

KIT2014, a Dual Inhaled PDE3/4 Inhibitor Enhancing Epithelial Function in the Airways

Laura Tasca¹, Valentina Sala¹, Xiao-Yann Huang³, Rosy Bonfante³, Bernadett Boda³, Samuel Constant³, Charlotte Dunne⁴, Natascha Hartl⁴, Katrin Radloff⁴, Nourhan Kawaji⁴, Dimitrios Goundis¹, Alessandra Ghigo^{1,2}

¹Kither Biotech, Via Nizza 52, Torino (TO), 10126, Italy

²Dipartimento di Medicina Molecolare, Università di Torino, Via nizza 52, Torino (TO), 10126, Italia

³Epithelix, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Switzerland

⁴Panthera Therapeutics GmbH, Neuendorfstrasse 20b, D-16761 Hennigsdorf

Summary

Inhaled mRNA therapies have emerged as a promising approach for genetic and rare diseases such as cystic fibrosis (CF), where only protein replacement can restore or compensate for dysfunctional or missing proteins. However, effective mRNA delivery, uptake and expression face several barriers, including mucus penetration, degradation, and endosomal escape. Add-on treatments that enhance the function of mRNA-based protein replacement therapies may further improve clinical efficacy.

KIT2014, a dual phosphodiesterase (PDE) 3/4 inhibitor developed by Kither Biotech, was evaluated as a novel add-on therapy for its ability to improve ciliary function, a key determinant of airway clearance, and to enhance the efficacy of inhaled mRNA-based treatments in diseases such as CF.

Using the MucilAir™-Pool model, KIT2014 was tested at multiple concentrations, assessing ciliary beating frequency (CBF) as the primary endpoint and epithelial integrity as the secondary endpoint. Apical administration of KIT2014 at 30 μ M (5.65 μ g/cm²) without prior mucus removal significantly increased CBF after 2 hours ($p < 0.05$) in fully differentiated human primary nasal epithelial cells, while preserving epithelial barrier integrity. All tested concentrations preserved transepithelial electrical resistance (TEER) after 6 hours of exposure.

These results demonstrate that KIT2014 significantly stimulates CBF while preserving epithelial integrity. This activity, combined with its ability to enhance CFTR gating, improve mucus hydration, reduce neutrophilic inflammation, and induce bronchodilation, provides a comprehensive approach to restoring airway function. This multimodal profile positions KIT2014 as a promising add-on therapy to enhance the performance of inhaled treatments, including future mRNA-based strategies for rare genetic diseases.

Key Message

KIT2014 stimulates ciliary beating while preserving epithelial integrity, supporting its potential to enhance the clinical efficacy of inhaled therapies for mucus-obstructed airways.

Introduction

The success of COVID-19 vaccines has accelerated the development of inhaled mRNA therapies, increasing interest in pulmonary delivery for various therapeutic applications. Compared to systemic administration, inhaled delivery offers advantages such as localized drug action, reduced systemic exposure, and potentially better patient adherence. However, mRNA delivery to the lungs remains challenging due to the respiratory tract's natural defences, particularly in diseased airways, where thick, viscous mucus limits drug access. In these conditions, mucus becomes highly adhesive, trapping inhaled therapeutics, including mRNA-loaded lipid nanoparticles (LNPs), and preventing cellular uptake. Beyond the mucus barrier, endosomal escape remains a key obstacle, as internalized nanoparticles often fail to release their mRNA cargo into the cytosol. These barriers have limited the success of prior inhaled protein replacement therapies, highlighting the need for innovative strategies to improve delivery and therapeutic efficacy¹.

Among the most promising targets for inhaled mRNA therapies are rare genetic respiratory diseases, such as CF, where restoring or replacing defective proteins can address the underlying cause of disease. In CF, where mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene impair chloride channel function, inhaled mRNA therapies, such as RCT2100² and ARCT-032³, are in clinical development to deliver functional CFTR transcripts directly to the lungs. This strategy is

particularly relevant for patients with Class I mutations, such as nonsense or frameshift variants, that abolish CFTR protein production and are unresponsive to current modulators; approximately 10–13% of CF patients fall into this group⁴.

