



Long-term effect of CFTR modulator therapy on airway nitric oxide

To the Editor:

The fraction of exhaled nitric oxide (F_{eNO}) is generally lower in individuals with cystic fibrosis (CF), compared to healthy controls. Two recent studies reported that the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator ivacaftor resulted in an increase in F_{eNO} after 4 weeks' therapy [1, 2], suggesting that changes in F_{eNO} have the potential to serve as biomarker of restored CFTR function. However, it is currently unknown whether ivacaftor results in a sustained increase in F_{eNO} and whether combination therapy of ivacaftor with the CFTR corrector lumacaftor also leads to changes in F_{eNO} . Therefore, the objective of this research was to document long-term effects of ivacaftor and lumacaftor-ivacaftor therapy on F_{eNO} in treated CF patients. The two prospective observational studies were approved by the local institutional review boards (Hospital for Sick Children's research ethics board #1000036224 and #1000057599, St Michael's Hospital research ethics board #13-089). Patients were included if they had a confirmed diagnosis of CF and were eligible for treatment with either therapy. F_{eNO} was measured before and 1, 3, 6, 12 and 24 months after initiation of therapy during regular outpatient visits. Sputum samples were collected in the ivacaftor cohort. The nonliquid phase (mucus plugs) of the sputum was processed by adding 0.1% dithiothreitol in Dulbecco's PBS (4:1, vol:wt), and the clear supernatant of the cell suspension was separated from the cells by centrifugation [3, 4]. No protease inhibitors were added. Samples were stored at -80°C before analysis of L-arginine metabolism using liquid chromatography-mass spectrometry (LC-MS), as reported [5]. Nitric oxide (NO) metabolites nitrate and nitrite were measured in sputum using Griess reagent [6] and myeloperoxidase (MPO) was measured using ELISA (R&D Systems, Minneapolis, MN, USA). Changes in outcomes between visits were assessed using paired t-tests and Wilcoxon signed-rank tests for skewed distributions, and correlations were assessed using Pearson's correlation coefficient.

34 patients treated with CFTR modulator therapy were included. The ivacaftor cohort consisted of eight paediatric (median (interquartile range (IQR)) age 12.5 (9.4–14.0) years, 63% female) and 12 adult (age 32.6 (24.6–42.8) years, 58% female) patients. In the lumacaftor-ivacaftor cohort there were 14 paediatric patients (age 14.5 (14.0–16.9) years, 57% female) and no adults.

At 1 month follow-up the ivacaftor cohort consisted of seven children and nine adult patients; at 1 year six children and five adults; and at 2 years six children and four adults. Forced expiratory volume in 1 s (FEV_1) at baseline was 78 (71–90)% predicted in the paediatric patients and 62 (47–74)% pred in the adult patients. FEV_1 was improved for the total group at 1 month, from 69.7% pred to 81.1% pred (change 11.4, 95% CI 6.1–16.8; $p<0.001$), and remained improved for the entire follow-up. Similar results were seen for forced vital capacity (FVC), with an improvement from 88.9% pred to 97.8% pred (change 8.9, 95% CI 5.0–12.8; $p<0.001$) at 1 month, which persisted throughout follow-up.

F_{eNO} was increased compared to baseline after 4 weeks on ivacaftor ($n=16$), as previously reported [1], and remained increased throughout follow-up (figure 1). When including all measurements, F_{eNO} weakly correlated with FVC ($r=0.29$, $p=0.009$) and FEV_1 ($r=0.41$, $p<0.001$; $n=81$), but changes in F_{eNO} did not correlate with changes in pulmonary function tests over time ($r=0.19$, $p=0.13$ for FEV_1 ; $r=0.14$, $p=0.28$ for FVC % pred; $n=64$).

