

Urinary leukotriene E₄/exhaled nitric oxide ratio and montelukast response in childhood asthma

Nathan Rabinovitch, MD,^a Nora J. Graber, MS,^b Vernon M. Chinchilli, PhD,^b Christine A. Sorkness, PharmD,^c Robert S. Zeiger, MD, PhD,^d Robert C. Strunk, MD,^e Leonard B. Bacharier, MD,^e Fernando D. Martinez, MD,^f and Stanley J. Szefer, MD,^a for the Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute* *Denver, Colo, Hershey, Pa, Madison, Wis, San Diego, Calif, St Louis, Mo, and Phoenix, Ariz*

Background: A subset of children with asthma respond better to leukotriene receptor antagonists than to inhaled corticosteroids. Information is needed to identify children with these preferential responses.

Objective: We sought to determine whether the ratio of urinary leukotriene E₄ (LTE₄) to fractional exhaled nitric oxide (FE_{NO}) delineates children with preferential responsiveness to montelukast compared with fluticasone propionate (FP) therapy.

Methods: Data from 318 children with mild-to-moderate asthma enrolled in 2 National Heart, Lung, and Blood Institute Childhood Asthma Research and Education Network studies (Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid [CLIC] and the Pediatric Asthma Controller Trial [PACT]) were analyzed. The association between LTE₄/FE_{NO} ratios at baseline and improved

lung function or asthma control days (ACDs) with montelukast and FP therapy was determined, and phenotypic characteristics related to high ratios were assessed.

Results: LTE₄/FE_{NO} ratios were associated with a greater response to montelukast than FP therapy for FEV₁ measurements (2.1% increase per doubling of ratio, $P = .001$) and for ACDs per week (0.3-ACD increase, $P = .009$) in the CLIC study. In PACT the ratio was associated with greater FEV₁ responsiveness to montelukast than FP therapy (0.6% increase, $P = .03$). In a combined study analysis, LTE₄/FE_{NO} ratios were associated with greater response to montelukast than FP therapy for FEV₁ (0.8% increase, $P = .0005$) and ACDs (0.3-ACD increase, $P = .008$). Children with LTE₄/FE_{NO} ratios at or above the 75th percentile were likely ($P < .05$) to be younger and female and exhibit lower levels of atopic markers and methacholine reactivity.

Conclusion: LTE₄/FE_{NO} ratios predict a better response to montelukast than FP therapy in children with mild-to-moderate asthma. (*J Allergy Clin Immunol* 2010;126:545-51.)

Key words: Asthma, biomarkers, fluticasone propionate, inhaled corticosteroids, leukotriene E₄, montelukast

From ^athe Department of Pediatrics, National Jewish Health and the University of Colorado Denver School of Medicine, Denver; ^bthe Department of Public Health Sciences, Pennsylvania State University, Hershey; ^cthe Schools of Pharmacy and Medicine and Public Health, University of Wisconsin, Madison; ^dthe Department of Pediatrics, University of California—San Diego, and the Department of Allergy, Kaiser Permanente, San Diego; ^ethe Department of Pediatrics, Washington University School of Medicine, St Louis; and ^fthe Department of Pediatrics, University of Arizona, Phoenix.

*Contributors to the Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute are listed in Appendix E1 in this article's Online Repository at www.jacionline.org.

Supported by NIH/NIEHS K23 grant no. ES015510-01 and grants HL064307, HL64288, HL064295, HL64287, and HL064305 from the National Heart, Lung, and Blood Institute. This study was carried out in part by the General Clinical Research Centers at Washington University School of Medicine (M01 RR00036), National Jewish Health (M01 RR00051), and the University of Wisconsin (M01 RR03186).

