ENaC inhibition in cystic fibrosis: potential role in the new era of CFTR modulator therapies

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ENaC inhibition with BI 1265162 is a promising strategy to optimise outcomes in patients with CF either eligible, or ineligible, for CFTR modulator therapy. Phase II clinical trials of BI 1265162 must now show this translates into clinical benefit. https://bit.ly/2OQ1IUI

Cite this article as: Mall MA. ENaC inhibition in cystic fibrosis: potential role in the new era of CFTR modulator therapies. *Eur Respir J* 2020; 56: 2000946 [https://doi.org/10.1183/13993003.00946-2020].

Introduction

Small-molecule cystic fibrosis transmembrane conductance regulator (CFTR) modulator drugs for cystic fibrosis are the first therapies since the disease was initially described by Fanconi *et al.* [1] in 1936 to target and partially restore the function of the CFTR Cl^- channel. CFTR modulator therapy is expected to have significant clinical benefits for many, but it does not result in a cure and is not appropriate or available for all patients with cystic fibrosis [2, 3]. In this review, evidence is described suggesting that inhibiting the epithelial Na^+ channel (ENaC) responsible for the Na^+/fluid absorption that contributes to airway surface dehydration and impaired mucociliary clearance (MCC) observed in cystic fibrosis airways may significantly improve clinical outcomes irrespective of the CFTR genotype, and may synergise with currently approved CFTR modulators to further improve clinical outcomes.

The CFTR modulator landscape

Significant progress has been made in the treatment of patients with cystic fibrosis with the introduction of CFTR modulator therapies, which consist of CFTR correctors that improve folding and trafficking of the common F508del-CFTR mutation and potentiators that improve the open probability of mutant CFTR channels at the apical cell membrane [4–6]. Current CFTR modulator drugs vary in efficacy in improving CFTR function and clinical outcomes. The potentiator ivacaftor was the first approved CFTR modulator for cystic fibrosis patients with at least one G551D-CFTR gating mutation and was shown to rescue mutant CFTR function to ∼50% of wild-type levels, which was associated with an improvement in mean absolute percentage predicted forced expiratory volume in 1 s ($ppFEV_1$) of ∼11% in the pivotal trial [3, 5, 7]. Subsequently developed corrector-potentiator combinations for patients homozygous for the common F508del-CFTR allele (lumacaftor/ivacaftor and tezacaftor/ivacaftor) or patients with one F508del allele and a residual function allele (tezacaftor/ivacaftor) showed smaller improvements in CFTR function (to ∼10–20% of wild-type levels), which were associated with more modest improvements in $ppFEV_1$ of ∼3–4% in F508del homozygous patients [6, 8, 9] and ∼7% in patients with one F508del allele in combination with a

Received: 31 March 2020 | Accepted after revision: 18 July 2020

residual function allele [10]. In addition, it has been shown that bacterial counts and inflammatory markers are reduced in sputum of patients treated with ivacaftor, but that bacteria are not eradicated over time [11, 12]. A triple-agent CFTR modulator drug (elexacaftor/tezacaftor/ivacaftor) has recently been approved in patients with a single F508del-CFTR allele. Elexacaftor, like tezacaftor, is a CFTR corrector, but acts additively at a second site in the F508del-CFTR protein to improve multiple folding defects [13]. Consistent with this additive effect at the molecular level, this triple-combination CFTR modulator therapy resulted in greater improvement in lung function and other clinical outcomes (an approximately 11–14% improvement in ppFEV$_1$ compared with control) in patients homozygous for F508del or compound heterozygous with a minimal function allele [14, 15] and is predicted to become the "gold standard" for the treatment of up to 90% of patients with cystic fibrosis (those with at least one F508del allele) [2]. However, for most patients with chronic cystic fibrosis lung disease, this improvement in pulmonary function is not necessarily a return to the normal range, and patients continue to have exacerbations, albeit at much reduced rates [14]. Improving clinical outcomes further in patients receiving elexacaftor/tezacaftor/ivacaftor has the potential to have a significant impact on the cystic fibrosis population (figure 1). In addition, the approximately 10% of cystic fibrosis patients for which CFTR modulator therapy is ineffective (those who do not carry at least one F508del allele) could benefit from a mutation-agnostic approach. Finally, while long-term safety of treatment with elexacaftor/tezacaftor/ivacaftor remains unknown, other therapeutic avenues should be considered.