PDE inhibitors have emerged as promising modulators of airway physiology, particularly in diseases characterized by airway obstruction and inflammation⁵. PDE3 and PDE4 are especially relevant for lung function due to their roles in degrading cyclic adenosine monophosphate (cAMP), a key regulator of airway smooth muscle tone, inflammation, and mucociliary clearance. PDE3 is predominantly expressed in airway smooth muscle, where its inhibition promotes bronchodilation and stimulates CFTR-mediated chloride secretion, enhancing mucus hydration. PDE4, mainly found in airway epithelial and inflammatory cells, regulates inflammation and ciliary function. Dual PDE3/4 inhibition synergistically combines these effects, offering bronchodilation, anti-inflammatory benefits, and improved mucociliary clearance.

KIT2014, a novel dual PDE3/4 inhibitor developed by Kither Biotech⁶, offers a promising strategy to enhance mucociliary clearance by simultaneously increasing CBF and improving mucus hydration. In fact, by inhibiting PDE3/4, KIT2014 elevates intracellular cAMP levels, which, in turn, stimulates CBF and concomitantly promotes CFTR gating. This latter leads to increased chloride ion secretion and subsequent mucus hydration, effectively reducing mucus viscosity and facilitating its clearance. Notably, this mechanism is active in both healthy and diseased settings such as CF. Beyond its effects on ciliary motility and mucus hydration, KIT2014 also demonstrates anti-inflammatory properties by reducing neutrophilic inflammation, along with bronchodilator effects. Altogether, these benefits create a favourable airway environment that can potentiate the clinical effectiveness of inhaled mRNA therapeutics. Currently, KIT2014 is undergoing clinical development, with Phase 1 trials in healthy volunteers now completed, underscoring its translational potential.

The objective of this study, therefore, was to evaluate the effects of KIT2014 on CBF, as part of a broader strategy to act as an add-on therapy to improve the clinical efficacy of inhaled CFTR-mRNA therapies.

Experimental Methods

This study employed the MucilAir™-Pool model⁷, by Epithelix, a fully differentiated human nasal epithelial cell system cultured at the air-liquid interface (ALI). The model consists of pooled cells from 14 different healthy nasal donors and recapitulates key physiological functions, including ciliary beating and mucus production. It provides a standardized, reproducible platform for evaluating mucolytic effects and respiratory therapeutics while minimizing donor variability.

A total of 18 MucilAir™-Pool tissues were used, distributed into treatment and control groups (N=9 each). KIT2014 was tested apically at three doses: Low (0.57 µg/cm², 3 µM), Medium (5.65 µg/cm², 30 µM), and High (56.47 µg/cm², 300 µM), in 10 µL PBS with Ca²⁺/Mg²⁺ (pH 7.5). Controls included Untreated (N=3), Vehicle (PBS, N=3), and Positive (Isoproterenol 50 µM, applied basally, N=3). In combined exposures, 500 µL MucilAir™ medium was added basally. An apical vehicle control was used to account for transient effects on CBF caused by apical fluid addition in ALI cultures. At T0, inserts were transferred to fresh plates with 500 µL basal medium and baseline CBF was measured before treatment. After apical application, tissues were incubated at 37°C, 5% CO₂, and 100% humidity, with CBF measured at 1 h (T1h), 2 h (T2h). CBF was analysed via high-speed video microscopy, focusing on active ciliated regions to avoid artifacts from mucus or debris. TEER was measured at T6h after apical addition of 200 µL medium using an epithelial voltohmmeter. All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using appropriate methods, with significance set at $p < 0.05$.

Results

KIT2014 induced a time and dose-dependent increase in CBF (Figure 1A and 1B). After 2 hours of apical exposure, the 5.65 µg/cm² dose (corresponding to 30 µM) significantly enhanced CBF to approximately 125% of vehicle control ($p < 0.05$), whilst 300 µM induced a peak increase in CBF at 1 hour, likely reflecting the typical kinetics of this assay. The positive control Isoproterenol (50 µM, basolateral), a β₂-adrenergic agonist known to elevate intracellular cAMP and stimulate ciliary motility, also significantly increased CBF, confirming the responsiveness of the system. Negative controls, including untreated tissues and vehicle-treated tissues, maintained CBF values within the expected

physiological range of approximately 8-11 Hz, demonstrating the functional integrity of the MucilAir™-Pool model and its suitability to assess ciliary responses. TEER measurements after 6 hours of exposure confirmed that KIT2014-treated tissues maintained epithelial barrier integrity, with values consistently above 200 $\Omega \cdot \text{cm}^2$ (Figure 1C). In contrast, apical exposure to 10% Triton X resulted in marked barrier disruption, validating the assay's sensitivity.

