Changes in Lung Clearance Index in Preschool-aged Patients with Cystic Fibrosis Treated with Ivacaftor (GOAL): A Clinical Trial

To the Editor:

Cystic fibrosis (CF) lung disease starts early in life and progresses throughout infancy and the preschool years (1, 2). Therapeutic strategies initiated early in life have the potential to reverse this process and maintain lung health. Ivacaftor is a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator that increases the channel's opening probability. Remarkable and sustained lung function improvements have been demonstrated in patients aged 6 years and older with gating CFTR mutations such as *G551D* on at least one allele (3, 4). A recent open-label trial provided evidence for safety and nutritional benefits in children aged 2–5 years, but lung function was not systematically assessed within this study (5). The lung clearance index (LCI) measured by multiple-breath washout has been shown to be a sensitive measure to capture lung function abnormalities in patients with CF and is feasible for use in preschool children because it requires minimal cooperation. LCI can also be used to detect treatment effects in interventional trials (6, 7). As part of GOAL (*G551D* Observational Study) (NCT 01521338), a multicenter longitudinal observational study to explore biomarkers as well as clinical and physiological characteristics of patients with CF with gating mutations initiated on ivacaftor therapy (8), we report the results of LCI in preschool patients assessed before and after ivacaftor treatment initiation.

Multiple-breath washout measurements based on nitrogen as the tracer gas using the EXHALYZER D (Eco Medics) were performed in preschool children with CF aged 3 to 5 years. Measurements were performed in accordance with recently published standards for preschool children (9) and modifications of the equipment to reduce dead space as described previously (2). Baseline was defined as the predose measurement immediately preceding the start of treatment; all other medications were kept constant throughout the study period. Changes in LCI from baseline to 1 month and from baseline to 6 months after the start of ivacaftor treatment were analyzed using nonparametric Wilcoxon signed-rank tests. Relative changes in LCI compared with baseline were evaluated using one-sample Kolmogorov-Smirnov tests.

This analysis includes five subjects with CF enrolled in the GOAL study in the United States and an additional four preschool-aged patients with CF started on ivacaftor at the Hospital for Sick Children in Toronto (SickKids). Baseline characteristics of the study population are displayed in Table 1. Median sweat chloride concentration at baseline was 103 mEq/L. All nine patients carried one copy of the *G551D* mutation, with seven patients being compound heterozygous for *F508del*. The average LCI at baseline was 10.6 (interquartile range [IQR], 8.9 to 11.2); seven of nine patients had LCI values above the upper limit of normal, reflecting increased ventilation inhomogeneity (2) (Figure 1A).

Mean sweat chloride concentrations decreased to 46 mEq/L 1 month after ivacaftor initiation (mean change [IQR], −56 [−61 to −47]). Within-patient LCI significantly improved 1 month after the first dose of ivacaftor (median LCI at 1 mo, 7.10 [6.6 to 8.5]; $P = 0.02$). The improvement in LCI was maintained 6 months after the first dose (median LCI, 7.3 [7.3 to 7.6]; $P = 0.03$). Assessing relative changes in LCI at 1 month and 6 months after ivacaftor treatment yielded similar results. LCI significantly improved from baseline at both 1 month (median change in LCI from baseline, −23.6% [IQR, −34.2 to −20.2]; $P < 0.001$) and 6 months post-treatment (median change in LCI from baseline, −24.6% [IQR, −31.4 to −20.4]; $P < 0.001$). Thus, rapid and sustained improvements in LCI were demonstrated with ivacaftor therapy that exceeded the between-test reproducibility and thus the physiologically relevant change for quarterly LCI measurements in health, which we recently demonstrated to be 15% in preschool children (10). The only two patients not showing an improvement in LCI had normal baseline values of LCI. The observed effect size was similar to what has been described in older patients with CF treated with ivacaftor (6, 11). This study also supports the concept that LCI is a suitable outcome measure to capture changes in lung function in preschool children on CFTR modulator therapy, and also that ivacaftor helps improve early airway disease even in young children. ■

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

Felix Ratjen, M.D., Ph.D.
Michelle Klingel, M.Sc.
University of Toronto
Toronto, Ontario, Canada

Philip Black, M.D.
Children's Mercy Hospital
Kansas City, Missouri

Michael R. Powers, M.D.
Oregon Health & Sciences University
Portland, Oregon

Hartmut Grasemann, M.D., Ph.D.
Melinda Solomon, M.D.
University of Toronto
Toronto, Ontario, Canada

Scott D. Sagel, M.D., Ph.D.
University of Colorado School of Medicine
Aurora, Colorado

Scott H. Donaldson, M.D.
North Carolina Children's Hospital
Chapel Hill, North Carolina

Steven M. Rowe, M.D.
University of Alabama at Birmingham
Birmingham, Alabama

Margaret Rosenfeld, M.D.
Seattle's Children's Hospital
Seattle, Washington

Supported by the Cystic Fibrosis Foundation.