ENaC: role in healthy and cystic fibrosis airways, and potential therapeutic target

In the conducting airways, ENaC is expressed at the apical membrane of airway epithelial cells and provides the limiting pathway for transepithelial Na$^+$ absorption that drives absorption of Cl$^-$ and water through the paracellular shunt pathway (figure 2) [16–18]. In healthy airways, coordinated Cl$^-$ secretion by CFTR and other Cl$^-$ channels and Na$^+$ absorption by ENaC is essential for proper volume regulation of airway surface liquid (ASL), which comprises the periciliary layer (PCL) and the overlying mucus layer [19, 20]. ENaC is regulated by several intracellular and extracellular mechanisms. Intracellular mechanisms include activation by convertase-type proteases, such as furin [21], and inhibition by CFTR [22, 23]. Extracellular activation mechanisms include proteolytic cleavage by neutrophil elastase and other proteases released from neutrophils and other inflammatory cells in the airways [24, 25], as well as by bacterial proteases released in airway infection [26, 27]. Therefore, ongoing inflammation and infection in cystic fibrosis patients with established lung disease, including those treated with CFTR modulators [11, 12], is likely to cause ongoing proteolytic cleavage and activation of ENaC in the airways.

Evidence suggests that ENaC is hyperactivated in cystic fibrosis as a result of CFTR dysfunction and/or proteolytic activation by host- and bacteria-derived proteases [22–29]. Hyperactivated ENaC in the airways of cystic fibrosis patients results in markedly increased Na$^+$ absorption [20, 30, 31], leading to increased paracellular Cl$^-$ and water absorption from the airway lumen, ASL volume depletion, hyperconcentration of mucus, reduced height of the PCL with compressed cilia, reduced MCC, mucus accumulation, airway plugging, bacterial colonisation, inflammation, progressive tissue damage and decline in lung function [25,

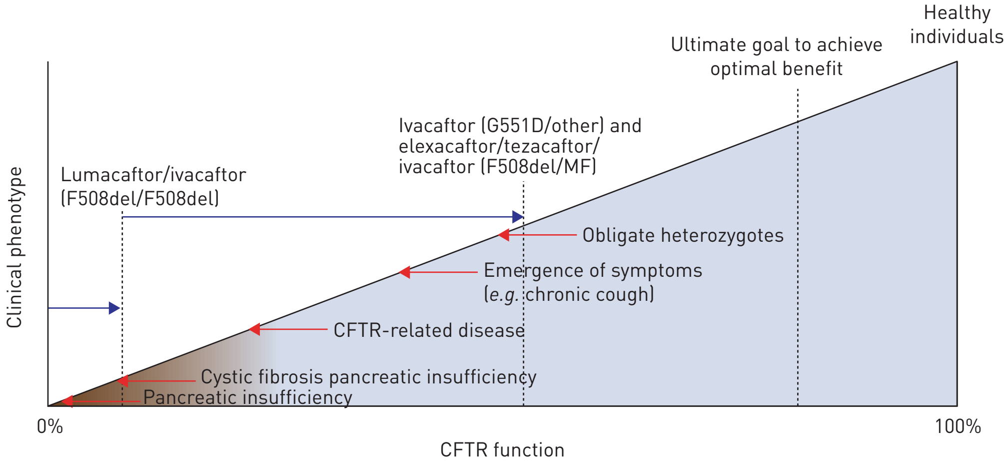

FIGURE 1 The relationship between clinical phenotype and cystic fibrosis transmembrane conductance regulator (CFTR) function, and levels of functional restoration of mutant CFTR by current CFTR modulator therapies in patients with cystic fibrosis. CBAVD: congenital bilateral absence of the vas deferens; MF: minimal function.

32]. The pathogenic role of increased ENaC function has been supported by the lung phenotype of mice with airway-specific overexpression of the β-subunit of ENaC (βENaC-Tg) that phenocopy cystic-fibrosis-like airway surface dehydration/mucus hyperconcentration and develop cystic-fibrosis-like lung disease [33, 34]. Collectively, these results support ENaC inhibition in the airways as an attractive target for cystic fibrosis therapy. In this context, it is noteworthy that patients with pseudohypoaldosteronism with loss-of-function mutations in the α- and β-subunits of ENaC have increased ASL volume and MCC rates [35], and cystic fibrosis patients with a mutation in the δ-subunit of ENaC causing reduced ENaC activity were found to have slow progression of lung disease [36].