Disclosure of potential conflict of interest: N. Rabinovitch has a patent application pending for LTE₄/FE_{NO} ratio from National Jewish Health. V. M. Chinchilli receives research support from the National Heart, Lung, and Blood Institute (NHLBI). C. A. Sorkness is on advisory boards for GlaxoSmithKline, Schering-Plough, AstraZeneca, and Novartis and receives research support from Schering-Plough and Sandoz. R. S. Zeiger has consultant arrangements with AstraZeneca, Aerocrine, Genentech, GlaxoSmithKline, Merck and Co, Schering-Plough, and MedImmune and receives research support from Aerocrine, Genentech, Merck and Co, GlaxoSmithKline, AstraZeneca, and TEVA Pharm. L. B. Bacharier receives honoraria from AstraZeneca, Genentech, GlaxoSmithKline, Merck, Schering-Plough, and Aerocrine. F. D. Martinez receives honoraria from MedImmune and Merck and receives research support from the National Institutes of Health (NIH). S. J. Szefer is a consultant for GlaxoSmithKline, Genentech, Merck, Boehringer-Ingelheim, Novartis, and Schering-Plough and receives research support from the NIH/NHLBI, the NIH/National Institute of Allergy and Infectious Disease, GlaxoSmithKline, and the NIEHS/US Environmental Protection Agency. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 19, 2010; revised June 25, 2010; accepted for publication July 7, 2010.

Reprint requests: Stanley J. Szefer, MD, National Jewish Health, 1400 Jackson St, Rm J304, Denver, CO 80206. E-mail: Szefer@njhealth.org.
0091-6749/\$36.00

© 2010 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2010.07.008

Clinicians currently have 2 main alternatives for initiation of pharmacotherapy in children with mild-to-moderate persistent asthma. These choices are inhaled corticosteroids (ICSs) or leukotriene receptor antagonists (LTRAs), especially montelukast. Population studies and clinical trials have established that ICS therapy is more effective than LTRAs on almost all major health outcomes.¹ However, there is a subset of patients who appear to respond better to LTRAs with greater improvements in lung function² and asthma control,³ highlighting the need to detect predictive characteristics to identify this population.

In this context, the National Heart, Lung, and Blood Institute (NHLBI) Childhood Asthma Research and Education (CARE) Network Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid (CLIC)^{2,3} and Pediatric Asthma Controller Trial (PACT)^{4,5} studies were designed to identify predictors of ICS (ie, fluticasone propionate [FP]) and montelukast responsiveness in school-aged children with mild-to-moderate disease. In these studies characteristics of children with better responses to FP therapy were defined in a reproducible manner. Greater differential responses to FP over montelukast therapy were associated in both the CLIC study and PACT, with greater methacholine reactivity and higher levels of fractional exhaled nitric oxide (FE_{NO}).^{2,5} Biological or physiological predictors of the subset of children who responded preferentially to montelukast therapy, however, could not be consistently identified.

Abbreviations used

ACD:	Asthma control day
CLIC:	Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid
FE _{NO} :	Fractional exhaled nitric oxide
FP:	Fluticasone propionate
ICS:	Inhaled corticosteroid
LTE ₄ :	Leukotriene E ₄
LTRA:	Leukotriene receptor antagonist
NHLBI:	National Heart, Lung, and Blood Institute
PACT:	Pediatric Asthma Controller Trial

A recent study of children with moderate-to-severe asthma predominantly receiving ICS therapy suggested that the ratio of 2 biomarkers, urinary leukotriene E₄ (LTE₄; a marker of cysteinyl leukotriene production and elimination) and FE_{NO}, could help identify children who would respond to the addition of montelukast.⁶ The authors of this study speculated that montelukast therapy might be most effective when the ratio of cysteinyl leukotriene inflammation (as measured by urinary LTE₄) is high relative to eosinophilic inflammation (as measured by FE_{NO}). This observation highlighted the increased predictive value of using a combination of biomarkers to predict therapeutic responses. The present analysis hypothesized that LTE₄/FE_{NO} ratios would be associated with a greater response to LTRAs than to ICS therapy in children with mild-to-moderate persistent asthma.

