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Moving the Dial on Airway Inflammation in Response to Trikafta in Adolescents with Cystic Fibrosis

Agathe Lepissier¹, Anne Sophie Bonnel^{2,3}, Nathalie Wizla⁴, Laurence Weiss⁵, Marie Mittaine⁶, Katia Bessaci⁷, Eitan Kerem⁸, Véronique Houdouin^{9,10}, Philippe Reix^{11,12}, Christophe Marguet^{13,14*}, Isabelle Sermet-Gaudelus^{1,2,10,15*}, and the MODUL-CF Pediatric French Network for Cystic Fibrosis

¹INSERM U1151, Institut Necker Enfants Malades, Paris, France; ²Hôpital Necker Enfants Malades, Assistance Publique Hôpitaux de Paris, Paris, France; ³Hôpital André Mignot, Le Chesnay, France; ⁴Hôpital Jeanne de Flandre, Lille, France; ⁵Hôpital de Hautepierre, Strasbourg, France; ⁶Hôpital des Enfants-CHU de Toulouse, Toulouse, France; ⁷American Memorial Hospital, Reims, France; ⁸Hadassah Hebrew University Medical Center, Jerusalem, Israel; ⁹Hôpital Robert Debré, Paris, France; ¹⁰Université Paris Cité, Paris, France; ¹¹Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron, France; ¹²UMR 5558 CNRS Equipe EMET Université Claude Bernard Lyon 1, Lyon, France; ¹³CHU de Rouen, Rouen, France; ¹⁴UMR 1311, Université de Normandie, Rouen, France; ¹⁵European Respiratory Network-Lung, Frankfurt, Germany

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*Co-last authors.

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Author Contributions: A.L. performed the experiments and wrote the manuscript. A.S.B. coordinated the study. N.W., L.W., M.M., K.B., V.H., and P.R. enrolled the patients and reviewed the manuscript. E.K. and C.M. enrolled the patients, participated to the design of the study, and edited the manuscript. I.S.-G. designed the study, obtained the funding, coordinated the study and the experiments, and wrote the manuscript.

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To the Editor:

Cystic fibrosis (CF) lung disease is characterized by excessive and sustained airway inflammation (1). The CFTR (cystic fibrosis transmembrane conductance regulator) highly effective modulator therapy (HEMT) elxacaftor, tezacaftor, and ivacaftor (ETI) demonstrated remarkable pulmonary improvement in patients with CF (2). Although the respiratory benefit reflects a better airway surface hydration and improved mucociliary clearance, data are missing regarding the effect of ETI on inflammation, specifically in patients with relatively mild lung disease. The aim of the current study is to assess the short-term effect of ETI on lung inflammation in adolescents with CF.

Methods

Study design. Patients with CF were enrolled in a multicenter real-life longitudinal study (NCT04301856). Data were collected at initiation (M0) and 1 month (M1). This included antibiotic treatment, pulmonary function tests, sweat tests, inflammatory markers in blood sampling (C-reactive protein [CRP], leukocytes, and polymorphonuclear neutrophil [PMN] counts), sputum cultures, and inflammatory biomarkers. The study was approved by the Centre Institutional Review Board. All parents gave informed consent.

Sputum sample preparation. Sputum was collected by sputum induction. Sputum samples were immediately placed on ice and frozen at -80°C . After thawing, 200 μl of sputum was processed as per laboratory protocol, and supernatant was frozen at -80°C until analysis (3, 4).

Sputum inflammatory biomarkers. Neutrophil elastase (NE) activity was measured using a fluorometric assay (Sigma-Aldrich). IL-1 β , IL-4, IL-6, IL-8, IFN- γ , and TNF- α (tumor necrosis factor α) concentrations were measured using V-PLEX Viral Panel 2 Human Kit, and V-PLEX Human IL-8 Kit (Meso Scale Discovery, Inc.). All tests were performed in triplicate.