Inhibition of ENaC was first demonstrated with amiloride, and in the kidney, this is an effective diuretic drug [37, 38]. In βENaC-Tg mice, which display a cystic-fibrosis-like lung phenotype, intrapulmonary treatment with amiloride was effective, leading to a reduction in mucus plugging, airway inflammation and pulmonary mortality when started as preventive therapy immediately after birth, *i.e.* when the lungs were structurally normal; however, no beneficial effects were observed in adult βENaC-Tg mice with established cystic-fibrosis-like lung disease [39]. In cystic fibrosis patients, probably due to amiloride's short half-life and limited potency, studies of the effect of inhaled amiloride on MCC and lung function resulted in only moderate and inconsistent improvements, even with multiple doses [40–42]. The amiloride derivative

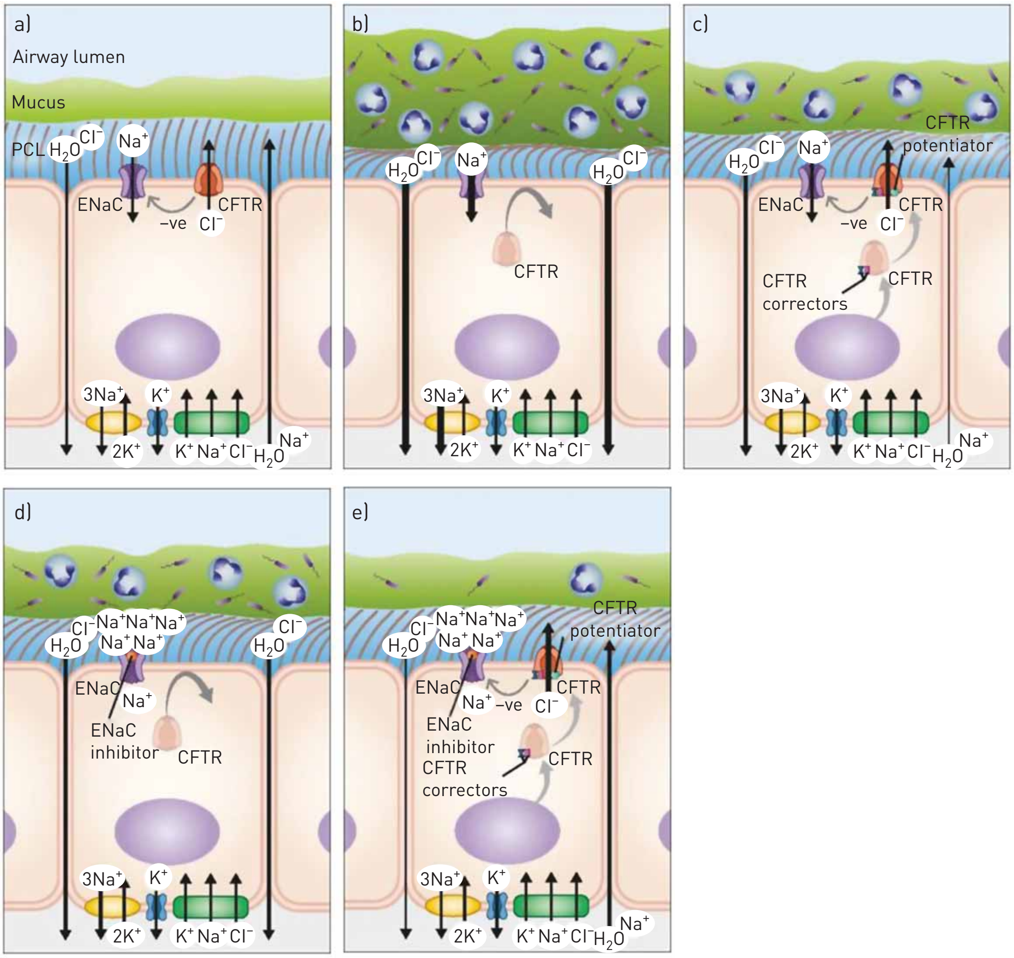

FIGURE 2 In healthy airways, a balance between cystic fibrosis transmembrane conductance regulator (CFTR)-mediated secretion and epithelial Na^+ channel (ENaC)-mediated absorption of NaCl and H_2O facilitates proper hydration of airway surfaces essential for effective mucociliary clearance (a). In cystic fibrosis airways, deficient CFTR-mediated Cl^-/fluid secretion and increased ENaC-mediated Na^+/fluid absorption leads to airway surface dehydration (reduced PCL and hyperconcentrated mucus), flattened cilia, impaired mucociliary clearance, bacterial colonisation and neutrophilic inflammation (b). Partial restoration of these pathological features by rescue of mutant CFTR function with CFTR modulators (correctors and potentiators) (c) or ENaC inhibition (d). Hypothesised synergy between CFTR modulation and ENaC inhibition, resulting in further improvement in airway surface hydration and reduction in pathological features in cystic fibrosis airways (e). Arrows indicate direction and magnitude of ion and water movement. -ve: negative regulation; PCL: periciliary layer.