METHODS

Study protocols

Methods and primary outcome results for the CLIC study^{2,3} and PACT^{4,5} have previously been extensively described. Appropriate institutional review board approval was obtained for each study before recruitment. Briefly, the CLIC study was a randomized crossover study of 127 children (6-17 years of age) with mild-to-moderate asthma. Blood for measurement of eosinophils, serum IgE, eosinophilic cationic protein, and FE_{NO} and urine for measurement of LTE₄ were collected at randomization (1 sample per child). Urinary LTE₄ was measured as described by Westcott et al.⁷ Urine was treated with 50 mg of murine antibody against the peptidoleukotrienes LTC₄, LTD₄, LTE₄, and N-acetyl LTE₄. The urine and antibody were allowed to incubate at room temperature for 4 to 5 hours. After filtering through a 10,000-μm cutoff filter, the LTE₄-antibody complex was separated by means of precipitation of the antibody with methanol. After evaporation of the methanol, an enzyme immunoassay was used for quantification. Urine LTE₄ levels were reported in picograms and standardized per milligram of creatinine. FE_{NO} levels were measured with a rapid-response chemiluminescent analyzer (flow rate, 50 mL/s; NIOX System, Aerocrine, Sweden), according to the guidelines of the American Thoracic Society.⁸ Total serum IgE (Pharmacia CAP system; Pharmacia, Uppsala, Sweden) and eosinophilic cationic protein (Pharmacia CAP system) levels were measured at a central site in serum from blood clotted at room temperature and then frozen and shipped.

Children were randomized either to active FP (100 μg twice daily administered by means of the Diskus device; GlaxoSmithKline, Research Triangle Park, NC) with montelukast placebo or to active montelukast (5 or 10 mg) by mouth nightly (based on age recommendations) with FP placebo for an 8-week period, at which time they were crossed over and treated for a second 8-week period. Asthma outcomes, including FEV₁ and asthma control days (ACDs), were collected at randomization and every 4 weeks subsequently.

PACT was a parallel randomized study of 285 children (aged 6-14 years) with mild-to-moderate asthma. Asthma outcome data, FE_{NO} values, and urine for LTE₄ were collected (1 sample per child) during a 2- to 4-week baseline characterization period and measured as described previously.^{7,8} Children

were randomized to one of 3 treatment arms (95 children in each arm): active FP (100 μg twice daily by means of Diskus), active montelukast (5 mg by mouth nightly), or active combination FP (100 μg plus 50 μg of salmeterol each morning by means of Diskus) and salmeterol (50 μg nightly by means of Diskus) with appropriate placebos. Children were treated for 12 months, with asthma control indices collected every 6 weeks. For this analysis, only the FP and montelukast arms were evaluated.

Statistical analysis

Demographic, biomarker, or asthma control differences between the CLIC study and PACT were compared by using χ^2 (for categorical) or Student *t* tests for continuous variables. Analyses for PACT were restricted to data collected during the first 8 weeks after randomization to compare PACT with the CLIC study and allow for a combined study analysis. The relationship between the log-transformed LTE₄/FE_{NO} ratio and the percentage change in FEV₁ from baseline or the change in average ACDs per week from the baseline period was evaluated. For comparison, values of LTE₄, FE_{NO}, and 1/FE_{NO} were also evaluated. FEV₁ measurements were performed 8 weeks after the start of each treatment phase in the CLIC study and 6 weeks after randomization in PACT, whereas mean ACDs were calculated from 4 to 8 weeks after the start of treatment in each study. An ACD was defined as a day without asthma symptoms, urgent care visits, hospitalizations, need for rescue albuterol (pre-treatment for exercise was permitted), or oral corticosteroids.

For CLIC data, the effect of each biomarker or biomarker ratio on the FEV₁ or ACD outcome was analyzed by treatment group by using a linear mixed-effects model to account for repeated measures as part of the study's crossover design, whereas the PACT data were analyzed with a linear regression model because it involved a parallel study design. For both studies, the estimates reported in the Results section represent regression slopes for the biomarkers, separately for montelukast and FP, with respect to the changes from baseline for the outcomes (FEV₁ and ACDs). Next, the difference between the estimated regression slopes was calculated as a measure of the preferential response to montelukast (montelukast – FP). A meta-analytic weighted average was used to obtain a combined estimate from both studies, in which the weight for each study was set equal to the inverse of the squared SE of the study estimate.

