In Vitro Pharmacology of KB407, an HSV-1-Based Gene Therapy Vector, for the Treatment of Cystic Fibrosis

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INTRODUCTION

Cystic fibrosis (CF), the most common inherited genetic disorder in the United States, is caused by mutations in the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR). Lack of functional CFTR in secretory airway epithelia results in defective Cl^-, bicarbonate, and thiocyanate secretion, coupled with enhanced Na^+ absorption and mucus production, leading to dehydration and acidification of the airway surface liquid[1-3]. CF is characterized by recurrent chest infections, increased airway secretions, and eventually, respiratory failure[4].

While FDA approval of four small molecule modulator therapies has been a boon to CF patients harboring the specific mutations responsive to these drugs, these modulators only treat a subset of the CF population. In particular need for effective drug intervention are the ~10% of CF patients harboring *CFTR* mutations that result in severely reduced or absent CFTR expression (class I mutations), as these patients suffer from the harshest and deadliest forms of CF[5]. Regrettably, no suitable therapies are approved for treating this most sensitive patient population. To this end, Krystal has developed KB407, a replication-defective herpes simplex virus type 1 (HSV-1) gene therapy vector encoding human CFTR, for molecular correction of CF.

MATERIALS & METHODS

Test Article

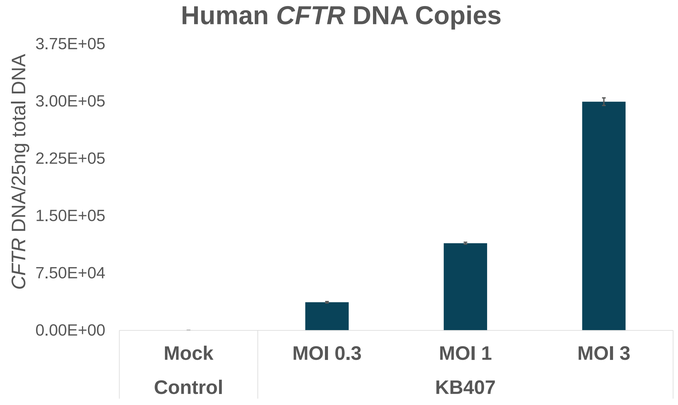
KB407: Krystal Biotech, Inc.'s propriety replication-incompetent, non-integrating HSV-1 vector expressing human CFTR.

Table 1. Critical Reagents

Reagent	Application	Source
Small airway epithelial cells	*In vitro* dose-ranging	Lonza (cat. no. CC-2933)
Organoid cultures	*Ex vivo* pharmacology[6]	Hubrecht Organoid Technology
Rhodamine 6G	R6G uptake assay[7]	Sigma (cat. no. 252433)
Anti-human CFTR	IF/western blot	R&D Systems (cat. no 25031)
Anti-human GAPDH	Western blot	Abcam (cat. no. ab9485)

RESULTS

In Vitro KB407 Dose-Ranging in CF Patient-Derived Small Airway Epithelial Cells (SAECs)



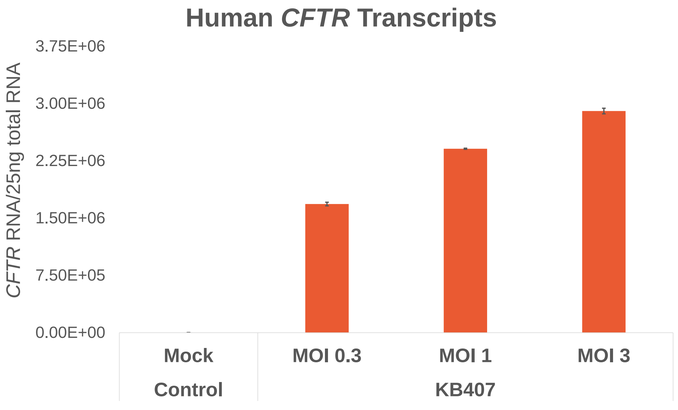


Figure 1. Dose-dependent increases in *CFTR* DNA and transcript levels upon KB407 infection of CF patient-derived SAECs. Data is presented as the average of three replicates ± standard error of the mean (SEM).