GS-9411, whilst 100-times more potent than amiloride and with a longer duration of action, failed Phase I trials as an inhaled ENaC blocker due to ENaC inhibition in the kidney, resulting in hyperkalaemia [43], which may have cardiac and neurological safety implications. These studies led to the hypothesis that if the poor pharmacodynamics and safety observed with amiloride and GS-9411 could be overcome with a newer-generation compound, ENaC inhibition would be a viable therapy to improve airway surface hydration and pulmonary outcomes in patients with cystic fibrosis, irrespective of CFTR genotype (*i.e.* a mutation-agnostic therapy), particularly in those with rare CFTR mutations that have no currently approved CFTR modulator therapy [42, 44–46]. Further, more recently, mucus hyperconcentration has also been implicated in the pathogenesis of a spectrum of other muco-obstructive lung diseases including chronic bronchitis and non-cystic fibrosis bronchiectasis, suggesting that ENaC inhibition may be beneficial far beyond cystic fibrosis [47–49].

Importantly, ENaC inhibition may act synergistically with CFTR modulators (figure 2e). CFTR can secrete or absorb Cl^- across epithelial surfaces depending on the electrochemical driving force that is determined by i) the intra- and extracellular Cl^- concentrations that are tightly regulated and result in a reversal potential for Cl^- (*i.e.* the membrane potential at which there is no net flow of Cl^- from one side of the membrane to the other, also known as the Nernst potential) in the range of −30 mV in cystic fibrosis airway epithelial cells, and ii) the membrane potential of the cell that is set by the relative conductances of Cl^-, Na^+ and K^+ channels and respective intra- and extracellular concentrations of these ions [17, 18, 50]. It is therefore predicted that if ENaC is inactive or not expressed in the same cell, cAMP-mediated stimulation will lead to a concomitant activation of apical CFTR and basolateral K^+ channels (reversal potential for K^+ approximately −90 mV) that drives the membrane potential more negative than the reversal potential of Cl^-, and thus generate a driving force for CFTR-mediated Cl^- secretion; however, if ENaC is active in the same cell (reversal potential for Na^+ approximately +60 mV), this will depolarise the membrane potential, reduce the driving force for Cl^- secretion, and may even result in CFTR-mediated Cl^- absorption as observed in the sweat duct [17, 18, 28, 50–52]. Regarding the combined use of CFTR modulators and ENaC inhibitors in patients with cystic fibrosis, this implies that inhibition of hyperactive ENaC in the apical membrane of airway epithelial cells may not only block ENaC-mediated Na^+/fluid absorption, but will also hyperpolarise the apical cell membrane and thus increase the driving force for Cl^-/fluid secretion *via* mutant CFTR channels that have been rescued and inserted into the apical plasma membrane by CFTR modulators. Whether this is reflected in synergistic effects on airway surface hydration, MCC and pulmonary outcomes in patients with cystic fibrosis and other muco-obstructive lung diseases needs to be assessed in clinical trials.

Clinical development of new ENaC inhibitors

Several new compounds designed for ENaC inhibition are currently in active preclinical development [44, 45, 53–57] (table 1). These new compounds employ different modes of action ranging from highly potent and durable inhibition of the ENaC channel pore, inhibition of ENaC-activating proteases, to inhibition of ENaC expression by antisense oligonucleotides or small interfering RNA. Of these, the new small-molecule ENaC inhibitor BI 1265162 is the only compound currently in Phase II development [60]. It has demonstrated efficacy in a preclinical investigation, with a markedly higher potency than amiloride (a 30–70-fold lower half maximal inhibitory concentration), and no effects on serum K^+ and plasma electrolytes have been observed [61]. In addition, BI 1265162 has shown safety in Phase I volunteer studies [62].

