CFTR Modulator Use Is Associated with Higher Hemoglobin Levels in Individuals with Cystic Fibrosis

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Abstract

Rationale: Understanding how cystic fibrosis transmembrane conductance regulator (CFTR) modulators influence comorbid conditions like anemia is of great interest to the cystic fibrosis community.

Objectives: To test the hypothesis that CFTR modulators are associated with higher hemoglobin (Hgb) levels.

Methods: Annualized Hgb and other laboratory, demographic, and anthropometric data were abstracted from the U.S. CF Foundation Patient Registry for adult and pediatric registrants before and after therapy with ivacaftor (IVA) or lumacaftor/ivacaftor (LUM/IVA) between January 2010 and December 2016. Univariate and multivariate linear mixed models were used to examine the effect of IVA on Hgb in patients with G551D-CFTR, and the effect of LUM/IVA on Hgb in F508del-CFTR homozygotes. Linear regression was used to characterize change in mean Hgb over time.

Results: A total of 1,347 registrants (707 males and 640 females) with G551D-CFTR and 12,582 F508del-CFTR homozygotes (6,640 males and 5,942 females) who had never undergone lung transplant and had contemporaneous data regarding Hgb and CFTR modulator use were identified. IVA was associated with average Hgb increases of 0.54 gm/dl (95% confidence interval [CI], 0.39–0.69; $P < 0.0001$) and 0.18 gm/dl (95% CI, 0.01–0.35; $P = 0.037$) for males and females, respectively, with G551D-CFTR. LUM/IVA was associated with average Hgb increases of 0.58 gm/dl (95% CI, 0.48–0.68; $P < 0.0001$) and 0.26 gm/dl (95% CI, 0.20–0.33; $P < 0.0001$) for male and female F508del-CFTR homozygotes, respectively. In multivariate models, IVA positively affected Hgb in males but not females, and LUM/IVA positively affected Hgb in both sexes.

Conclusions: IVA and LUM/IVA use are both associated with higher Hgb levels in patients with CF.

Keywords: cystic fibrosis; ivacaftor; lumacaftor; hemoglobin; anemia

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Several high-profile clinical trials have demonstrated that drugs that correct and/or potentiate the function of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel improve lung function and reduce pulmonary

exacerbation (PEx) risk in individuals with cystic fibrosis (CF) who have certain CFTR mutation profiles (1–3). Given that CFTR is widely expressed throughout the human body (4–8), it is reasonable to expect that CFTR modulators could confer health

benefits referable to organs outside the aerodigestive tract. Indeed, recent “real world” reports have described amelioration of CF-related diabetes (CFRD) in a F508del/G551D-CFTR patient (9) and improved insulin secretion in five patients with

G551D-CFTR (10) treated with ivacaftor (IVA). Researchers have observed weight gain and lower rates of *Pseudomonas aeruginosa*- and *Staphylococcus aureus*-positive sputum cultures in IVA-treated patients with G551D-CFTR (11–14). *In vitro* studies also suggested that CFTR modulators might be salubrious through noncanonical mechanisms, including improved immune cell function (15, 16) and reduced tone of smooth muscle found in arterial and bronchial walls (17).

Anemia with or without iron deficiency has been recognized in the CF population for decades (18–20), but it continues to be only partially understood as an extrapulmonary complication. Depending on the definition being considered, approximately 10–30% of CF cohorts are anemic (18, 19, 21). In adults with CF, lower hemoglobin (Hgb) values are associated with more severe lung function impairment, malnutrition, iron deficiency, and an increased likelihood of having CF-related diabetes (CFRD) (22). Oral iron supplementation does not improve Hgb levels in anemic adults with CF despite a modest correction of blood iron stores (23), an observation that suggests that additional factors contribute to anemia.