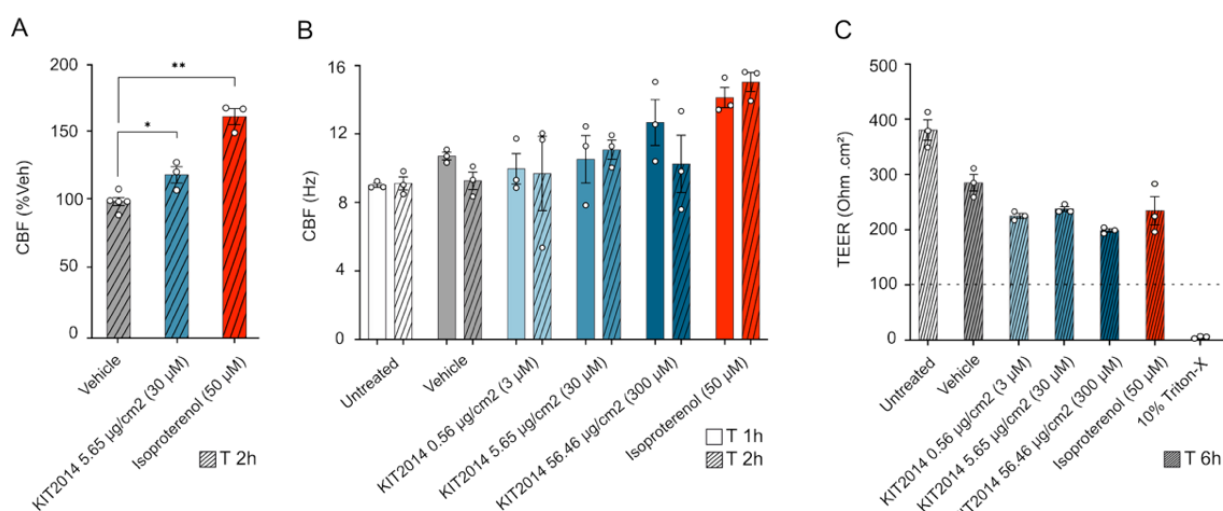


Figure 1: (A) Bar graph showing CBF expressed as a percentage of vehicle control after 2 hours of treatment, and (B) bar graph showing CBF values expressed in Hertz (Hz) across different treatment groups after 1 and 2 hours of treatment. KIT2014 increased CBF across all tested concentrations compared to vehicle control, reaching statistical significance at 5.65 $\mu\text{g}/\text{cm}^2$ (30 μM) when expressed at percentage of vehicle ($p < 0.05$). Isoproterenol, applied basally as a positive control, also significantly increased CBF when expressed at percentage of vehicle ($p < 0.01$). (C) Bar graphs showing TEER measurements ($\Omega \cdot \text{cm}^2$) across treatment groups. After 6 hours, all KIT2014 concentrations preserved TEER values above 200 $\Omega \cdot \text{cm}^2$, indicating preserved epithelial integrity. The 10% Triton X positive control caused marked loss of barrier function, validating assay sensitivity. Data are presented as mean $\pm$ SEM, with $n = 3$ per condition.

Collectively, these findings demonstrate that KIT2014 effectively stimulates CBF without compromising epithelial integrity, supporting its potential as an add-on for inhaled therapeutics targeting conditions characterized by impaired mucus clearance.

Discussion

This study highlights the therapeutic potential of KIT2014, a dual PDE3/4 inhibitor, as an add-on for inhaled treatments targeting mucus-obstructed airways. KIT2014 significantly increased CBF in a dose-dependent manner without compromising epithelial barrier integrity. These effects were observed in fully differentiated human airway epithelial cultures grown at the ALI with intact apical mucus, closely reproducing the *in vivo* airway environment. Preserving these physiological conditions is critical, as mucus penetration remains one of the main challenges for inhaled therapeutics in diseases such as CF. Under these conditions, the enhancement of CBF by KIT2014 represents a clinically relevant response. Beyond stimulating ciliary motility, KIT2014 promotes CFTR-mediated chloride secretion, improves mucus hydration, and enhances chloride transport even under limited CFTR expression. Combined with its anti-inflammatory and bronchodilator properties⁶, this multimodal profile supports its potential as an adjunct to inhaled mRNA therapies for inflammatory and obstructive airway diseases.