Importantly, preclinical work supports the potential mutation-agnostic property of BI 1265162 [61]. Using highly differentiated human airway epithelial cell cultures grown at an air–liquid interface, it was found that transepithelial fluid absorption from the apical surface to the basolateral compartment was reduced by BI 1265162, with or without CFTR modulators, in both cystic fibrosis and normal airway cultures. This work also supports the hypothesis of a potential synergistic effect with CFTR modulators. Addition of lumacaftor/ivacaftor alone to F508del/F508del cystic fibrosis cultures partially restored mucus transport velocity to approximately 50% of that observed in normal airway cultures; however, addition of BI 1265162 to lumacaftor/ivacaftor further improved mucus transport velocity in cystic fibrosis cultures to a level similar to normal airway cultures. Taken together, the above evidence suggests that BI 1265162 is a promising candidate as a monotherapy for cystic fibrosis patients for whom CFTR modulators are ineffective and in combination with CFTR modulators providing synergistic effects. However, no ENaC inhibitor development, so far, has translated into clinical success [44] and several clinical development programmes of ENaC inhibitors for patients with cystic fibrosis (VX-371; QBW276; Novartis and SPX-101; Spyryx (an indirect inhibitor)) have recently been terminated (table 1). The clinical development of VX-371 for primary ciliary dyskinesia (NCT02871778) has also recently been terminated (clinicaltrials.gov NCT02871778). Failure of ENaC inhibitors to progress in clinical development may be due to inadequate dosing and/or deposition by inhalation in cystic fibrosis patients with chronic lung disease characterised by heterogeneous airway mucus plugging. These ENaC inhibitors demonstrated

TABLE 1 Preclinical and clinical development of ENaC inhibitors for cystic fibrosis

Drug	Company	Type	MoA	Comments	References
Preclinical					
Benzamil/phenamil (second-generation amiloride derivatives)		Small molecule	Direct inhibition	Discontinued due to rapid clearance, short half-life and epithelial permeability in lungs in sheep	[58]
NVP-QBE 170	Novartis	Small molecule	Direct inhibition		
QUB-TL1		Small molecule	Channel-activating protease inhibitor		[54]
MK 104	Mucokinetica	Small molecule	Channel-activating protease inhibitor		[55, 56]
ENaC inhibitory ASO	Ionis	Molecular inhibition	ASO		[57]
ETD001	Enterprise Therapeutics	Small molecule	Direct inhibition		
Phase I					
GS-9411 (third-generation amiloride derivative)	Parion	Small molecule	Direct inhibition	Discontinued due to SAE of acute hyperkalaemia	[43]
BI 443651	Boehringer Ingelheim	Small molecule	Direct inhibition	Discontinued due to palatability issues	clinicaltrials.gov NCT02706925
AZD5634	AstraZeneca	Small molecule	Direct inhibition	In Phase Ib	clinicaltrials.gov NCT02950805
ARO-ENaC	Arrowhead Pharmaceuticals	Molecular inhibition	Small interfering RNA	In Phase I/IIa	clinicaltrials.gov NCT04375514
IONIS-ENaCRx	Ionis	Molecular inhibition	ASO	In Phase I/IIa	clinicaltrials.gov NCT03647228
Phase II					
Amiloride		Small molecule	Direct inhibition	Discontinued due to limited therapeutic efficacy in lungs	[41]
Camostat	Novartis	Small molecule	Prostatin inhibitor (channel-activating protease; major regulator of ENaC)	Discontinued due to adverse events/ tolerability issues	[59]
VX-371	Vertex/Parion	Small molecule	Direct inhibition	Discontinued in combination with ivacaftor/lumacaftor due to lack of efficacy	clinicaltrials.gov NCT02709109
SPX-101	Spyryx	Peptide analogue	SPLUNC-1 analogue, promoting ENaC channel internalisation	Discontinued due to lack of efficacy	clinicaltrials.gov NCT03229252
QBW276	Novartis	Small molecule	Direct inhibition	Development terminated due to strategic reasons	clinicaltrials.gov NCT02566044
BI 1265162	Boehringer Ingelheim	Small molecule	Direct inhibition		clinicaltrials.gov NCT04059094

ASO: antisense oligonucleotide; ENaC: epithelial sodium channel; MoA: mode of action; SAE: serious adverse event; SPLUNC-1: short palate, lung, and nasal epithelium clone protein.