Whether CF-related anemia is caused by absolute and/or functional iron deficiency or restricted erythropoiesis is not altogether clear, but CFTR function might be important for the production and/or longevity of red blood cells (RBCs) that contain Hgb. In normal individuals, RBC and epithelial cell plasma membranes have a similar abundance of CFTR (24), whereas CFTR abundance in RBC plasma membranes from patients with CF is reduced by ~70% (25). In a zebrafish model, the PDZ binding domain of CFTR, but curiously not its chloride channel activity, was found to be indispensable for Wnt-dependent hematopoiesis (26). This finding constitutes the most direct evidence for the involvement of CFTR in RBC production.

In the present study, we leveraged the U.S. CF Foundation Patient Registry (CFFPR) (27) and the recent introduction and now mainstream use of CFTR modulators in clinical practice to determine whether IVA and lumacaftor (LUM) combined with IVA (LUM/IVA) are associated with higher Hgb values in CF. We hypothesized that pharmacologic restoration of CFTR function by CFTR

modulators would independently improve Hgb concentrations in patients with CF.

This work was previously presented as an abstract at the 2018 North American Cystic Fibrosis Conference in Denver, Colorado (28).

Methods

Study Population

This was a retrospective study of children and adults with CF who were followed at >120 accredited care centers in the United States and consented (primarily or via caregivers) to have their personal health information collected in the CFFPR. The history, organization, and accuracy of data in the CFFPR have been described by others (28, 29). Data collected between January 1, 2010 and December 31, 2016 were analyzed. Complete blood counts (CBCs), which included white blood cell (WBC) counts, Hgb levels, and platelet counts, were first tabulated in the CFFPR in 2010. Data used in this study came from annualized profiles for each registrant. Registrants with any history of lung transplant (TXP) or who were not candidates for IVA or LUM/IVA therapy based on their CFTR genotype, or who had missing Hgb and/or drug prescription data were excluded. For each registrant with concurrent assessments of Hgb and IVA or LUM/IVA use in the CFFPR, data from the last year before initiation of treatment were considered to be baseline. This approach ensured that each registrant had at least one pre- and one post-treatment Hgb measurement.

Outcomes of Interest

The main outcome of interest was the estimated effect of IVA or LUM/IVA use on Hgb concentration, expressed as gm/dl, in males and females with one or two G551D-CFTR alleles or two F508del-CFTR alleles, respectively. The main outcome of interest was assessed in univariate and then multivariate analyses to adjust for covariates that could influence Hgb levels. These covariates were chosen *a priori* based on biological plausibility and/or clinical relevance and included the following: age (yr), height (cm), weight (kg), Global Lung Initiative (GLI) forced expiratory volume in 1 second (FEV₁)% predicted, infection with *Pseudomonas aeruginosa* and/or methicillin-resistant *Staphylococcus aureus*

(MRSA), hemoptysis (any severity), CFRD, pancreatic enzyme replacement therapy, oxygen use, CF vitamin use, WBC count, platelet count, number of clinic visits per year, and any PEX during a given year.

Statistical Analyses

Given that Hgb concentrations are lower in females than in males in the general population (30, 31), the final study cohort was divided according to sex and treatment type. Unpaired Student's *t* tests with Welch's approximation for unequal variances were used to compare all continuous variables except for numbers of pre- and post-treatment Hgb values per patient and numbers of pre- and post-treatment years of follow-up, in which case Wilcoxon rank-sum tests were used to compare medians. Chi-squared (χ^2) tests were used to compare proportions of patient groups with respect to categorical variables. Generalized linear mixed models (GLMMs) were constructed in which subject was a random effect to account for repeated measures and within-subject variability, IVA or LUM/IVA was a fixed effect, and predefined covariates were included simultaneously as fixed effects. The variance-covariance structure of the random effect was assumed to be unstructured. The statistical significance of fixed effects was evaluated using the Wald χ^2 test. Standard error (SE) calculations were clustered by subject. Two-way ANOVA was used to determine whether five quantiles of mean year-to-year Hgb difference contained similar numbers of patients. Linear regression was used to characterize variation in the mean Hgb level over time. All analyses were performed using STATA MP 15.1 (StataCorp). Graphs were created using GraphPad Prism 7.01 for Windows (GraphPad Software). A two-sided *P* value < 0.05 was considered statistically significant.