A mild decrease at 1 month was seen in sputum L-arginine, a substrate for NO synthases (NOS) (baseline 14.7 (8.1–40.7) $\mu\text{mol}\cdot\text{L}^{-1}$, median change -5.4 , $p=0.04$; $n=13$), and an insignificant decrease for the NOS

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Ivacaftor results in a sustained increase in F_{eNO} in children and adults with CF. The increase in F_{eNO} may be related to changes in airway NO metabolism by myeloperoxidase. Lumacaftor-ivacaftor therapy does not have an immediate effect on F_{eNO} . <http://bit.ly/2mzqhds>

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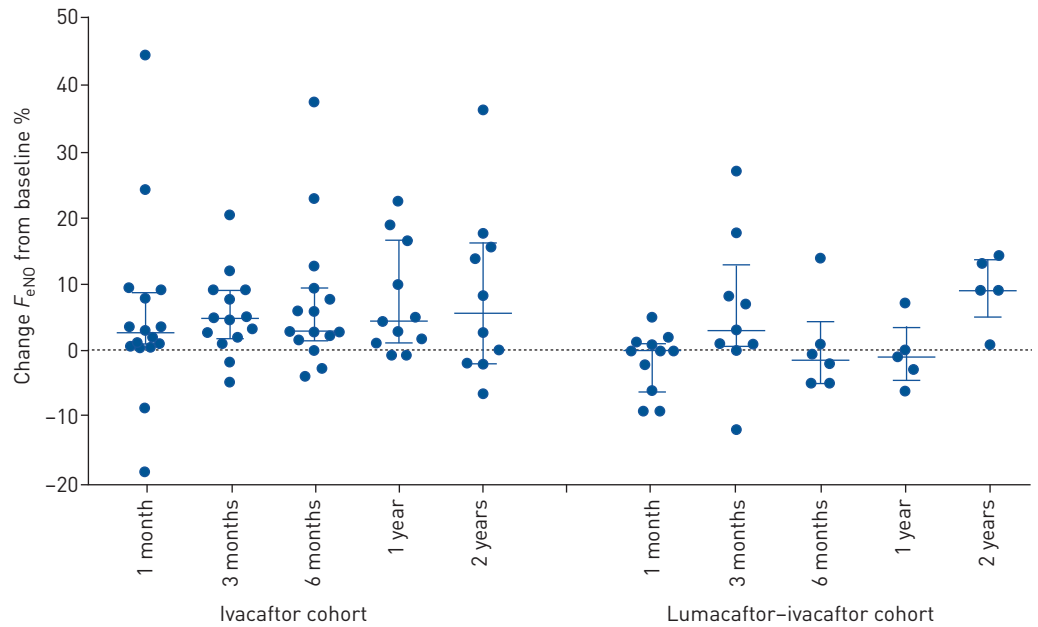


FIGURE 1 Changes in the exhaled nitric oxide fraction (F_{eNO}) in patients with cystic fibrosis treated with ivacaftor or lumacaftor-ivacaftor. Each data point represents an individual measurement. Also shown are median and interquartile range changes from before treatment baseline. Median F_{eNO} was increased from baseline at all time points in ivacaftor-treated patients. A significant increase in F_{eNO} in the lumacaftor-ivacaftor-treated patients was seen only at 2 years follow-up.

inhibitor asymmetry dimethylarginine (ADMA) (baseline 0.08 (0.01 – 0.26) $\mu\text{mol}\cdot\text{L}^{-1}$, median change -0.01 ; $p=0.06$). While there was a correlation between F_{eNO} and sputum L-arginine/ADMA ratio, an index of NOS impairment ($r=0.31$, $p=0.03$; $n=51$), which was supportive of previous observations [5], L-arginine/ADMA remained unchanged during follow-up.

MPO was decreased at 1 month (baseline 27.0 (10.7 – 52.2) $\mu\text{g}\cdot\text{mL}^{-1}$, median change -4.8 ; $p=0.03$) in the total group ($n=9$), but at no other time point. There was no change in sputum levels of nitrite or nitrate in the total group, but reduced nitrite at 4 weeks in adults (baseline 22.8 (21.0 – 37.2) $\mu\text{mol}\cdot\text{L}^{-1}$, median change -9.1 , $p=0.03$). Nitrite/nitrate ratio was decreased from baseline at 4 weeks ($p=0.06$), but at no other visit and in adults only ($n=7$). There was a significant reverse correlation between sputum MPO and F_{eNO} ($r=-0.34$, $p=0.02$; $n=45$), suggesting that a decrease in MPO content may contribute to the increase in F_{eNO} with ivacaftor therapy.