improvement of MCC in the sheep model, but lack of translation of this preclinical model to patients with cystic fibrosis may be related to the fact that the sheep do not have airway mucus plugging and structural lung damage commonly present in patients with cystic fibrosis. Therefore, the dose of inhaled ENaC inhibitor that improves MCC in the sheep model may not be sufficient to achieve therapeutically active ENaC inhibition in cystic fibrosis patients. To address this issue in more detail, more recent cystic fibrosis animal models such as the cystic fibrosis pig and cystic fibrosis ferret, which feature cystic-fibrosis-like lung disease including heterogeneous mucus plugging, chronic airway infection, inflammation and bronchiectasis, may be utilised to better understand issues related to target engagement and the potential role of ENaC inhibitors as a therapeutic option in cystic fibrosis lung disease [63, 64]. Whether the hurdle of sufficient delivery of inhaled BI 1265162 to mucus-obstructed airways can be overcome in patients with cystic fibrosis remains to be seen in clinical trials. In addition, systemic side effects such as hyperkalaemia, short study duration, non-study-related exacerbations and lack of sensitivity of traditional endpoints such as ppFEV$_1$ to detect treatment benefits may impede the clinical development of an inhaled ENaC inhibitor therapy.

How could these hurdles be overcome in the clinical development of a new ENaC inhibitor therapy for cystic fibrosis? First, a successful ENaC inhibitor will have to be highly potent, delivered to the airways in a sufficient dose and have a long duration of action to provide effective ENaC inhibition and maximal treatment effect. Second, the new ENaC blocker should result in minimal off-target effects and systemic exposure. In this context, the following translational challenges that have ended the clinical programmes of earlier ENaC inhibitors have been addressed in order to minimise BI 1265162's risk of failure in Phase II studies. The risk of underdosing was minimised by basing the dose for the Phase II trial in cystic fibrosis patients on fluid absorption data in the rat model (in which BI 1265162 was instilled into the trachea) in addition to MCC data in the sheep model (in which BI 1265162 was nebulised) [61], factoring in the expected lung deposition using the Respimat device in humans [65]. Of note, the improvements in MCC observed with BI 1265162 in the sheep model [61] were comparable to the improvement obtained in cystic fibrosis patients with the G551D gating mutation after starting CFTR modulator therapy with ivacaftor [66]. These data suggest that inhaled BI 1265162, if delivered successfully to the cystic fibrosis lung, has the potential to provide similar benefits on MCC in cystic fibrosis patients with genotypes that do not respond to CFTR modulators. In the subsequent Phase I studies [62], BI 1265162 did not result in drug-related hyperkalaemia and was well tolerated when administered as single or multiple doses up to 1200 µg daily. Moving into Phase II, lung clearance index (LCI) derived from multiple breath washout has been included as an additional endpoint [60]; LCI reflects ventilation inhomogeneity in the lung [67], correlates with mucus plugging and other morphological changes of the airways [68] and is more sensitive than spirometry (ppFEV$_1$) to detect functional abnormalities in the small airways typically affected in cystic fibrosis [67]. Crucially, BI 1265162 is an ENaC inhibitor that is considerably more potent than amiloride and has, at least preclinically, been shown to work as a mutation-agnostic monotherapy and in synergy with CFTR modulation [61].

Conclusion

Despite the exciting breakthroughs in the development of CFTR modulator therapy, this therapy is not effective for all patients with cystic fibrosis, as only patients with at least one F508del allele can benefit [2]. Additionally, functional restoration of the underlying ion/fluid transport defect and clinical outcomes are still suboptimal in those patients who are eligible for CFTR modulator therapy. Whereas systemic delivery of CFTR modulators can improve CFTR function in multiple affected organs, potential benefits of inhaled ENaC blockers are likely limited to the lungs. However, ENaC inhibition may become a viable option for patients for whom existing CFTR modulator therapy is ineffective, and it has the potential to act synergistically with CFTR modulation in those patients for whom it is. ENaC inhibition is therefore a promising strategy to optimise therapeutic benefit. Clinical trials with novel, long-acting ENaC inhibitors such as BI 1265162 will now have to demonstrate that ENaC inhibition is safe and translates into clinical benefit in people with cystic fibrosis.

Conflict of interest: M.A. Mall reports grants from German Federal Ministry of Education and Research, German Research Foundation and Einstein Foundation Berlin, during the conduct of the study; personal fees from Bayer, Boehringer Ingelheim, Polyphor, Arrowhead Pharmaceuticals, ProQR Therapeutics, Spyryx Biosciences, Vertex Pharmaceuticals, Santhera, Galapagos, Sterna Biologicals, Enterprise Therapeutics and Celtaxys, outside the submitted work. In addition, M.A. Mall has a patent on the Scnn1b-transgenic mouse with royalties paid, and a patent on use of sodium channel blockers for early therapy of obstructive lung diseases issued.

Support statement: Funding was received from Boehringer Ingelheim. Funding information for this article has been deposited with the Crossref Funder Registry.

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