

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

Kyle Salka, M.S.
Maria Arroyo, M.D.
Samira Naime, M.D.
Elizabeth Chorvinsky, B.Sc.
Children's National Health System
Washington, DC
and
George Washington University
Washington, DC

Maria J. Gutierrez, M.D.
Johns Hopkins University
Baltimore, Maryland

Dinesh K. Pillai, M.D.
Geovanny F. Perez, M.D.
Gustavo Nino, M.D., M.Sc.*
Children's National Health System
Washington, DC
and
George Washington University
Washington, DC

*Corresponding author (e-mail: gnino@childrensnational.org).

References

1. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: more than just signaling the alarm. *J Clin Invest* 2019;129:1441–1451.
2. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med* 2017;377:936–946.
3. Berry CE, Billheimer D, Jenkins IC, Lu ZJ, Stern DA, Gerald LB, et al. A distinct low lung function trajectory from childhood to the fourth decade of life. *Am J Respir Crit Care Med* 2016;194:607–612.
4. Perez GF, Pancham K, Huseni S, Preciado D, Freishtat RJ, Colberg-Poley AM, et al. Rhinovirus infection in young children is associated with elevated airway TSLP levels. *Eur Respir J* 2014;44:1075–1078.
5. Vu LD, Siefker D, Jones TL, You D, Taylor R, DeVincenzo J, et al. Elevated levels of type 2 respiratory innate lymphoid cells in human infants with severe RSV bronchiolitis. *Am J Respir Crit Care Med* [online ahead of print] 25 Jun 2019; DOI: 10.1164/rccm.201812-2366OC.
6. Wolf S, Perez GF, Mukharesh L, Isaza N, Preciado D, Freishtat RJ, et al. Conditional reprogramming of pediatric airway epithelial cells: a new human model to investigate early-life respiratory disorders. *Pediatr Allergy Immunol* 2017;28:810–817.
7. Lee H-C, Headley MB, Iseki M, Ikuta K, Ziegler SF. Cutting edge: inhibition of NF- κ B-mediated TSLP expression by retinoid X receptor. *J Immunol* 2008;181:5189–5193.
8. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations *in vivo*. *Am J Respir Crit Care Med* 2014;190:1373–1382.
9. Anderson EL, Kobayashi T, Iijima K, Bartemes KR, Chen CC, Kita H. IL-33 mediates reactive eosinophilopoiesis in response to airborne allergen exposure. *Allergy* 2016;71:977–988.
10. Snelgrove RJ, Gregory LG, Peiró T, Akthar S, Campbell GA, Walker SA, et al. Alternaria-derived serine protease activity drives IL-33-mediated asthma exacerbations. *J Allergy Clin Immunol* 2014;134:583–592, e6.
11. Han M, Rajput C, Hong JY, Lei J, Hinde JL, Wu Q, et al. The innate cytokines IL-25, IL-33, and TSLP cooperate in the induction of type 2 innate lymphoid cell expansion and mucous metaplasia in rhinovirus-infected immature mice. *J Immunol* 2017;199:1308–1318.
12. Stier MT, Bloodworth MH, Toki S, Newcomb DC, Goleniewska K, Boyd KL, et al. Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal lymphopoietin. *J Allergy Clin Immunol* 2016;138:814–824, e11.
13. Liptzin DR, Landau LI, Taussig LM. Sex and the lung: observations, hypotheses, and future directions. *Pediatr Pulmonol* 2015;50:1159–1169.
14. Tsilingiri K, Fornasa G, Rescigno M. Thymic stromal lymphopoietin: to cut a long story short. *Cell Mol Gastroenterol Hepatol* 2017;3:174–182.
15. Malinczak CA, Fonseca W, Rasky AJ, Ptaschinski C, Morris S, Ziegler SF, et al. Sex-associated TSLP-induced immune alterations following early-life RSV infection leads to enhanced allergic disease. *Mucosal Immunol* 2019;12:969–979.