Statistical analyses. Data were expressed as median (interquartile range). Comparisons were analyzed using the Fisher test, paired Student's *t* test, ANOVA, or simple regression analysis. FEV₁ change at M1 was quantified by its relative change from baseline.

Results

Patient population. Seventy-six European White adolescents (33 girls, median [interquartile range] age, 15 [3] yr), were enrolled from seven centers across France. Forty-six patients were p.Phe508del homozygous and had received Orkambi (lumacaftor/ivacaftor) before enrollment; the 30 remaining patients were compound heterozygous for F508del and a minimal function mutation and had not been previously treated with CFTR modulators.

Most of the patients presented with mild lung disease, as assessed by a median predicted FEV₁ (ppFEV₁) of 76% (Table 1). Patients colonized with *Pseudomonas aeruginosa* ($n = 18$), *Stenotrophomonas maltophilia*, or *Achromobacter xylosoxidans* ($n = 17$) had a significantly decreased ppFEV₁ (71% [18%] vs. 80% [20%]; $P = 0.04$). Thirty-one patients received oral or intravenous (IV) antibiotic therapy at initiation of ETI because of bronchial exacerbation.

Lung function and clinical parameters. Patients presented a significant improvement after 1 month of ETI treatment relative to baseline in both weight (+1.1 [2.9] kg) and FEV₁ (+24% [31%]),

Table 1. Change in Clinical, Respiratory, and Bacteriological Endpoints and Inflammatory Biomarkers

Parameters	M0	M1	P Value
Clinical and microbiological endpoints			
Weight, kg	50 (11.7)	51.9 (10.2)	<0.0001
Sweat chloride, mEq/L	92.5 (29.5)	44.0 (30)	<0.0001
FEV ₁ , L	2.4 (0.9)	3.1 (1.1)	<0.0001
FEV ₁ , pp	76.0 (21)	96.0 (24)	<0.0001
FVC, L	3.3 (1.1)	3.7 (1.2)	<0.0001
FVC, pp	90.0 (16)	102.0 (18)	<0.0001
FEF _{25–75} , L	2.3 (1.4)	3.3 (1.7)	<0.0001
FEF _{25–75} , pp	58.0 (40.5)	86.5 (46.7)	<0.0001
Bronchial colonization			
<i>Staphylococcus aureus</i>	55	49	0.007
<i>Pseudomonas aeruginosa</i>	17	12	<0.0001
<i>Achromobacter xylosoxidans</i> or <i>Stenotrophomonas maltophilia</i>	17	3	0.01
Blood inflammatory biomarkers			
Leukocytes, nb/μl	7,465 (3,192)	6,400 (2,595)	<0.0001
Polymorphonuclear neutrophil count, nb/μl	4,035 (2,810)	3,100 (1,846)	<0.0001
CRP, mg/L	0.5 (3.4)	0.0 (0.2)	0.0002
Sputum inflammatory biomarkers			
Neutrophil elastase, μg/ml	23.4 (100.8)	2.1 (4.6)	<0.0001
TNF-α, pg/ml	12.4 (36.75)	2.6 (11.4)	0.15
IFNγ, pg/ml	3.8 (9.1)	4.0 (8.4)	0.25
IL-1β, pg/ml	132.2 (482.1)	22.9 (76.5)	0.02
IL-4, pg/ml	0.3 (0.5)	0.2 (0.4)	0.75
IL-6, pg/ml	7.0 (28.1)	4.9 (17.4)	0.28
IL-8, pg/ml	25,682.2 (70,448.2)	10,969.9 (28,618.9)	0.006

Definition of abbreviations: CRP = C-reactive protein; FEF_{25–75} = forced mid-expiratory flow; M0 = month 0; M1 = month 1; nb = number; pp = percentage predicted; TNF = tumor necrosis factor.