Regulatory Approval

The study protocol was approved by the Committee for the Protection of Human Subjects at Dartmouth College (#28756). CFFPR data were released to A.H.G. after the protocol (PRR119) was reviewed by the CFFPR committee.

Results

The process by which the final study cohort was identified is outlined in the STROBE

diagram in Figure 1. The initial CFFPR dataset contained information on 35,442 registrants collected from 2010 to 2016. We excluded data from 2,477 people who had undergone lung TXP at any time before or during the 7-year study period. From the remaining cohort of 32,965 individuals who had never had a lung TXP, 1,679 people were excluded because they lacked Hgb data. An additional 16,204 patients were excluded because they were not candidates for IVA or LUM/IVA treatment based on their CFTR genotype profile. Comparisons of the clinical and biochemical characteristics of patients who were or were not drug treatment candidates based on their genotype profile are provided in Table E1. Of the 15,082 patients who were IVA or LUM/IVA candidates, 1,153 did not have any documentation of IVA or LUM/IVA use and were also excluded. The final study cohort included 13,929 registrants, 1,347 (707 males and 640 females) of whom had one or two G551D-CFTR alleles, and 12,582 (6,640 males and 5,942 females) of whom had two F508del-CFTR alleles.

The median number of pre-IVA Hgb values per patient was 3 (interquartile range [IQR] = 3) for G551D-CFTR females and 3 (IQR = 2) for G551D-CFTR males. The median number of post-IVA Hgb values per patient was 4 (IQR = 2) for G551D-CFTR females and males. The median number of years of data collection before initiation of IVA was 3 (IQR = 3) for male and 3 (IQR = 4) for female patients with G551D-CFTR. The median number of years of data collection after initiation of IVA for both male and female patients with G551D-CFTR was 5 (IQR = 1). The median number of pre- and post-LUM/IVA Hgb values was 5 (IQR = 2) and 2 (IQR = 1), respectively, for both male and female F508del-CFTR homozygotes. The median number of years of data collection before initiation of LUM/IVA for male and female F508del-CFTR homozygotes was 7 (IQR = 1) and 7 (IQR = 0), respectively. The median number of years of data collection after initiation of LUM/IVA for both male and female F508del-CFTR patients was 2 (IQR = 1). The median total number of years of follow-up per patient was 6 (IQR = 0).

The baseline characteristics of the final study population are listed in Table 1. Within the G551D-CFTR cohort, age, lung function, prevalence of comorbid

Figure 1. STROBE diagram of the study population, including numbers of patients who were excluded because of a history of lung transplant (TXP), lack of hemoglobin (Hgb) data, cystic fibrosis transmembrane conductance regulator (CFTR) genotype profile for which ivacaftor (IVA) or lumacaftor/ivacaftor (LUM/IVA) treatment was not indicated, and lack of drug treatment data. The final study cohort is described with respect to type of drug treatment and sex.