Copyright © 2020 by the American Thoracic Society



Pharmacologic Inhibition of Vacuolar H⁺ATPase Attenuates Features of Severe Asthma in Mice

To the Editor:

Asthma can be defined as “an abnormal state of the airways which causes the airways to narrow too much and too quickly in response to a wide variety of provoking stimuli” (1), and is associated with numerous genetic and environmental interactions. Because of the complexity and heterogeneity of asthma, some patients may experience spontaneous bronchoconstriction, and especially those with severe asthma are less likely to achieve complete reversibility of airflow obstruction (2). Many patients with asthma also have comorbidities such as obesity and metabolic dysfunction, further complicating the disease phenotype and making treatment more difficult (3). The factors that determine these associations are not well understood. Current therapies that target airway narrowing and inflammation are not entirely comprehensive or universally effective, and many patients remain symptomatic. Despite advances in biological therapies, the decision criteria that clinicians use when selecting a biologic for each patient remain uncertain. Therefore, alternative novel therapies are needed to provide new avenues in the pursuit of severe asthma control. In this study, we tested the therapeutic efficacy of bafilomycin A1 (Baf) compared with fluticasone propionate (FP) in a steroid-insensitive mouse model of severe asthma. Baf, a macrolide produced by *Streptomyces griseus* ssp. *sulphureus*, is a highly selective inhibitor of vacuolar-type H⁺-ATPase (V-ATPase) (4, 5). One function of V-ATPases is to acidify the intracellular environment of lysosomes. In binding to V-ATPases, Baf inhibits autophagy flux by interfering with the fusion of the autophagosome with the lysosome (5, 6). Autophagy is a homeostatic process that is dependent on lysosome fusion to degrade and recycle cellular components, and dysregulation of autophagy is known to be associated with severe asthma pathogenesis (7). We further verified our findings in primary human smooth muscle cells *in vitro*, where we looked at overcoming steroid insensitivity by Baf treatment.

Methods

Female BALB/c mice were subjected to a model of house dust mite (HDM, 100 μ g) allergen in complete Freund's adjuvant (CFA, 100 μ l) (subcutaneous) sensitization and challenge with intranasal

Supported by the funding from the Rebecca Cooper Research Foundation.

Author Contributions: Conception and design: P.S. Data analysis and interpretation: K.D.M., A.K., S.G., and P.S. Performance of experiments: K.D.M. and A.K. Writing of the original draft: K.D.M. and P.S. Reviewing and editing of the manuscript: K.D.M., A.K., M.H., S.G., and P.S.