In addition to the predictive models, baseline characteristics of both CLIC and PACT children with high LTE₄/FE_{NO} ratios (≥ 75 th percentile) were compared with other children by using a Cochran-Mantel-Haenszel test for categorical variables and 1-way ANOVA for continuous variables. For these analyses, 273 CLIC and PACT participants in total were examined because of missing values of either LTE₄ or FE_{NO}. For all analyses, statistical significance was reported for *P* values of less than .05.

RESULTS

In total, data from 318 children participating in the CLIC study (all children) or PACT (montelukast and FP arms only) were analyzed. At baseline, children in PACT were younger, had earlier age of onset of asthma symptoms (*P* < .0001, Table I), and exhibited higher blood eosinophil levels (*P* = .01) and PC₂₀ values (*P* = .04) than children in the CLIC study (Table I). Although FEV₁ measurements were similar in the CLIC study and PACT, baseline ACDs were significantly lower in the PACT cohort (*P* < .0001, Table I). In addition, mothers of children in the PACT cohort were less likely to have smoked during pregnancy (*P* = .001, Table I). Biomarker levels were similar in both studies (Table I).

The distribution of biomarker levels and their logarithmically values is presented in Table E1 (available in this article's Online Repository at www.jacionline.org). For the CLIC study, 8 of the 127 subjects were missing LTE₄ values only, 14 were missing FE_{NO} values only, and 1 was missing both LTE₄ and FE_{NO} values. For PACT, 13 of the 191 subjects were missing LTE₄ values only,

TABLE I. Baseline characteristics for CLIC and PACT participants

Baseline characteristic	CLIC	PACT (only includes those participants randomized to MT or FP)	P value* comparing CLIC and PACT	CLIC and PACT combined
Randomized participants (no.)	127	191	—	318
Age (y)	11.8 ± 3.35	9.7 ± 2.21	<.0001	10.5 ± 2.91
Male sex, n (%)	75 (59.1)	114 (59.7)	.91	189 (59.4)
White, no. (%)	95 (74.8)	149 (78.0)	.51	244 (76.7)
Hispanic or Latino, no. (%)	31 (24.4)	36 (18.9)	.23	67 (21.1)
BMI (kg/m ²)	20.9 ± 4.95	19.9 ± 4.93	.06	20.3 ± 4.96
Age at onset asthma symptoms (y)	4.8 ± 3.70	3.2 ± 2.78	<.0001	3.8 ± 3.27
Mother smoked while pregnant, no. (%)	25 (19.7)	14 (7.3)	.001	39 (12.3)
Household member smokes indoors, no. (%)	22 (17.3)	23 (12.0)	.19	45 (14.2)
Parental asthma diagnosis, no. (%)	57 (49.6)	96 (50.3)	.10	153 (50.0)
Eczema, no. (%)	40 (31.5)	79 (41.4)	.07	119 (37.4)
Positive aeroallergen skin test responses (of 8)	2.9 ± 2.24	2.5 ± 2.05	.09	2.6 ± 2.13
Serum IgE (kU/L [log2])	7.1 ± 2.08	7.2 ± 2.15	.88	7.1 ± 2.11
Blood eosinophils (%)	4.9 ± 3.41	6.0 ± 3.89	.01	5.6 ± 3.73
Serum eosinophilic cationic protein (μg/L [log2])	4.0 ± 1.18	4.0 ± 1.42	.99	4.0 ± 1.33
Urinary LTE ₄ (pg/mg creatinine [log2])	6.7 ± 0.67	6.6 ± 0.74	.39	6.6 ± 0.71
FE _{NO} (ppb [log2])	4.7 ± 1.39	4.7 ± 1.36	.87	4.7 ± 1.37
Prebronchodilator FEV ₁ (% predicted)	95.8 ± 13.26	97.8 ± 12.86	.19	97.0 ± 13.03
Prebronchodilator FEV ₁ /FVC (%)	80.1 ± 7.84	80.1 ± 7.86	.97	80.1 ± 7.84
Average ACDs/wk	2.2 ± 2.33	0.3 ± 0.23	<.0001	1.1 ± 1.76
Average night awakenings/wk	0.3 ± 0.55	0.0 ± 0.08	<.0001	0.1 ± 0.37
Methacholine PC ₂₀ (mg/mL)	2.7 ± 3.80	2.0 ± 2.57	.04	2.3 ± 3.13

BMI, Body mass index; FVC, forced vital capacity; MT, montelukast.