Data are given as median (interquartile range) or bronchial colonization refers to the chronic identification of bacteria in sputum.

and 52 patients demonstrated a decrease in sweat test below 60 mmol/L (Table 1). Five patients with chronic colonization for *P. aeruginosa* and 14 with *A. xylosoxidans* or *S. maltophilia* demonstrated negative cultures 1 month after ETI initiation. The change in ppFEV₁ was not significantly different in patients previously treated with Orkambi (+21% [32%] vs. +29% [23%] for patients naive to CFTR modulators) or those receiving antibiotics at ETI initiation (+21% [24%] vs. +28% [30%] in untreated patients). ppFEV₁ was significantly increased in patients with *P. aeruginosa*, *A. xylosoxidans*, or *S. maltophilia* in sputum at M0 (+32% [28%] vs. +20% [23%] in noncolonized patients; $P = 0.03$).

Inflammation biomarkers. At baseline, blood concentrations of CRP, leukocytes, and PMN count were within the normal range in contrast to the sputum inflammatory markers, which were elevated (5). ppFEV₁ was negatively correlated with sputum biomarkers, including NE ($P = 0.0006$) and IL-8 ($P = 0.009$), as well as with blood CRP ($P = 0.00004$), leukocytes ($P = 0.00004$), and PMN counts ($P = 0.01$).

All blood and sputum inflammatory biomarkers decreased at M1. The difference was significant for NE, IL-1β, and IL-8 in sputum and CRP, leukocytes, and PMN count in blood (Figure 1 and Table 1). FEV₁ change was significantly correlated with that of sputum NE ($P = 0.02$), blood CRP ($P = 0.04$), leukocytes ($P = 0.0007$), and PMN count ($P = 0.0002$), and nearly at the significant concentration for sputum IL-8 ($P = 0.07$). Interestingly, some patients did not experience respiratory improvement despite a significant change in inflammatory biomarkers. There was no correlation between changes in sweat test and blood or sputum inflammatory

biomarkers. Change in NE at 1 month was not significantly affected by previous treatment with Orkambi (−16 [89] μg/ml vs. −17 [98] μg/ml), antibiotic course at initiation (−16 [80] μg/ml vs. −19 [123] μg/ml). Although baseline NE was more elevated in patients with *P. aeruginosa*, *A. xylosoxidans*, or *S. maltophilia* (66 [111] μg/ml vs. 17 [83] μg/ml; $P = 0.1$), negativation of sputum culture for these bacteria at M1 did not significantly impact NE change (−52 [136] μg/ml vs. −81 [68] μg/ml; not significant). A similar profile was observed for the other biomarkers.

Discussion

This study in adolescents with relatively mild lung CF disease shows a decrease in sputum and blood inflammatory markers at 1 month after ETI initiation.

There are contradictory reports regarding the effect of HEMT on inflammation (6–8). Our observation that NE, IL-8, and IL-1β decrease significantly in sputum in parallel to CRP and PMN count in blood provides some evidence that ETI blunts neutrophil-derived inflammation in the short term. Once in the CF lung, PMNs acquire a modified phenotype that promotes NE release either free in the airway or bound to PMNs (4). As previously reported for circulating PMNs (9), our results suggest that ETI may also reverse this dysregulation in the lung. Importantly, those changes occurred regardless of whether patients were chronically infected with *P. aeruginosa*, *A. xylosoxidans*, or *S. maltophilia* or had been treated with antibiotics at initiation, suggesting an antiinflammatory effect, independent of infection.