Median (IQR) FEV_1 for the lumacaftor-ivacaftor cohort was 80.1 (68.6 – 93.8)% pred at baseline and was not significantly different at any time point after initiation of therapy. There was no change in FVC % pred during follow-up.

Median (IQR) F_{eNO} before lumacaftor-ivacaftor (10 (8 – 15) ppb) was similar to ivacaftor baseline values, but did not change within the first year of treatment (figure 1). However, increased F_{eNO} from baseline was found for five patients at the 2-year follow-up (median increase 9 ppb, 95% CI 2.8 – 15.6 ppb; $p=0.02$). Changes in F_{eNO} did not correlate with changes in FEV_1 ($r=0.14$, $p=0.35$).

Alterations in L-arginine/NO metabolism and reduced NO availability are thought to be clinically important in CF, as low NO may contribute to airways obstruction and favour colonisation with certain pathogens. While the exact reasons for the decreased F_{eNO} in CF are currently not known, studies have shown that 4 weeks of therapy with the CFTR potentiator ivacaftor resulted in an increase of F_{eNO} [1, 2, 7]. We here present data showing that the increase in F_{eNO} on ivacaftor therapy is not short-lived or transient, but rather sustained over a 2-year follow-up. Furthermore, using LC-MS analyses in sputum we demonstrate that the long-term increase in F_{eNO} is not due to changes in L-arginine availability or L-arginine metabolism in the airways. However, our data suggest that changes in airway metabolism of NO by MPO may be a cause of the observed increase in F_{eNO} .

Previous studies have shown that the relative quantities of NO, NO_2^- and NO_3^- provide mechanistic insight into NO metabolism in the CF lung and that NO catabolism within the airway prior to exhalation may contribute to reduced F_{eNO} in CF. NO oxidised by MPO results in NO_2^- and continued oxidation by

MPO and other oxidative processes results in NO_3^- formation [8, 9]. We observed a decrease in sputum MPO levels, decreased nitrite and thus relatively more nitrate (no change in total $\text{NO}_2^- + \text{NO}_3^-$), which suggests that liberation of NO from NO_2^- stored in airway secretions may contribute to the observed increase in F_{eNO} at 4 weeks of therapy. Although changes in MPO were not significant at other time points, a potential role of MPO for the observed changes in NO metabolism was supported by a reverse correlation of MPO sputum levels and F_{eNO} . However, our observations are based on relatively low numbers and do not exclude the possibility that other mechanisms such as changes in airway pH, inflammation and increased NO synthase expression or function may contribute to the long-term effect of ivacaftor therapy on airway NO. In addition, the decreasing number of CF adults during follow-up may introduce a bias towards positive changes in F_{eNO} over time, as the effect of ivacaftor on airway NO may be more pronounced in children compared to adults with CF [1].

In contrast to ivacaftor, lumacaftor–ivacaftor therapy did not result in a consistent change in F_{eNO} . This may be related to differences in efficacy, as lumacaftor–ivacaftor it is known to have less significant effects on respiratory outcomes in patients homozygous for F508del, compared to ivacaftor monotherapy in patients with gating mutations [10]. The lack of effect on F_{eNO} in children within the first year of treatment was consistent with a recent observation in four adults with CF [7]. However, F_{eNO} was significantly increased from baseline in a small number of our paediatric patients at 2 years follow-up (n=5). Whether longer term treatment with lumacaftor–ivacaftor results in a sustained effect on F_{eNO} will need to be shown in future studies.

Ivacaftor results in a sustained increase in F_{eNO} in children and adults with CF. The increase in F_{eNO} with ivacaftor may be related to changes in airway NO metabolism by MPO. Lumacaftor–ivacaftor did not have an immediate effect on F_{eNO} in treated children, supporting findings in adults with CF.

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