Originally Published in Press as DOI: 10.1164/rccm.201802-0243LE on April 3, 2018

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Table 1. Demographic Characteristics of the Study Population at the Baseline Visit

Characteristic	GOAL (*n* = 5)	SickKids (*n* = 4)	GOAL + SickKids (*n* = 9)
Age, yr	4.2 (4.0 to 5.1)	4.8 (3.0 to 5.8)	4.2 (3.9 to 5.2)
Female, *n* (%)	3 (60%)	2 (50%)	5 (56%)
Genetics, first allele, *n* (%)			
G551D	5 (100%)	4 (100%)	9 (100%)
Genetics, second allele, *n* (%)			
F508del	3 (75%)	4 (80%)	7 (78%)
1717-1G→A	1 (11%)	1 (20%)	0 (0%)
R1066C	1 (11%)	0 (0%)	1 (25%)
Height *z*-score	0.52 (0.47 to 1.06)	−0.49 (−1.08 to 0.14)	0.34 (−0.92 to 0.52)
Weight *z*-score	0.55 (−0.13 to 0.88)	−0.19 (−0.80 to −0.02)	−0.09 (−0.30 to 0.55)
BMI *z*-score	−0.12 (−0.73 to 0.62)	−0.28 (−0.55 to 0.39)	−0.12 (−0.55 to 0.62)
LCI	10.6 (8.9 to 11.2)	8.9 (7.6 to 11.6)	9.8 (8.2 to 11.2)
Sweat chloride	105 (103 to 107)	98 (94 to 103)	103 (96 to 105)
Pancreatic insufficiency, *n* (%)	5 (100%)	4 (100%)	9 (100%)
Infections, *n* (%)			
Staphylococcus aureus	2 (50%)*	1 (25%)	3 (38%)*
Haemophilus influenzae	2 (50%)*	0 (0%)	2 (25%)*
Pseudomonas aeruginosa	0 (0%)*	0 (0%)	0 (0%)*
Concomitant medications, *n* (%)			
Dornase alfa	2 (40%)	1 (25%)	3 (33%)
Hypertonic saline	2 (40%)	0 (0%)	2 (22%)
Oral antibiotics	0 (0%)	1 (25%)	1 (11%)

Definition of abbreviations: BMI = body mass index; GOAL = G551D Observational Study; LCI = lung clearance index; SickKids = Hospital for Sick Children in Toronto.
All continuous variables are presented as medians with interquartile ranges.
*One GOAL subject did not have microbiology assessments performed at baseline.

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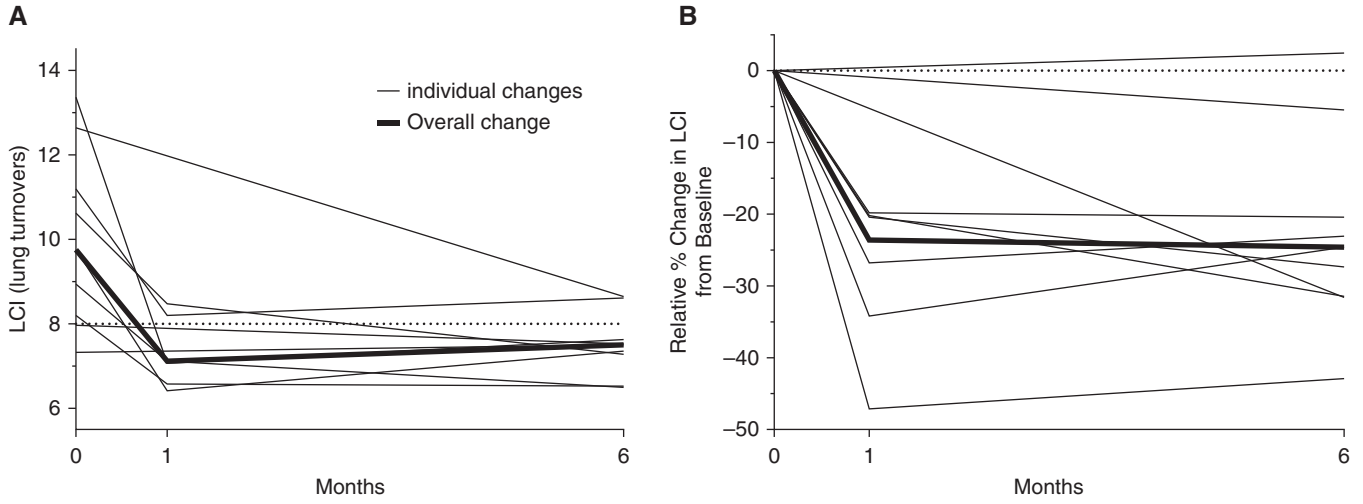

Figure 1. (*A*) Absolute and (*B*) relative median changes per subject in lung clearance index (LCI) in preschool children treated with ivacaftor. Absolute LCI significantly improved 1 month after the first dose of ivacaftor (median [interquartile range (IQR)] LCI at 1 mo, 7.1 [6.6 to 8.5]; $P = 0.02$). The improvement in LCI was maintained 6 months after the first dose (median [IQR] LCI, 7.5 [7.3 to 7.6]; $P = 0.03$). Similarly, relative LCI significantly improved from baseline at both 1 month (median [IQR] change in LCI from baseline, −23.6% [−34.2 to −20.2]; $P < 0.001$) and 6 months after treatment (median [IQR] change in LCI from baseline, −24.6 [−31.4 to −20.4]; $P < 0.001$). The dotted lines represent an upper limit of normal of 8 in *A* and a change of 0% in *B*.