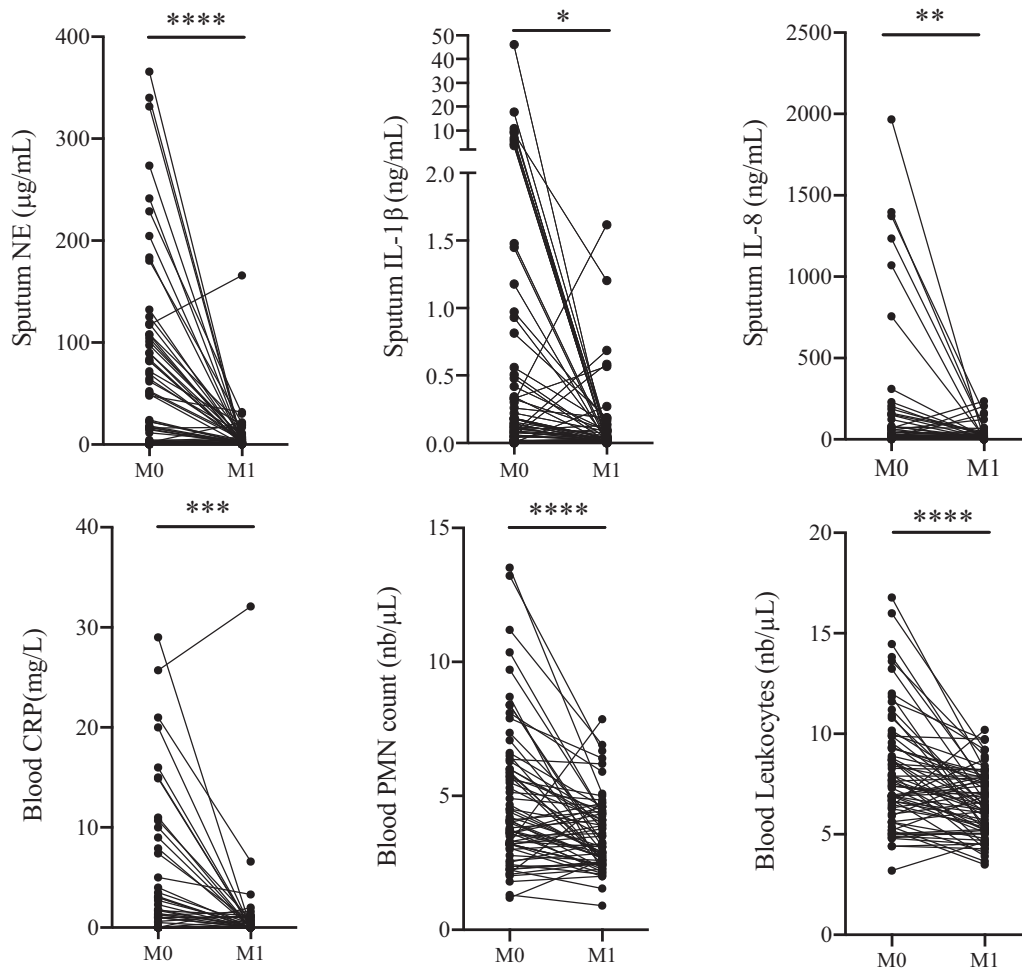


Figure 1. Concentration of sputum NE, IL-1 β , IL-8, and blood CRP, PMN, and leukocyte counts at baseline (M0) and M1 treatment with elxacaftor, tezacaftor, and ivacaftor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. CRP = C-reactive protein; M0 = month 0; M1 = month 1; NE = neutrophil elastase; PMN = polymorphonuclear neutrophil.

Given the strong association between neutrophilic inflammation and progression of lung damage in early life, our results may suggest that HEMT may prevent or at least attenuate longer-term pulmonary decline (10). An important point is that, at the individual level, the improvement of ppFEV₁ was not directly associated with the variation of the inflammatory markers. This can be the result of irreversible damage. Conversely, patients with remaining significant amounts of NE may require adjunctive anti-elastase therapy. This illustrates the complexity of understanding the exact targets of HEMT on the inflammatory cascade in CF airways and how restoration of CFTR function may affect inflammation and vice versa. This also outlines the necessity of longitudinal studies in the CF population to assess whether this antiinflammatory effect is sustained. ■

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Correspondence and requests for reprints should be addressed to Isabelle Sermet-Gaudelus, M.D., Ph.D., Paris, France. Email: isabelle.sermet@aphp.fr.