Table 1. Clinical and biochemical characteristics of the final study cohort

	Baseline Patient Characteristics*			
	Any G551D (n = 1,347)		F508del x 2 (n = 12,582)	
	Male (n = 707)	Female (n = 640)	Male (n = 6,640)	Female (n = 5,942)
Age, yr	16.2 (13.3)	15.9 (13.2)	17.3 (11.9) [†]	16.4 (11.3)
Height, cm	138.2 (40.6) [†]	131.1 (35.3)	145.8 (34.1) [†]	137.9 (30.0)
Weight, kg	44.4 (28.2) [†]	38.0 (22.5)	46.5 (24.3) [†]	39.9 (19.2)
White, n (%)	602 (85)	587 (92)	6,454 (97)	5,756 (97)
GLI FEV ₁ % predicted	78.8 (24.3)	79.4 (23.8)	78.9 (23.3) [†]	80.2 (22.6)
GLI FVC% predicted	90.2 (18.9)	89.2 (19.6)	90.3 (18.4)	90.3 (18.7)
<i>Pseudomonas aeruginosa</i> , n (%)	383 (54)	351 (55)	3,272 (49)	3,015 (51)
MRSA, n (%)	195 (28)	192 (30)	2,448 (37) [†]	2,403 (40)
Hemoptysis, n (%)	7 (1)	6 (1)	359 (5)	202 (3)
CFRD, n (%)	97 (14)	90 (14)	1,634 (25) [†]	1,579 (27)
PERT, n (%)	616 (87)	545 (85)	6,591 (99)	5,889 (99)
Vitamin use, n (%)	604 (85)	535 (84)	6,522 (98)	5,859 (98)
Any supplemental feeding, n (%)	453 (64) [†]	357 (56)	5,610 (84) [†]	4,800 (81)
Medicare, n (%)	57 (8)	53 (8)	705 (11)	683 (11)
Medicaid, n (%)	265 (37)	250 (39)	3,436 (52)	3,131 (53)
Oxygen therapy, n (%)				
None	547 (77)	476 (74)	4,306 (65)	3,904 (66)
Continuously	10 (1)	14 (2)	124 (2)	143 (3)
Nocturnal and/or exertion	12 (2)	10 (1)	102 (1)	133 (2)
During exacerbation	25 (4)	25 (4)	301 (5)	317 (5)
As needed	6 (1)	2 (1)	55 (1)	62 (1)
Missing	11 (1)	7 (1)	77 (1)	50 (1)
Unavailable	96 (14)	106 (17)	1,675 (25)	1,333 (22)
White blood cell count, ×10 ³ /mcl	9.4 (3.5) [†]	9.8 (3.3)	9.6 (3.4) [†]	9.8 (3.3)
Hemoglobin, gm/dl	13.6 (2.2) [†]	12.9 (2.3)	13.9 (1.9) [†]	12.9 (1.7)
Platelet count, ×10 ³ /mcl	301 (82) [†]	323 (75)	292 (82) [†]	314 (78)
Clinic visits in year, n	4.3 (2.4) [†]	5.0 (2.7)	4.6 (2.5) [†]	5.3 (2.9)
Any pulmonary exacerbation in year, n (%)	415 (59)	372 (58)	6,065 (91)	5,475 (92)
Any hospitalization in year, n (%)	319 (45) [†]	309 (48)	4,612 (69) [†]	4,388 (74)
Death in year, n (%)	16 (2)	15 (2)	115 (2)	124 (2)

Definition of abbreviations: CF = cystic fibrosis; CFRD = cystic fibrosis–related diabetes; CFTR = cystic fibrosis transmembrane conductance regulator; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; GLI = Global Lung Initiative; LUM/IVA = lumacaftor/ivacaftor; MRSA = methicillin-resistant *Staphylococcus aureus*; PERT = pancreatic enzyme replacement therapy.

*Data from the last year before first prescription of IVA or LUM/IVA as documented in the CF Foundation Patient Registry.

[†]P < 0.05 for comparison between males and females with the same CFTR genotype profile.

conditions, and patterns of lung infection were similar between the sexes, but males were taller and heavier than females and were more commonly observed to use supplemental nutrition. In the homozygous F508del-CFTR cohort, males were taller and heavier than females, and they were also slightly older, more likely to use supplemental nutrition, and less likely to have CFRD or MRSA infection than females. GLI FEV₁% predicted was somewhat lower in homozygous F508del-CFTR males than females despite the similitude of GLI forced vital capacity (FVC)% predicted.