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The Role of the Human Immune System in Chronic Hypoxic Pulmonary Hypertension

To the Editor:

Pulmonary hypertension (PH) of classes 1–3 is associated with perivascular infiltration of mast cells, B cells, T cells, macrophages, and dendritic cells; the formation of tertiary lymphoid tissues; and the generation of autoantibodies (1, 2). In rats, adaptive and innate immune cells, including mast cells and B cells, are causally linked to PH in that their deficiency or depletion attenuates the disease (3, 4). Although the emerging recognition of (auto)immunity in PH has fueled the exploration of antiinflammatory and immunosuppressive treatment strategies (5), the underlying mechanisms remain obscure. Regrettably, immunotargeted therapies work poorly in murine PH, preventing a mechanistic dissection by means of knockout or transgenic mouse models. Mast cell stabilizers have proven effective in rat models of PH and yielded promising results in a first clinical trial (4, 6); yet, mast cells do not seem relevant in murine PH (7). This species dependence may be attributable to distinct features of the murine immune response (8). The use of mouse models is further hampered by the fact that, in contrast to rats and humans, mice develop only mild PH with little muscularization of the precapillary arterioles and minimal smooth muscle cell proliferation (9). We hypothesized that these features may be causally linked in that differences in the murine immune response account for the mild PH phenotype and poor efficacy of immunotargeted therapies.

Methods

We compared the development of chronic hypoxic PH and responsiveness to immunotargeted therapy in three mouse strains: Balb/c mice, immunodeficient NOD scid γ (NSG) mice, and NSG mice engrafted with human hematopoietic $CD34^+$ progenitor cells (humanized mice; all from The Jackson Laboratory), which differentiate into multilineage, mature human blood and immune cells. The protocol was approved by the Animal Care Committee of St. Michael's Hospital. Before experiments were conducted, human cells accounted for 67.7% ± 13.7% (mean ± SEM) of $CD45^+$ leukocytes in the peripheral blood of the humanized mice. For each strain, female mice (20–22 wk old) were exposed to either normoxia, chronic hypoxia (5 wk, 10% O_2), or hypoxia and treatment with the mast cell stabilizer cromolyn (40 mg/kg i.p. daily). Right ventricular systolic pressure (RVSP) and heart rate were assessed by microtip catheter, tricuspid annular plane systolic excursion and pulmonary artery acceleration time were assessed by echocardiography, and right ventricular (RV) hypertrophy was assessed using the Fulton index. Perivascular infiltration of mast cells, T cells, B cells, and macrophages in lung tissue was analyzed by immunofluorescence or immunohistochemistry using antimouse tryptase, CD3, CD19, or CD68 antibodies in Balb/c and NSG mice, and the corresponding antihuman antibodies in humanized mice (all from Abcam). From hematoxylin and eosin– and α-smooth muscle actin–stained histological sections, the medial wall thickness and the fraction of nonmuscularized, partially muscularized, or fully muscularized vessels in pulmonary arterioles less than 50 μm in diameter were quantified in blinded analyses. Data are means ± SEM. Statistical differences ($P < 0.05$) were tested by Pearson's χ^2 test or one-way ANOVA with Dunnett's *post hoc* test.

Results

Relative to Balb/c and NSG mice, chronic hypoxia in humanized mice markedly increased the RVSP, wall thickening, and muscularization of small pulmonary arterioles (Figure 1A). Normoxic baseline data and heart rate did not differ between strains. The Fulton index increased, and tricuspid annular plane systolic excursion and pulmonary artery acceleration time decreased in chronic hypoxia, but showed no difference between

Supported by grants-in-aid from the Canadian Institutes of Health Research (CIHR) and the Heart and Stroke Foundation of Canada to W.M.K., and an Erwin Schroedinger Fellowship from the Fonds zur Förderung der wissenschaftlichen Forschung (Austrian Science Fund) to D.Z. Y.H. is supported by a grant for Excellent Young Scholars from the Third Military Medical University, and K.A.C. is supported by a CIHR New Investigator Award and an Early Researcher Award from the Ministry of Ontario.

Author Contributions: Y.H., D.Z., W.G., S.B., N.M.G., and G.K. conducted experiments. Y.H., K.A.C., and W.M.K. designed experiments and analyzed the data. Y.H. and W.M.K. drafted the manuscript, and all authors edited the original manuscript.

Originally Published in Press as DOI: 10.1164/rccm.201711-2175LE on April 9, 2018