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Targeting Pulmonary Hypertension Caused by Pulmonary Fibrosis: A Promising Natural Killer T Cell-based Therapy

Zi-Hao Wang and Qiong Zhou

Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

ORCID IDs: 0000-0002-1069-4099 (Z.-H.W.); 0000-0002-3120-3017 (Q.Z.).

To the Editor:

We read the article by Jandl and colleagues, recently published in the *Journal*, with immense interest (1). In this study, Jandl and colleagues revealed the important role of natural killer T (NKT) cells in pulmonary hypertension (PH) caused by pulmonary fibrosis. Compared with patients who have interstitial lung disease (ILD) without PH and donor lung controls, patients with PH-ILD had significantly increased type I collagen deposition in lung tissues and decreased CD3⁺CD56⁺ NKT-like cells in pulmonary arteries (1). In a bleomycin-induced mouse model of pulmonary fibrosis, the authors showed potent antifibrotic efficacy of KRN7000

(α -Galactosylceramide; α -GalCer) (1), the classic agonist for invariant NKT (iNKT) cells (2).

The authors then detected decreased STAT1 expression in pulmonary artery smooth cells (PASCs) and isolated pulmonary arteries from patients with PH-ILD. Coculture of α -GalCer-treated peripheral blood mononuclear cells and PASCs significantly decreased (transforming growth factor (TGF)- β -induced type I collagen deposition in PASCs. Jandl and colleagues found that CXCL9 expression was decreased in the plasma and lung tissue of patients with PH-ILD. Furthermore, the authors demonstrated that short-term treatment with α -GalCer significantly increased CXCL9 secretion in human NKT-like cells. *In vitro* CXCL9 treatment also inhibited TGF- β -induced type I collagen deposition in human PASCs. The strengths of this study lie in the use of patient samples and mouse models for *in vivo* and *in vitro* experiments, as well as the potential for the translational treatment of PH-ILD with α -GalCer.

Human CD3⁺CD56⁺ NKT-like cells are known to be heterogeneous, containing CD4⁺, CD8⁺, TCR $\gamma\delta$ ⁺, mucosal-associated invariant T-type cells, and a few iNKT cells (3). Also, murine CD3⁺NK1.1⁺ cells contain mucosal-associated invariant T-type cells and activated CD8⁺ T cells. iNKT cells in both mice and humans are defined as CD3⁺ T cells with semiinvariant T cell receptors (TCRs) that utilize invariant TCR- α chains based on mouse V α 14-J α 18 and human V α 24-J α 18 and recognize CD1d-presented lipids such as α -GalCer (4). In humans, they can be detected with anti-V α 24 antibodies or CD1d tetramers loaded with α -GalCer or an equivalent. In mice, there are no useful antibodies to V α 14 or invariant TCRs, and a convincing way to define them is by using CD1d tetramers loaded with α -GalCer or a homolog. Markers such as CD56 and CD161 in humans and NK1.1 in mice are also activation markers expressed on activated conventional T cells (5). Therefore, the expression of CD56 and NK1.1 molecules is present not only on NKT cells but also on other effector cells. A growing number of studies have shown that NKT cell-based therapy holds great promise in different diseases, especially cancer (6). The study by Jandl and colleagues is the first to demonstrate the essential role of NKT cells in the pathobiology of PH. However, the study still has some limitations. As the authors mentioned, NKT cell subpopulations should also be analyzed using more generally recognized markers, such as CD1d tetramers loaded with α -GalCer. Notably, this research enriches the field of NKT cells and provides a new NKT cell-based target for controlling the progression of PH. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Correspondence and requests for reprints should be addressed to Qiong Zhou, M.D., Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Email: zhouqiongjt@126.com.

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