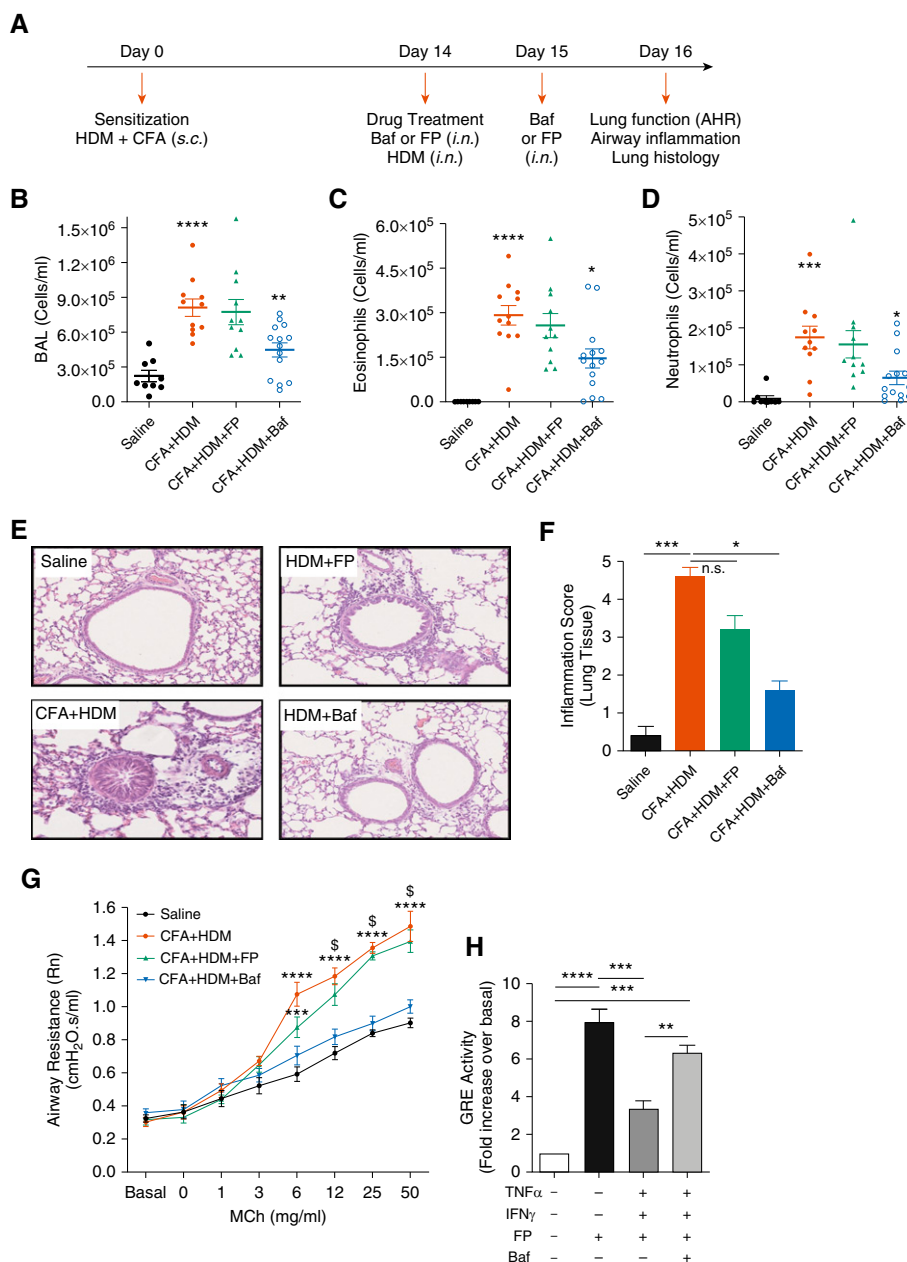


Figure 1. Effect of bafilomycin A1 (Baf) on features of severe allergic asthma in mice. (A) Schematic showing mouse model of severe asthma induced by house dust mite (HDM) and complete Freund's adjuvant (CFA) administration in mice (see METHODS). Fluticasone propionate (FP, 10 μ g) or Baf (3 mg/kg) treatment was given intranasally (i.n.) on the designated days 1 hour before HDM allergen challenge. (B) Airway inflammation was assessed in the BAL fluid by counting the total cell influx. Total immune cells in the BAL fluid were significantly increased in the CFA+HDM-treated animals ($****P < 0.0001$) compared with saline-treated mice, in which immune cells were significantly reduced with Baf treatment ($**P = 0.0065$) but not with FP. (C and D) Cell differentials revealed greater eosinophil (C) and neutrophil (D) influx with HDM challenge ($****P < 0.0001$ and $***P = 0.0009$), whereas reductions were observed with Baf treatment ($*P = 0.0106$ [C] and $*P = 0.0233$ [D]) but not FP. (E and F) Hematoxylin and eosin staining was performed to highlight increased tissue inflammatory cell infiltration in HDM-challenged mice ($***P = 0.0004$ [F]) compared with saline-treated mice. Tissue inflammation was substantially reduced with Baf treatment ($*P = 0.0255$ [F]) but not completely cleared in FP-treated mice ($P = 0.666$ [F]). (G) Lung function data show differences in airway resistance (Rn) derived using a constant phase model in response to increasing concentrations of the contractile agonist methacholine (MCh). HDM-exposed mice showed greater airway hyperresponsiveness (AHR) than saline- and BafA1-treated mice at 6, 12, 25, and 50 mg/ml MCh ($****P < 0.0001$). Baf treatment resulted in lower airway resistance than CFA+HDM, whereas FP-treated mice showed higher airway resistance compared with saline-treated mice ($***P = 0.0001$ at 6 mg/ml MCh and $^{\$}P < 0.0001$ at 12, 25, and 50 mg/ml). (H) FP alone increased glucocorticoid response element (GRE) activity in human airway smooth muscle cells ($****P < 0.0001$), while FP-induced GRE activity was significantly reduced by TNF- α +IFN- γ treatment when compared to FP alone ($****P = 0.0004$). Combination treatment (Baf+FP) significantly recovered GRE activity when compared to FP+TNF- α +IFN- γ treated cells ($**P = 0.0068$), and was no different when compared to FP alone ($P = 0.1793$). Mouse data represent $n = 8$ –12 mice, whereas for cell-based data we used $n = 3$ –4. One- or two-way ANOVA was used with Bonferroni's multiple comparisons test when appropriate, and nonparametric analyses (Kruskal-Wallis test) were used for inflammation scores. n.s. = not significant; s.c. = subcutaneous.