To explore KIT2014's ability to enhance the efficacy of inhaled mRNA therapies for CFTR replacement, additional studies will be conducted in ALI airway models using Panthera's dual-platform system. This approach integrates PTXmRNA®, an mRNA therapeutic technology, with PTXΔLNP®, an advanced lipid nanoparticle system designed for targeted pulmonary delivery (Figure 2). CFTR mRNA will be delivered using the PTXmRNA® platform, which provides optimized 5' and 3' untranslated regions for human lung cells, together with codon optimization, advanced capping, and uridine replacement strategies. Delivery will be achieved using LNPs from the PTXΔLNP® collection, characterized by optimized surface charge, demonstrated lung tropism *in vivo*⁸, and efficient transfection of human pulmonary cells *in vitro*. Finally, to overcome the thick mucosal barrier characteristic of CF patients, small lipid nanoparticles containing optimized amounts of PEG lipid will be tested for the transfection of human bronchial epithelial cells in the presence or absence of mucus⁹.

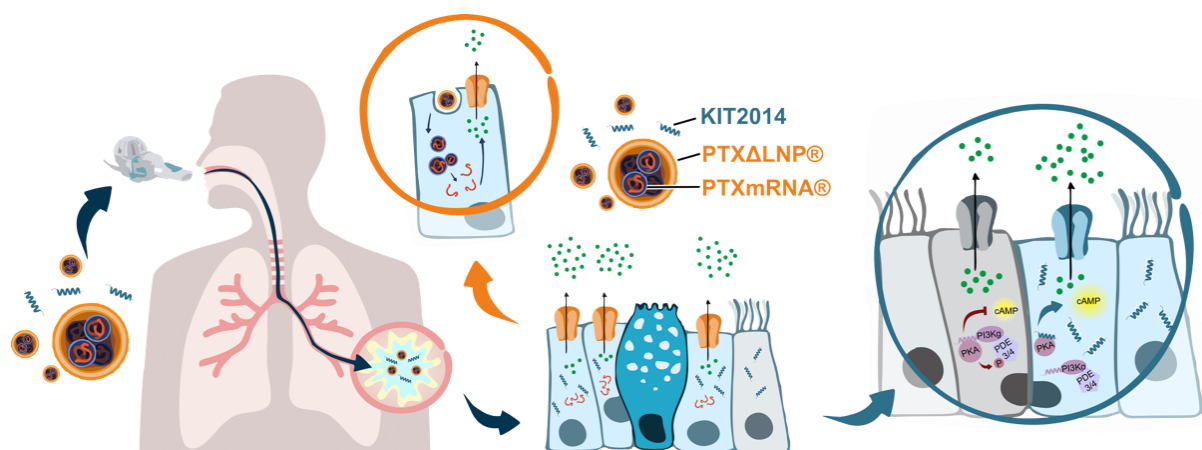


Figure 2: This schematic illustrates the proposed combined therapeutic strategy developed by Kithier Biotech and Panthera Therapeutics for targeted lung delivery of mRNA and peptide-based therapies. The inhaled formulation contains KIT2014 peptide and PTXmRNA®, encoding CFTR protein, encapsulated within Panthera's PTXΔLNP® lipid nanoparticles. Upon inhalation, the therapy reaches the lungs and targets airway epithelial cells. The PTXΔLNP® nanoparticles facilitate the cellular uptake of mRNA, which is then released and translated into functional CFTR proteins to restore chloride transport and improve epithelial function. Simultaneously, KIT2014 enhances therapeutic efficacy by inhibiting PDE3/4, leading to increased intracellular cAMP levels. This in turn activates protein kinase A (PKA), which boosts CBF by promoting ciliary motor activity and enhances CFTR gating, improving chloride secretion and water transport across the epithelium. Together, these mechanisms enhance mucus hydration and improve mucociliary clearance, synergistically acting on top of channel replacement by CFTR-mRNA.

Conclusions

Our findings support KIT2014 as a dual-action add-on for inhaled therapies, enhancing ciliary beating and CFTR-mediated chloride secretion to improve mucus hydration and clearance in CFTR-compromised tissues. Proof-of-concept studies will further evaluate its potential in combination with LNP-based mRNA therapies, with the aim of optimizing treatment regimens for respiratory diseases characterized by mucus obstruction. Together, these results provide a strong foundation for developing KIT2014 as a versatile and effective add-on to inhaled genetic medicines and other respiratory therapies.