RESULTS (CONTINUED)

In Vitro CFTR Protein Analysis in KB407-Transduced CF Patient-Derived SAECs

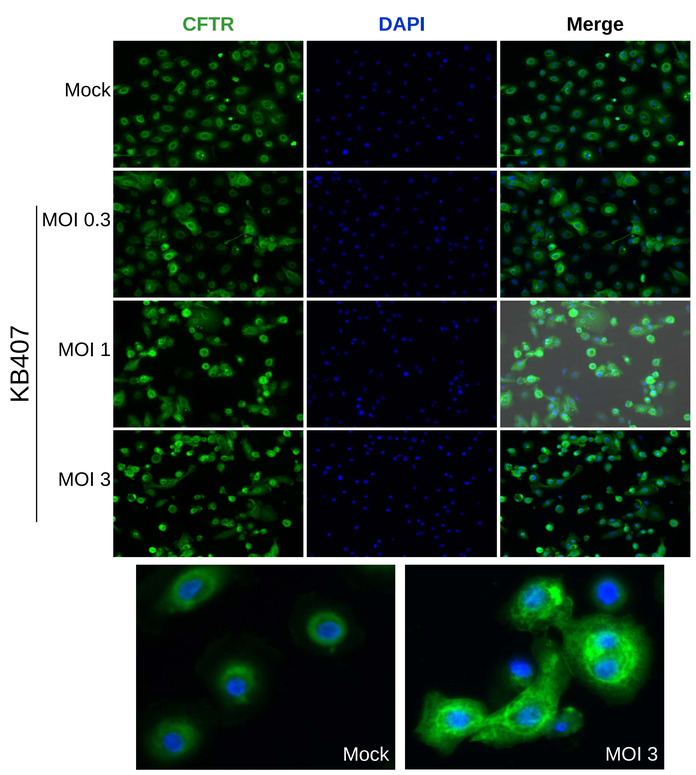
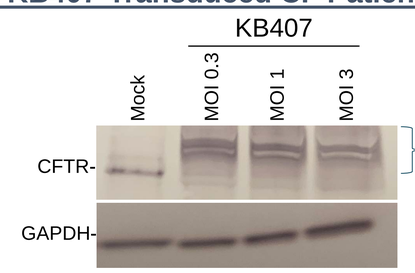


Figure 2. CFTR protein expression and relative cellular localization upon KB407 infection of CF patient-derived SAECs.



Appearance of a high molecular weight doublet is suggestive of proper post-translational modification, maturation, and trafficking of the exogenous protein[8].

Figure 3. Intracellular CFTR protein expression upon KB407 infection of CF patient-derived SAECs.

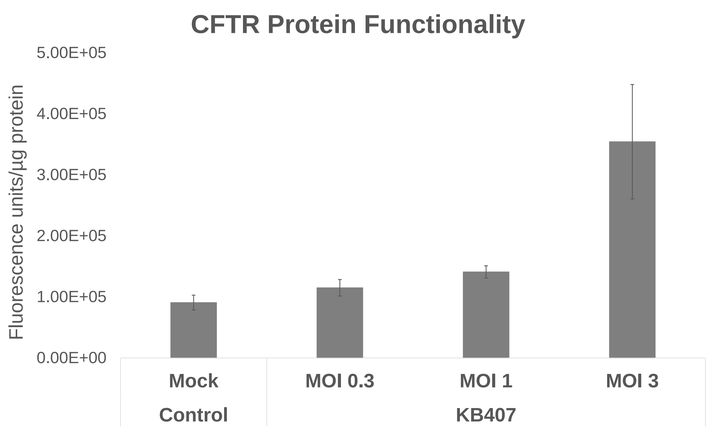


Figure 4. Dose-depend increase in CFTR-mediated R6G uptake upon KB407 infection in CF patient-derived SAECs. Data is presented as the average of three replicates ± SEM.