HDM (100 µg HDM in 35 µl saline) to induce severe asthma in the presence and absence of Baf (3 mg/kg) or FP (10 µg) delivered intranasally (Figure 1A). Lung function measurements were performed using flexiVent (Scireq) with increasing concentrations of methacholine (MCh). Airway inflammation was assessed by counting the total immune cell influx in BAL fluid. Multiplexing laser bead technology was used to measure BAL cytokine expression, quantitative PCR was used to measure mRNA of key cytokines, and hematoxylin and eosin staining was used to evaluate inflammation in the lung tissue as previously described (8). Primary human airway smooth muscle (ASM) cells were transfected with glucocorticoid response element (GRE) reporter constructs as previously described (9). Steroid insensitivity was induced by a combined treatment of TNF-α (10 ng/ml) and IFN-γ (500 IU/ml) for 6 hours (10) in the presence or absence of FP (100 nM) ± Baf (20 nM) (2 h before TNF-α+IFN-γ treatment). The cells were then lysed and the luciferase activity was measured. One-way or two-way ANOVA was used when appropriate with Bonferroni's multiple comparisons test. Nonparametric analysis (Kruskal-Wallis test) with Dunn's multiple comparisons was used when applicable and data were expressed as ± SEM. Prism V8.0.1 software (GraphPad) was used for analysis, and $P < 0.05$ was considered statistically significant.

Results

Using the model of severe asthma shown in Figure 1A, we measured the effects of Baf or FP on airway inflammation and hyperresponsiveness. Total immune cells were increased in the BAL of CFA+HDM mice ($****P < 0.0001$) compared with saline-treated mice, which failed to be attenuated with FP treatment but was significantly reduced in CFA+HDM+Baf mice ($**P = 0.0065$) (Figure 1B). Differential cell counting revealed that Baf was able to reduce the number of eosinophils ($*P = 0.0106$)

(Figure 1C) and neutrophils ($*P = 0.0233$) (Figure 1D) compared with CFA+HDM, whereas FP did not reduce these numbers. We also found that CFA+HDM+Baf decreased lung inflammation ($P < 0.05$), whereas FP was not effective in reducing tissue inflammation in this model (Figures 1E and 1F). BAL cytokine analysis revealed increases in the protein concentrations of IL-4, IL-5, IL-13, and CXCL1 (KC) ($****P < 0.0001$) in CFA+HDM mice compared with saline-treated mice (Table 1). Baf treatment significantly reduced the expression of IL-4 ($**P = 0.0022$), IL-5 ($****P < 0.0001$), and KC ($**P = 0.0038$) compared with CFA+HDM treatment, whereas FP reduced the levels of IL-5 ($**P = 0.0087$) but did not change the concentrations of IL-4, IL-13, and KC (Table 1). The fold change of IL-4 ($**P = 0.0023$), IL-5 ($*P = 0.0166$), and IL-13 ($***P = 0.0006$) lung tissue mRNA was increased in HDM+CFA mice compared with saline-treated mice. IL-13 was slightly reduced with Baf treatment, whereas FP treatment resulted in a greater reduction in IL-4 and IL-5 mRNA (Table 1). Sensitization with CFA and HDM challenge induced airway hyperresponsiveness (AHR) to inhaled MCh in mice compared with saline and CFA+HDM+Baf animals ($****P < 0.0001$ at 6, 12, 25 and 50 mg/ml MCh). Baf treatment prevented AHR, whereas FP-treated mice still experienced significant AHR ($***P = 0.0001$ at 6 mg/ml MCh and $*P < 0.0001$ at 12, 25, and 50 mg/ml) (Figure 1G). In ASM cells, GRE-reporter activity was enhanced in the presence of FP alone ($****P < 0.0001$), whereas TNF-α+IFN-γ treatment rendered cells steroid insensitive ($***P = 0.0004$). Baf treatment was able to overcome steroid insensitivity in ASM cells ($**P = 0.0068$) (Figure 1H).

