to asthmatic hyperresponsiveness (4). Accordingly, inhibition of TMEM16A is considered a potential therapeutic target for asthma, other chronic obstructive lung diseases, and PAH.

Dr. Danahay and colleagues have mentioned their unpublished observations about the lack of any bronchospasm during the inhalation of the nebulized ETX001 in a conscious sheep model. However, inhaled drugs such as nitric oxide have strong pulmonary vasoactive effects when they come into close contact with the precapillary vessels. Some inhaled drugs such as iloprost may also be taken up into the systemic circulation where they come into contact with all the organs (5). Therefore, inhaled medications are not completely restricted to the airways.

In addition, in patients independent of CFTR genotype, activation of TMEM16A by denufosol failed to demonstrate any benefit to patients with CF. In a multicenter, randomized, parallel group, double-blind, placebo-controlled trial, the aerosol induced a cough or coughing in more than half of the patients. This adverse effect could be associated with additional mucus production but also with airway obstruction (6).

The proposed activation of TMEM16A as a druggable target in patients with CF poses a number of difficult questions. Enhancement of TMEM16A activity represents an option to improve chloride channel function in CF; however, it also bears risks for clinical complications such as bronchial obstruction, pulmonary hypertension, and disturbances in gut motility. Therefore, any prospective clinical trial should pay special attention to such potential adverse effects. ■

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Reply to Olschewski *et al.*

From the Authors:

Though we appreciate Dr. Olschewski and colleagues' perspectives on the potential for unwanted effects of increasing the activity of TMEM16A, it should be noted that this is based on studies in which the channel has either been genetically ablated or inhibited with low-potency nonselective blockers. Although these studies provide some guidance around TMEM16A function, an understanding of the effects of positive channel modulation requires potent and selective pharmacological modulators that enhance TMEM16A activity. Through the identification and careful preclinical characterization of TMEM16A potentiators such as ETX001, we have been able to address the potential safety implications of increasing the activity of the channel in addition to developing a deeper understanding of the potential therapeutic benefit (1). Contrary to the concerns outlined by Dr. Olschewski and colleagues, we have recently reported that ETX001 has no effect on airway or vascular smooth muscle function as well as no effect on either airway goblet cell formation or function (2). The local instillation of ETX001 into the airways of rats showed no effects on lung function and did not affect airway smooth muscle tone in isolated human bronchi. Importantly, ETX001 did not affect vascular smooth muscle contraction using freshly isolated human pulmonary artery preparations where compound exposure levels were constant and far in excess of the effective concentration required to give 50% of the maximal response for the channel (2). In addition, ETX001 has been designed to have a short

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systemic half-life to further limit any potential for systemic adverse effects.

The apparent inconsistency between the previously published data and pharmacological data generated using potent and selective TMEM16A potentiators may reflect *1*) the imperfect translation of genetically manipulated models, *2*) the limitations of using nonselective pharmacological inhibitors to characterize ion channel function, and *3*) concluding that positive modulation will deliver the opposite phenotype to inhibition. For example, data reported from pulmonary arterial hypertension models using a Tmem16a knockout and supported by pharmacological studies using the nonselective TMEM16A blocker benzbromarone failed to translate into clinical efficacy (3). In this study, a paradoxical increase in mean pulmonary artery pressure was reported in benzbromarone-treated patients with pulmonary arterial hypertension (3). In addition, although some Tmem16a knockdown studies have reported a reduction in blood pressure, overexpression of the channel did not cause an increase in pressure (4).

Dr. Olschewski and colleagues correctly note the failure of the inhaled P2Y2 agonist, denufosol, to demonstrate clinical benefit in patients with cystic fibrosis. The reasons for this are likely multifactorial and may include poor pulmonary pharmacokinetics, rapid degradation by ectonucleotidases, potential receptor desensitization, and emptying of intracellular calcium stores (5, 6). It should be noted that contrary to Dr. Olschewski's letter, cough and sputum production did not differ between the placebo and denufosol-treated cohorts in the TIGER2 (Transport of Ions to Generate Epithelial Rehydration 2) study. In contrast to P2Y2 agonists, which act indirectly through elevation of intracellular calcium, compounds such as ETX001 selectively enhance the activity of TMEM16A in response to physiologically regulated changes in intracellular calcium (1).

ETD002, a first-in-class TMEM16A potentiator, has successfully completed its Investigational New Drug enabling safety studies with clinical studies expected to commence in 2020. As with all drug candidates in clinical development, the potential for adverse events will be carefully monitored. ■

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Whither the Bicarbonate Era

To the Editor:

For metabolic acidosis, beyond treating the underlying cause, correcting hypoxemia, and establishing good perfusion, sodium bicarbonate is often given at variable arbitrary thresholds of depressed blood pH. Recently, Zanella and colleagues (1) employed extracorporeal removal of chloride by electrodialysis in healthy pigs made acidemic by either lactic acid infusion or hypoventilation (CO_2 retention). By physically drawing off chloride and establishing a local separation of charge, blood electroneutrality at the membrane is immediately reestablished by the hydrolysis of water to yield a hydroxyl ion that instantly combines with CO_2 to form bicarbonate. The authors show the feasibility of quantitatively increasing bicarbonate in this fashion for both forms of acidosis without the associated and unwanted hypernatremia and volume loading that can occur with intravenous sodium bicarbonate administration. The accompanying editorialists (2) proclaim the postbicarbonate era with this study that illustrates a major tenet of the Stewart approach to acid–base chemistry and its superiority over other approaches to understanding acid–base physiology and pathophysiology. In Stewart's paradigm, H^+, OH^-, HCO_3^-, and CO_3^{2-} are relegated to the status of dependent variables; that is, they can only be formed from the differential movements and exchanges of independent strong ions (Na^+, K^+, and Cl^-) that disturb electroneutrality, which is immediately corrected by the hydrolysis of water and reaction with CO_2. Although the heuristics of the Stewart analysis are valid, I remain unconvinced by the claim that what the mathematics of this approach reveal demands that physiology must follow these rules and conclusions. The assumption that only strong ions and their differential movement from one space to another alters H^+ and HCO_3^- concentrations because the math is

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