Multiple distinctions were apparent between males and females irrespective of the CFTR genotype profile (Table 1). WBC and platelet counts were higher and Hgb concentrations were lower in females. Females had higher numbers of clinic visits

and hospitalizations per year compared with males, but the same number of PEx per year as males.

Unadjusted, IVA was associated with 0.54 gm/dl (95% confidence interval [CI], 0.39–0.69; P < 0.0001) and 0.18 gm/dl (95% CI, 0.01–0.35; P = 0.037) increases in the mean Hgb level for G551D-CFTR males and females, respectively (Figure 2, left panel). Similarly, after initiation of LUM/IVA, 0.58 gm/dl (95% CI, 0.48–0.68; P < 0.0001) and 0.26 gm/dl (95% CI, 0.20–0.33; P < 0.0001) increases in the mean Hgb level were observed in male and female F508del-CFTR homozygotes, respectively (Figure 2, right panel). The complete output from both univariate GLMMs can be found in Table E2.

The estimated adjusted effects of IVA and other covariates on the mean Hgb

concentration in patients with G551D-CFTR are presented in Figure 3 and Table E3. This multivariate GLMM showed that even after adjustment for potential confounders, a positive effect of IVA was still observed for G551D-CFTR males (0.23 gm/dl; 95% CI, 0.05–0.41; P = 0.013), but not females (−0.07 gm/dl; 95% CI, −0.30 to 0.16; P = 0.552). Subgroup analyses (Figures E1–E4) showed that IVA was not associated with higher Hgb among G551D-CFTR females with CFRD, *P. aeruginosa* infection, fewer than eight clinic visits per year, and age < 35 years, whereas a positive effect of IVA on Hgb was identified among G551D-CFTR males with these attributes and G551D-CFTR females without them.

In the homozygous F508del-CFTR population, a modest positive effect of

Figure 2. Comparison of estimated hemoglobin differences by sex associated with ivacaftor (IVA) or lumacaftor/ivacaftor (LUM/IVA) in patients with cystic fibrosis and one or two G551D-CFTR alleles or two F508del-CFTR alleles, respectively, using CF Foundation Patient Registry data collected between 2010 and 2016. Lines denote the 95% confidence interval of the estimated mean hemoglobin value. * $P < 0.05$ for same-treatment, same-sex comparison. CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator.

LUM/IVA on Hgb was seen in males (0.10 gm/dl; 95% CI, 0.01–0.20; $P = 0.026$) and females (0.16 gm/dl; 95% CI, 0.08–0.23; $P < 0.0001$), even after adjustment for covariates (Figure 4, Table E4). Higher body weight (kg) and GLI FEV₁% predicted were predictors of higher Hgb readings, while older age, any use of supplemental oxygen, and history of PEx in a given year were predictors of lower Hgb readings for both sexes (Table E4). Age-group responses to LUM/IVA are depicted in Figure E5.

We determined that modulator-related improvements in Hgb were time dependent in patients with G551D-CFTR (Figures 5A and 5B) and F508del-CFTR × 2 patients (Figures 5C and 5D). Coefficients of linear regression equations describing the variation in mean Hgb readings over time are given in Table E5. To exclude the possibility that our thesis about higher Hgb levels and CFTR modulator use was informed by subgroups with disproportionately robust Hgb responses, we confirmed that quantiles of mean year-to-year Hgb difference contained similar numbers of patients (Tables E6–E9). We did not observe any significant change in mean Hgb from 2010–2016 for males with class III–V CFTR mutations other than G551D-CFTR for whom modulator therapy was not indicated (Table E10, Figure E6). However, we found a modest downward trend in mean Hgb

from 2010–2016 for females with class III–V CFTR mutations other than G551D-CFTR (Table E10, Figure E6).