Discussion

Severe asthma remains difficult to treat with current therapies; therefore, potential targets for effective treatment of severe asthma are being investigated (7, 11). We recently demonstrated that autophagy can be targeted in chronic asthma (8). In this study, we show that treatment with BafA1 alleviates airway inflammation and prevents the

Table 1. Cytokine/Chemokine Protein and mRNA Expression in BAL and Lung Tissue

BAL Cytokine Protein Expression				
Cytokine/chemokine	Saline pg/ml (±SEM)	CFA+HDM pg/ml (±SEM)	CFA+HDM+FP pg/ml (±SEM)	CFA+HDM+Baf pg/ml (±SEM)
IL-4	5.2 (2.86)	269* (27.8)	194.2 (15.9)	139.8 [†] (24.8)
IL-5	6.6 (2.23)	187.4* (12.8)	121 [†] (18.2)	72.8* (9.8)
IL-13	1 (0.55)	124.8* (24.6)	73.8 (8.4)	78.6 (9.4)
KC (CXCL1)	8.6 (4.5)	375.4* (35.5)	302.2 (49.4)	142.2 [†] (48.5)

Lung tissue cytokine mRNA levels				
Cytokine	Saline fold change (±SEM)	CFA+HDM fold change (±SEM)	CFA+HDM+FP fold change (±SEM)	CFA+HDM+Baf fold change (±SEM)
IL-4	1.0 (0.16)	20.7 [†] (4.1)	10.3 (2.6)	17.8 (1.8)
IL-5	1.0 (0.15)	8.7 [†] (1.7)	3.3 (2.2)	7.7 (1.1)
IL-13	1.5 (0.63)	316 [§] (53.5)	284.3 (61.3)	215 (29.0)

Definition of abbreviations: Baf = bafilomycin; CFA = complete Freund's adjuvant; FP = fluticasone propionate; HDM = house dust mite; KC = keratinocyte chemoattractant.

* $P < 0.0001$.

[†] $P < 0.01$.

[‡] $P < 0.05$.

[§] $P < 0.001$.

development of AHR associated with HDM challenge and the steroid-resistant severe asthma phenotype *in vitro* and *in vivo*. Bafilomycins inhibit V-ATPase and at high concentrations are capable of blocking late-phase autophagy and inhibiting the formation of autophagolysosomes (12). At lower concentrations, bafilomycins have been shown to target both early and late phases of autophagy, and demonstrate antiviral properties with the absence of host cytotoxicity (5, 13). Pretreatment with BafA1 (to inhibit autophagy) followed by a TAS2R (taste 2 receptor) agonist mitigated TAS2R-induced cell death in human ASM cells (14). TAS2R agonists have previously been shown to promote ASM relaxation, inhibit mitogen-induced cell growth, and mitigate features of allergic airways disease (15, 16). TGF- β (transforming growth factor β) induces autophagy and fibrosis simultaneously, as shown in human atrial myofibroblasts and ASM (8, 17). It was also previously shown that BafA1 treatment can reduce a TGF- β -induced fibrogenic response in atrial fibroblasts (17). The findings of this study highlight the therapeutic potential of BafA1 in severe asthma. Our results indicate that exploring the role of autophagy modulation in severe asthma therapy could potentially lead to novel treatment opportunities for severe asthma. ■

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

Kielan D. McAlinden, Ph.D.*
Anudeep Kota, M.S.*
Mehra Haghi, Ph.D.
University of Technology Sydney
Sydney, New South Wales, Australia

Saeid Ghavami, Ph.D.
University of Manitoba
Winnipeg, Manitoba, Canada

Pawan Sharma, Ph.D.†
Thomas Jefferson University
Philadelphia, Pennsylvania

ORCID ID: 0000-0002-2904-2306 (P.S.).

*These authors contributed equally to this work.

†Corresponding author (e-mail: pawan.sharma@jefferson.edu).