*P values were calculated by using the χ^2 test (for categorical variables) or Student *t* test (for continuous variables).

9 were missing FE_{NO} values only, and 0 were missing both LTE₄ and FE_{NO} values. Median values for FE_{NO} were measured at 26.4 ppb, which was consistent with asthma. Median levels were 100 pg/mg for LTE₄, 0.04 ppb for 1/FE_{NO}, and 3.9 pg/mg/ppb for the LTE₄/FE_{NO} ratio.

Relationship between biomarker predictors and treatment response

FEV₁ response. Table II summarizes the association between each of the biomarker predictors and the FEV₁ percentage change from baseline. In the CLIC study LTE₄/FE_{NO} ratios were associated with a significant montelukast response from baseline (1.7% increase per doubling of the ratio, *P* = .02) and a greater montelukast than FP response (2.1% increase, *P* = .001). FE_{NO} values were significantly associated with a greater FEV₁ response to FP than montelukast (1.8% increase for each doubling of biomarker at baseline, *P* = .004). In PACT LTE₄/FE_{NO} ratios were inversely associated with FP response (0.6% decrease, *P* = .001) and positively associated with a greater montelukast than FP response (0.6% increase, *P* = .03). In addition, FP response was associated with LTE₄ (2.8% increase, *P* = .03) or FE_{NO} (0.4% increase, *P* = .02) values and inversely associated with 1/FE_{NO} values (7.6% decrease, *P* = .02). In the combined study meta-analysis LTE₄/FE_{NO} ratios were significantly associated with a greater montelukast than FP response (0.8% increase, *P* = .0005) and inversely associated with FP response (0.6% decrease, *P* = .0008). LTE₄ values were associated with a significant montelukast (2.4% increase, *P* = .01) or FP (2.4% increase, *P* = .01) response but not a differential response (*P* = .68). FE_{NO} values were associated with FP response (0.4% increase, *P* = .01) and a greater FP than montelukast response (0.6% increase,

P = .01), whereas 1/FE_{NO} values were inversely associated with FP response (7.1% decrease, *P* = .02).

ACD response. Table III summarizes the association between each of the biomarker predictors and changes in average ACDs per week from baseline. In the CLIC study LTE₄/FE_{NO} ratios were associated with a greater montelukast than FP response (0.3-ACD increase per doubling of the ratio, *P* = .009), and FE_{NO} values were associated with a greater FP than montelukast response (0.3 ACD increase, *P* = .01), whereas 1/FE_{NO} values were inversely associated with FP response (4.9-ACD decrease, *P* = .02). There were no significant associations observed in PACT for any of the predictors.

In the combined study meta-analysis LTE₄/FE_{NO} ratios were significantly associated with a greater response to montelukast than FP therapy (0.3-ACD increase, *P* = .008). In addition, FE_{NO} values were associated with a significant FP response (0.4-ACD increase, *P* = .009) and a greater FP than montelukast response (0.3-ACD increase, *P* = .009), whereas 1/FE_{NO} values were inversely associated with FP response (5.2-ACD decrease, *P* = .01).