References

- (1) Huang, C.; Li, H.; Duan, X.; Zhang, P.; Qi, S.; Du, J.; Song, X.; Tong, A.; Yu, G. Inhaled Non-Viral Delivery Systems for RNA Therapeutics. *Acta Pharm. Sin. B* **2025**, *15* (5), 2402–2430. <https://doi.org/10.1016/j.apsb.2025.03.033>.
- (2) Davies, J. C.; Polineni, D.; Boyd, A. C.; Donaldson, S.; Gill, D. R.; Griesenbach, U.; Hyde, S. C.; Jain, R.; McLachlan, G.; Mall, M. A.; Alton, E. W. F. W. Lentiviral Gene Therapy for Cystic Fibrosis: A Promising Approach and First-in-Human Trial. *Am. J. Respir. Crit. Care Med.* **2024**, *210* (12), 1398–1408. <https://doi.org/10.1164/rccm.202402-0389ci>.
- (3) Geller, D. E.; Crowley, C.; Froehlich, J.; Schwabe, C.; O'Carroll, M. WS10.03 Inhaled LUNAR®-CFTR mRNA (ARCT-032) Is Safe and Well-Tolerated: A Phase 1 Study. *J. Cyst. Fibros.* **2024**, *23*, S19. [https://doi.org/10.1016/s1569-1993\(24\)00166-8](https://doi.org/10.1016/s1569-1993(24)00166-8).
- (4) Fajac, I.; Sermet, I. Therapeutic Approaches for Patients with Cystic Fibrosis Not Eligible for Current CFTR Modulators. *Cells* **2021**, *10* (10), 2793. <https://doi.org/10.3390/cells10102793>.
- (5) Sherpa, R. T.; Koziol-White, C. J.; Panettieri, R. A. Advancing Obstructive Airway Disease Treatment: Dual PDE3/4 Inhibition as a Therapeutic Strategy. *Cells* **2025**, *14* (9), 659. <https://doi.org/10.3390/cells14090659>.
- (6) Ghigo, A.; Murabito, A.; Sala, V.; Pisano, A. R.; Bertolini, S.; Gianotti, A.; Caci, E.; Montresor, A.; Premchandrar, A.; Pirozzi, F.; Ren, K.; Della Sala, A.; Mergioti, M.; Richter, W.; De Poel, E.; Matthey, M.; Calder, S.; Cardone, R. A.; Civiletti, F.; Costamagna, A.; Quinney, N. L.; Butnarusu, C.; Visentin, S.; Ruggiero, M. R.; Baroni, S.; Crich, S. G.; Ramel, D.; Laffargue, M.; Tocchetti, C. G.; Levi, R.; Conti, M.; Lu, X.-Y.; Melotti, P.; Sorio, C.; De Rose, V.; Facchinetti, F.; Fanelli, V.; Wenzel, D.; Fleischmann, B. K.; Mall, M. A.; Beekman, J.; Laudanna, C.; Gentzsch, M.; Lukacs, G. L.; Pedemonte, N.; Hirsch, E. A PI3Kγ Mimetic Peptide Triggers CFTR Gating, Bronchodilation, and Reduced Inflammation in Obstructive Airway Diseases. *Sci. Transl. Med.* **2022**, *14* (638). <https://doi.org/10.1126/scitranslmed.abl6328>.
- (7) Mercier, C.; Jacqueroix, E.; He, Z.; Hodin, S.; Constant, S.; Perek, N.; Boudard, D.; Delavenne, X. Pharmacological Characterization of the 3D MucilAir™ Nasal Model. *Eur. J. Pharm. Biopharm.* **2019**, *139*, 186–196. <https://doi.org/10.1016/j.ejpb.2019.04.002>.
- (8) Radloff, K.; Gutbier, B.; Dunne, C. M.; Moradian, H.; Schweska, M.; Gossen, M.; Ahrens, K.; Kneller, L.; Wang, Y.; Moga, A.; Gkionis, L.; Keil, O.; Fehring, V.; Tondera, D.; Giese, K.; Santel, A.; Kaufmann, J.; Witzernath, M. Cationic LNP-Formulated mRNA Expressing Tie2-Agonist in the Lung Endothelium Prevents Pulmonary Vascular Leakage. *Mol. Ther. Nucleic Acids* **2023**, *34*, 102068. <https://doi.org/10.1016/j.omtn.2023.102068>.
- (9) Tafech, B.; Rokhforouz, M.; Leung, J.; Sung, M. M.; Lin, P. J.; Sin, D. D.; Lauster, D.; Block, S.; Quon, B. S.; Tam, Y.; Cullis, P.; Feng, J. J.; Hedtrich, S. Exploring Mechanisms of Lipid Nanoparticle-Mucus Interactions in Healthy and Cystic Fibrosis Conditions. *Adv. Healthc. Mater.* **2024**, *13* (18). <https://doi.org/10.1002/adhm.202304525>.