Discussion

This retrospective study of Hgb data collected in the CFFPR between 2010 and 2016 represents the first detailed assessment of this important hematologic parameter in a large cohort of patients with CF ($N = 13,929$). In the last year before CFTR modulator initiation, mean Hgb level, body weight, and height were higher in males than in females irrespective of CFTR genotype profile. Also of note, females had uniformly higher WBC and platelet counts, less prevalent use of supplemental nutrition, and a greater number of clinic visits per year before treatment. Unadjusted for potential confounders, IVA and LUM/IVA were associated with statistically significant improvements in Hgb. Covariate adjusted, the relationships between CFTR modulator use and Hgb were more modest, but they remained statistically significant in F508del-CFTR homozygotes and G551D-CFTR males.

The fact that Hgb levels in venous blood are generally higher in males than in females is a feature common to mammals (32), so we were not surprised to observe the same distinction in our analyses of

data from patients with CF. More interesting, however, is that CFTR functional restoration by IVA and LUM/IVA had a more robust association with improved Hgb in males than in females (Figure 2, Table E2). Investigators have rationalized the evolutionarily conserved phenomenon of relative anemia among females as being a consequence of menstruation, which reduces not only red cell mass but also iron stores (33); however, this cannot be the sole explanation given that this sex-related Hgb disparity is also observed in nonmenstruating, nonplacental mammals (32). A compelling theory proposed by Murphy (32) is that estrogens and androgens differentially influence the afferent microcirculation supplying the renal juxtaglomerular apparatus, a structure that is involved in sensing tissue oxygen delivery and blood volume (34). Although CFTR is widely distributed in the kidney (35), the function of the juxtaglomerular apparatus is not known to depend on CFTR. Because CFTR is normally found in erythrocyte plasma membranes and is reduced in abundance in erythrocytes from patients with CF (24, 25), our findings suggest that CFTR modulators might favorably affect erythrocyte development, function, and/or survival, and that these effects could differ in magnitude between the sexes. It is curious that males more commonly used supplemental nutrition than females despite similar frequencies of pancreatic enzyme replacement therapy and CF vitamin use (Table 1), but we cannot conclude that supplemental nutrition independently factored into sex-related Hgb differences. Higher pretreatment WBC and platelet counts in females (Table 1) could attest to sex-specific differences in the inflammatory milieu given that WBC and platelet counts increase during periods of physiologic stress (36, 37).

We acknowledge the possibility that restoring CFTR function favorably but indirectly affects Hgb by alleviating “inflammatory block,” a phenomenon in which erythropoiesis is restricted by a hepcidin-mediated reduction in blood iron content, impairment of erythrocyte maturation by multiple cytokines, and shortened erythrocyte survival due to macrophage activation (38). Consistent with this theory are simultaneous observations of lower serum iron levels and higher serum

Figure 3. Covariate-adjusted estimates of the effects of ivacaftor (IVA) on hemoglobin in males (green circles) and females (orange circles) with G551D-CFTR from the multivariate generalized linear mixed model in Table E3. Lines denote the 95% confidence interval of the estimated mean hemoglobin (gm/dl). CF = cystic fibrosis; CFRD = CF-related diabetes; GLI FEV₁ = Global Lung Initiative forced expiratory volume in 1 second; MRSA = methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; PERT = pancreatic enzyme replacement therapy; PEx = pulmonary exacerbation; WBC = white blood cell.

hepcidin-25 and IL-6 levels in adults with CF (39). However, mitigation of inflammation, as evidenced by a reversal in these relationships among serum iron, hepcidin-25, and IL-6 during PEx treatment, did not improve anemia in the same subjects, but this could have been a consequence of the short duration of observation (39). Assuming that higher WBC and platelet counts reflect a heightened inflammatory state and that inflammatory block causes anemia in CF, we

might have expected to see a more dramatic Hgb response to CFTR modulators among females who had higher baseline WBC and platelet counts than males (Table 1), but in fact, the opposite was true (Figure 2). In the general population, WBC and platelet counts tend to be higher in females than in males (31), so the more robust Hgb increase associated with CFTR modulators in males might have less to do with sex-specific differences in systemic inflammation and more to do with differences between

men and women with respect to iron homeostasis, blood loss through menstruation, and/or nutritional status. Alternatively, higher WBC and platelet counts in females with and without CF might speak to similarly (albeit modestly) heightened levels of systemic inflammation. Clearly, more work is needed to gain a mechanistic understanding of why CFTR modulators improve Hgb levels in patients with CF.