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

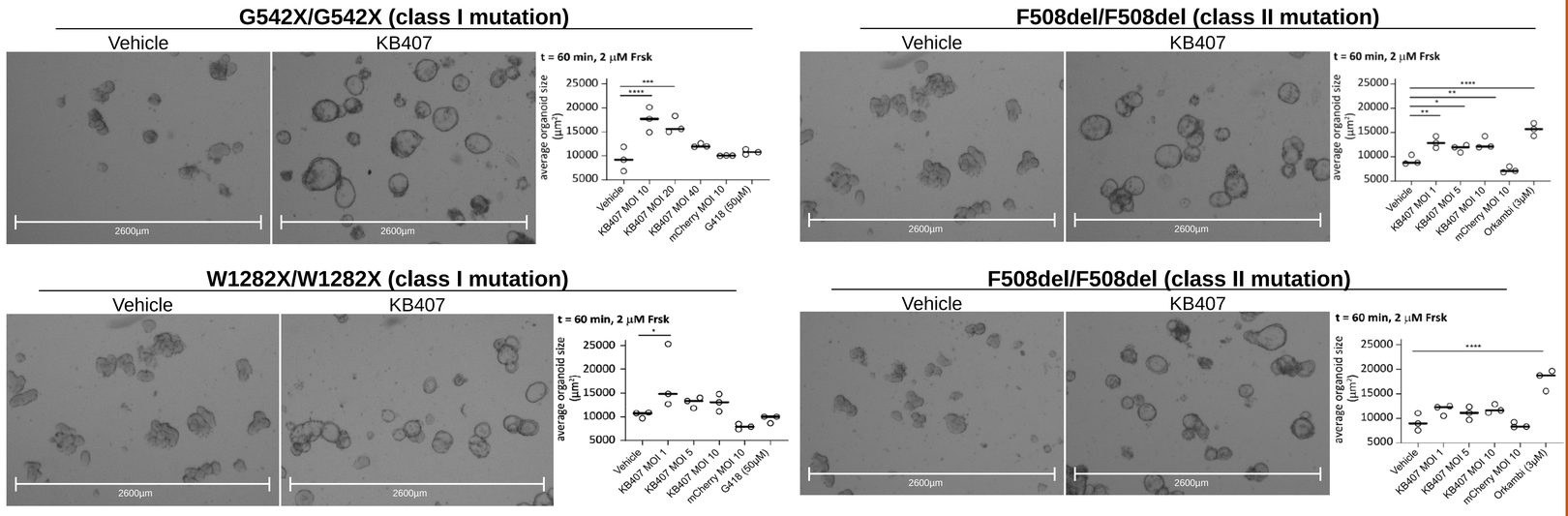


Figure 5. KB407-mediated functional correction to cystic phenotype of CF patient-derived intestinal organoids (PDOs) 48 hours post-infection, as assessed by a forskolin-induced swelling (FIS) assay. Organoids were strained with calcein green and imaged before and every 10 minutes after 2µM forskolin (Frsk) addition for 60 minutes. G418 or Orkambi were used as positive controls where appropriate; vehicle alone or HSV-mCherry were used as negative controls. Representative brightfield images show PDO morphology 24 hours after vehicle or KB407 treatment. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

CONCLUSIONS

- KB407 infects primary CF SAECs in a dose-dependent manner, resulting in robust expression of human *CFTR* at the transcript and protein levels.
- The vector efficiently produces functional, full-length CFTR protein that properly traffics to the cell membrane.
- KB407 transduction leads to a striking alteration of organoid morphology from a compact budding CF phenotype to a cystic organoid phenotype exhibiting wild-type characteristics, irrespective of the underlying *CFTR* mutation, within 24 hours of infection at MOIs ranging from 1 to 40.
- The corrected cystic morphology of multiple CF PDOs exposed to low doses of KB407 suggests that high levels of exogenous CFTR expressed in a minority of cells is sufficient to establish disease correction.
- These data support KB407 as a novel gene therapy for the treatment of cystic fibrosis.

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