References

1. Woolcock AJ. Strategies for the management of asthma. *Respirology* 1996;1:79–83.
2. Boulet LP. Irreversible airway obstruction in asthma. *Curr Allergy Asthma Rep* 2009;9:168–173.
3. Chapman DG, Irvin CG, Kaminsky DA, Forgione PM, Bates JH, Dixon AE. Influence of distinct asthma phenotypes on lung function following weight loss in the obese. *Respirology* 2014;19:1170–1177.
4. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozana A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* 2016;12:1–222.
5. Yeganeh B, Ghavami S, Kroeker AL, Mahood TH, Stelmack GL, Klonisch T, et al. Suppression of influenza A virus replication in human lung epithelial cells by noncytotoxic concentrations bafilomycin A1. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L270–L286.
6. Ghavami S, Mutawe MM, Schaafsma D, Yeganeh B, Unruh H, Klonisch T, et al. Geranylgeranyl transferase 1 modulates autophagy and apoptosis in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L420–L428.
7. Poon AH, Chouiali F, Tse SM, Litonjua AA, Hussain SN, Bagloli CJ, et al. Genetic and histologic evidence for autophagy in asthma pathogenesis. *J Allergy Clin Immunol* 2012;129:569–571.
8. McAlinden KD, Deshpande DA, Ghavami S, Xenaki D, Sohal SS, Oliver BG, et al. Autophagy activation in asthma airways remodeling. *Am J Respir Cell Mol Biol* 2019;60:541–553.
9. Moodley T, Wilson SM, Joshi T, Rider CF, Sharma P, Yan D, et al. Phosphodiesterase 4 inhibitors augment the ability of formoterol to enhance glucocorticoid-dependent gene transcription in human airway epithelial cells: a novel mechanism for the clinical efficacy of roflumilast in severe chronic obstructive pulmonary disease. *Mol Pharmacol* 2013;83:894–906.
10. Tliba O, Damera G, Banerjee A, Gu S, Baidouri H, Keslacy S, et al. Cytokines induce an early steroid resistance in airway smooth muscle cells: novel role of interferon regulatory factor-1. *Am J Respir Cell Mol Biol* 2008;38:463–472.
11. Martin LJ, Gupta J, Jyothula SS, Butsch Kovacic M, Biagini Myers JM, Patterson TL, et al. Functional variant in the autophagy-related 5 gene promoter is associated with childhood asthma. *PLoS One* 2012;7:e33454.
12. Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct Funct* 1998;23:33–42.
13. Yuan N, Song L, Zhang S, Lin W, Cao Y, Xu F, et al. Bafilomycin A1 targets both autophagy and apoptosis pathways in pediatric B-cell acute lymphoblastic leukemia. *Haematologica* 2015;100:345–356.
14. Pan S, Sharma P, Shah SD, Deshpande DA. Bitter taste receptor agonists alter mitochondrial function and induce autophagy in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2017;313:L154–L165.
15. Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, et al. Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 2010;16:1299–1304.
16. Sharma P, Yi R, Nayak AP, Wang N, Tang F, Knight MJ, et al. Bitter taste receptor agonists mitigate features of allergic asthma in mice. *Sci Rep* 2017;7:46166.
17. Ghavami S, Cunningham RH, Gupta S, Yeganeh B, Filomeno KL, Freed DH, et al. Autophagy is a regulator of TGF- β 1-induced fibrogenesis in primary human atrial myofibroblasts. *Cell Death Dis* 2015;6:e1696.

Copyright © 2020 by the American Thoracic Society



Ciliary Localization of the Intraflagellar Transport Protein IFT88 Is Disrupted in Cystic Fibrosis

To the Editor:

In healthy airways, inhaled microbial pathogens are trapped in mucus and removed by the directional movement of cilia, a process known as “mucociliary clearance” (MCC) (1). This mechanism relies on the

Supported in part by a grant from the Lundbeck Foundation to the Innovation Centre, Denmark, and Stanford University to fund the Lundbeck Foundation Clinical Research Fellowship (E.M.S.), by the Stanford Maternal and Child Health Research Institute TIP award (C.M.), and by the Ross Mosier CF Research Laboratories fund. S.T.C. was supported by the University of Copenhagen Excellence Programme for Interdisciplinary Research (2016 Funds), Independent Research Fund Denmark (6108-00457), the Novo Nordisk Foundation (NNF15OC0016886), and the Carlsberg Foundation (CF18-0532 and CF15-0425).

Author Contributions: All authors contributed to the study concept and design. C.M. recruited the subjects and collected the nasal brush biopsies. E.M.S., E.K.V., S.T.C., and C.M. performed microscopic analyses and imaging processing. E.M.S. calculated base/axoneme ratios. C.M. performed the statistical analysis. E.M.S. drafted the manuscript, and all authors critically revised it, gave final approval of it, and consented to publishing it.

This letter has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.