Interestingly, the covariate-adjusted Hgb estimates for G551D-CFTR females treated with IVA were the only ones that were found to be statistically insignificant. This may be due in part to the smaller sample size and/or other unmeasured confounders. However, there is evidence that pregnancy rates among women with CF and G551D-CFTR treated with IVA more than doubled between 2010 and 2014 (40). It is conceivable that this trend masked improvements in Hgb attributable to IVA, given that Hgb decreases during the first and second trimesters of pregnancy.

The human body increases RBC mass via complex mechanisms in response to chronic hypoxemia, as exemplified by people who live at high altitudes (41). Lack of secondary erythrocytosis (and instead the presence of anemia) in hypoxemic patients with CF was recognized decades ago (42) and again reported more recently (21). By including patterns of supplemental oxygen use as a covariate in Hgb GLMMs, we intended to explore this paradox for the first time in a large cohort of patients with CF, although the frequency with which patients used supplemental oxygen, as tabulated in the CFFPR, was admittedly a proxy for hypoxemia severity. The CFFPR does not capture oxyhemoglobin saturation data, so we were unable to include that in our models. Nonetheless, we present strong evidence in the multivariate Hgb GLMMs for ineffective erythropoiesis in CF despite hypoxemia, a potent stimulus red blood cell production. Absolute and/or functional iron deficiency affects many patients with CF (19, 22, 43, 44), and erythropoiesis is restricted by one or both of these states (45). Because the CFFPR does not gather data on biomarkers of iron homeostasis and prescription of iron supplements, blood transfusions, and/or erythropoietin, we could not test the hypothesis that our observation of higher Hgb levels associated with CFTR

modulators was influenced by these confounders.

Other predictive relationships between covariates and Hgb from the GLMMs (Figures 3 and 4, Tables E3 and E4) should be mentioned. Higher Hgb levels were associated with higher GLI FEV₁% predicted. Thus, our results are congruent with the observation of von Drygalski and Biller (18) that lung function was better in nonanemic patients with CF ≥ 16 years of age. In certain presumably healthy

populations, higher values of biomarkers of iron sufficiency (46) and Hgb (47) are correlated with higher FEV₁, so our finding and that of von Drygalski and Biller may not be unique to individuals with CF. To the best of our knowledge, this is the first assessment of relationships between Hgb and WBC count, and Hgb and platelet count in CF. In the Hgb GLMMs for patients with G551D-CFTR, the WBC count tended to vary directly with Hgb, whereas the platelet count varied indirectly with Hgb (Table E3). The

same relationship was true among F508del-CFTR homozygotes only with respect to Hgb and WBC count (Table E4). We did not question whether CFTR modulators discretely influenced the WBC and platelet counts.

