediated CFTR expression, in CF colorectal epithelial cells 48 hours post-transduction



Genotype of cells used in study

- F508del/F508del
- 3527/Q493X
- F508del/R117H
- F508del/D1152H



IL-10, IL-12p70, IP-10, GM-CSF, IFN-λ1/2/3 also assessed, no significant induction (data not shown)

CF, cystic fibrosis; MOI, multiplicity of infection.
Data Courtesy of Dr. Gerard Kaiko, U. Newcastle.

Summary

✓	Expression and localization of CFTR in CF primary small airway cells
✓	Post-translation glycosylation of CFTR protein
✓	Functional correction in 3D organoid model
✓	KB407 is stable after nebulization
✓	KB407 expresses human *CFTR* in airways of mice and NHPs upon nebulization
✓	No adverse findings in GLP toxicology study
✓	KB407 transduces ciliated and secretory cells (both club and goblet cells) in NHPs, suggesting that each cell type is amenable to KB407 transduction through their apical membranes upon nebulization
✓	Human *CFTR* transcripts found to be 106%-440% of the endogenous levels in NHPs upon KB407 administration, suggesting transgene expression at physiologically relevant levels
✓	No evidence of significant cytokine/chemokine induction in transduced CF patient cells, limiting likelihood of significant inflammation after KB407 nebulization in treated patients
✓	Investigational New Drug (IND) application accepted by FDA in July 2022

CF, cystic fibrosis; GLP, good laboratory practice; NHPs, nonhuman primates.