inflammatory diseases, or secondary bacterial or viral infection, may indicate aspergillosis. However, radiological presentation can be atypical for invasive fungal disease in COVID-19 pneumonia, resembling influenza. The quantity of mycological arguments or the variety of assays is not decisive, although the quality of clinical specimens is conclusive for proving invasive aspergillosis. ■

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TMEM16A Potentiation: Possible Drawbacks

To the Editor:

Dr. Danahay and colleagues present an interesting and timely study of TMEM16A potentiation to increase the epithelial fluid secretion and thereby enhance mucus clearance in cystic fibrosis (CF) (1). They report that the novel compound ETX001 potentiates the opening of TMEM16A channels and augments the magnitude of the chloride current. In human cell and animal models, the ETX001 effect was independent of CFTR function. The authors conclude that the novel potentiator could also be suitable for patients with CF without mutation in CFTR. However, when previous investigations of TMEM16A are considered, this approach may raise concerns with respect to safety.

TMEM16A, a protein encoded by the gene ANO1, is a calcium-activated chloride channel robustly expressed not only in epithelial cells but also in smooth muscle cells of airways, pulmonary and systemic vessels, gastrointestinal smooth muscle cells, and the endothelial cells of pulmonary arteries (2). The wide distribution of the channel indicates diversity in its physiological functions, such as secreting chloride and regulating vascular and gastrointestinal tone. In addition, in the pacemaker cells of the gut, TMEM16A is important for peristalsis generation. Under physiological conditions, TMEM16A is active at the resting membrane potential, and the open probability is dependent on the intracellular calcium concentration. The chloride current is voltage-dependent and exhibits a greater current amplitude in a depolarized state than at hyperpolarization.

In addition to its physiological function, TMEM16A can be upregulated by, for example, ET-1 (endothelin-1), the transcription factor HIF-1α, or IL, factors that have been found to be important players in the pathology of pulmonary arterial hypertension (PAH). We have recently reported that TMEM16A is strongly upregulated in remodeled pulmonary arteries from patients with idiopathic PAH and that this change causes the depolarization of pulmonary arterial smooth muscle cells as well as a contraction of small pulmonary arteries (3). These detrimental changes were reversed by TMEM16A silencing or its pharmacological inhibition. We have shown that such inhibition reduced the increased pulmonary vascular tone in both *ex vivo* and *in vivo* settings and reversed pulmonary arterial remodeling, causing amelioration of pulmonary hypertension and right ventricular strain in two independent PAH animal models (3). TMEM16A overactivation was observed in inflammatory lung diseases, in which it was strongly upregulated in secretory cells and airway smooth muscle cells, significantly contributing

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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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