In addition to the aforementioned caveats regarding interpretation of our findings, our study has a number of limitations. First, we had to exclude 2,832 (~8.0%) of the 35,442 patients in the original dataset due to missing data. Their lack of data could have invalidated one or more of our conclusions. Second, we cannot presume that CFTR functional restoration would be associated with higher Hgb readings in the sizable cohort that was excluded based on genotype profile (Figure 1). These nondrug candidates differed in many respects from individuals who had CFTR modulator options (Table E1). Most of these distinctions in nondrug candidates could individually or collectively inform better overall health and higher Hgb levels on that basis. Third, our findings are only relevant to Caucasians with CF, a fact that is consistent with the demography of the CFTR genotype profiles examined (48–50) but does not address racial differences in Hgb (30, 51). Fourth, we did not study patients who had undergone lung TXP, because Hgb levels in this population could be affected by myriad factors, including immunosuppressive drugs and infection. Fifth, our Hgb GLMMs incorporated a finite set of covariates that we believed could reasonably augment or diminish any effect of CFTR modulators on Hgb, but our reasoning is subject to critique. Thus, we could have residual confounding and unmeasured confounders. For example, better nutrition could have led to improved Hgb during the study period in ways not accounted for by our choices of covariates. However, we are justified in exploring a range of Hgb covariates because precious little was known about CF-related anemia before our study. Sixth, we assumed that medication prescription tabulated in the CFFPR was synonymous with medication use. Drug prescription data in the CFFPR can deviate from those contained in the electronic medical record, but this is uncommon (27). Finally, given our study design, we can only speculate about a mechanism by which CFTR modulation improves Hgb. Nonetheless, our observations stand to assist the CF research community by offering a clinically relevant pretext for

Figure 4. Covariate-adjusted estimates of the effects of lumacaftor/ivacaftor (LUM/IVA) on hemoglobin in males (green circles) and females (orange circles) with two F508del-CFTR alleles from the multivariate generalized linear mixed model in Table E4. Lines denote the 95% confidence interval of the estimated mean hemoglobin effect (gm/dl). CF = cystic fibrosis; CFRD = CF-related diabetes; GLI FEV₁ = Global Lung Initiative forced expiratory volume in 1 second; MRSA = methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; PERT = pancreatic enzyme replacement therapy; PEx = pulmonary exacerbation; WBC = white blood cell.

Figure 5. (A–D) Change in mean hemoglobin over time in G551D-CFTR males (A), G551D-CFTR females (B), F508del-CFTR × 2 males (C), and F508del-CFTR × 2 females (D). Dotted lines denote the 95% confidence interval of the best-fit regression lines for hemoglobin as function of time in years for populations that were (green dots and lines) and were not (orange dots and lines) treated with a modulator. CFTR = cystic fibrosis transmembrane conductance regulator; IVA = ivacaftor; LUM = lumacaftor; SD = standard deviation.

in vitro studies of CFTR and erythrocyte biology.

It is very reasonable to question why the magnitude of the modulator-associated Hgb increase was smaller in patients with G551D-CFTR treated with IVA than in F508del-CFTR homozygotes treated with LUM/IVA (Figure 2), given that IVA reduces sweat chloride levels more potently than LUM/IVA (52, 53). This observation can be rationalized if CFTR modulation improves Hgb by a mechanism other than chloride channel activity, a possibility that is supported by data from *in vitro* experiments (26). It is also possible that patients with

G551D-CFTR were healthier in respects not accounted for by our covariate-adjusted GLMMs, and accordingly, they might have had a less robust Hgb response. We also found that G551D-CFTR females ≥ 35 years old were the only age-restricted subgroup to have a statistically significant increase in Hgb associated with IVA (Figure E4). It is plausible that G551D-CFTR women <35 years old might lose enough iron through menstruation to develop iron-deficiency anemia, or they might experience the physiologic anemia of pregnancy more often, such that IVA does not appear to improve Hgb levels in this population.

Additionally, female patients with G551D-CFTR who survived to ≥35 years of age but did not receive IVA might have had other unmeasured reasons to be more anemic than their contemporaries who received IVA.

To summarize, we have leveraged data from the CFFPR to demonstrate that the clinical use of IVA and LUM/IVA in patients with CF and CFTR genotype profiles in whom these drugs restore CFTR function is independently associated with higher Hgb concentrations. We provide estimates of the magnitude of this effect with and without adjustment for factors that could influence Hgb. Importantly, we also

confirm that IVA and LUM/IVA increase Hgb to a lesser extent in females than in males with CF. Future energies should be spent to better understand this discrepancy and the basis for the phenomenon. ■

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