Demographic characteristics of children with high LTE₄/FE_{NO} ratios

Table IV relates the demographic characteristics measured at baseline of children with high (≥75th percentile) versus lower LTE₄/FE_{NO} ratios. Children with high ratios were more likely to be younger (*P* < .0001) and female (*P* = .03). Children with high LTE₄/FE_{NO} ratios were less likely to be atopic (ie, exhibit ≥1 positive aeroallergen skin test response; *P* < .0001) and had fewer positive aeroallergen skin test responses (*P* < .0001), lower serum IgE values (*P* = .0002), lower percentage of blood eosinophils (*P* < .0001), lower serum eosinophilic cationic protein

TABLE II. Relationship between $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios and percentage change in FEV_1^*

	CLIC (n = 127)			PACT (n = 191)			CLIC + PACT (meta-analytic results; n = 318)		
	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)
Log2 ($\text{LTE}_4/\text{FE}_{\text{NO}}$)									
Estimate	1.7	–0.4	+2.1	0.0	–0.6	+0.6	0.1	–0.6	+0.8
SE	0.71	0.70	0.63	0.18	0.18	0.25	0.17	0.17	0.23
P value†	.02	.58	.001	.92	.001	.03	.55	.0008	.0005
Log2 LTE_4									
Estimate	1.9	2.3	–0.5	2.5	2.8	–0.3	2.4	2.4	–0.4
SE	1.35	1.35	1.27	1.29	1.29	1.8	0.93	0.93	1.04
P value†	.17	.09	.72	.06	.03	.87	.01	.01	.68
Log2 FE_{NO}									
Estimate	–0.9	0.9	–1.8	0.0	0.4	–0.4	–0.1	0.4	–0.6
SE	0.72	0.72	0.61	0.18	0.18	0.26	0.17	0.17	0.24
P value†	.22	.21	.004	.92	.02	.12	.76	.01	.01
1/(log2 FE_{NO})									
Estimate	3.1	–3.4	+6.5	–0.1	–7.6	+7.5	0.2	–7.1	+7.3
SE	9.07	9.06	7.85	2.86	3.18	4.28	2.73	3.00	3.76
P value†	.74	.71	.41	.98	.02	.08	.94	.02	.05

MT, Montelukast.

*CLIC = Second 4 weeks of data in each treatment phase; PACT = first 6 weeks of data after randomization to a treatment.

†P values were computed by using a regression model to see whether the estimate (slope) is significantly different from zero. Comparison of CLIC data and CLIC plus PACT data uses a mixed linear model to account for repeated measures among CLIC subjects.

TABLE III. Relationship between $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios and change in ACDs per week*

	CLIC (n = 127)			PACT (n = 191)			CLIC + PACT (meta-analytic results; n = 318)		
	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)
Log2 ($\text{LTE}_4/\text{FE}_{\text{NO}}$)									
Estimate	0.2	–0.1	+0.3	0.9	–0.2	+1.2	0.2	–0.1	+0.3
SE	0.18	0.18	0.12	0.70	0.69	0.98	0.17	0.17	0.12
P value†	.60	.19	.009	.18	.73	.23	.16	.54	.008
Log2 LTE_4									
Estimate	0.6	0.7	–0.1	0	–0.4	+0.3	0.2	0.1	0
SE	0.35	0.35	0.27	0.35	0.34	0.49	0.21	0.24	0.24
P value†	.09	.05	.73	.89	.27	.50	.30	.61	.98
Log2 FE_{NO}									
Estimate	0	0.4	–0.3	–0.2	0.9	–1.0	0	0.4	–0.3
SE	0.17	0.17	0.12	0.68	0.67	0.95	0.16	0.16	0.12
P value†	.84	.05	.01	.82	.19	.28	.99	.009	.009
1/(log2 FE_{NO})									
Estimate	–3.0	–4.9	+2.0	2.8	–15.0	+17.9	–2.8	–5.2	+2.1
SE	2.15	2.15	1.52	10.60	12.32	16.25	2.11	2.12	1.51
P value†	.17	.02	.20	.79	.22	.27	.19	.01	.16

MT, Montelukast.

*CLIC = Second 4 weeks of data in each treatment phase; PACT = weeks 4 to 8 of data after randomization to a treatment.

†P values were computed by using a regression model to see whether the estimate (slope) is significantly different from zero. Comparison of CLIC data and CLIC plus PACT data uses a mixed linear model to account for repeated measures among CLIC subjects.

values ($P = .004$), and higher methacholine PC_{20} values ($P = .02$) compared with those with lower $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios. The $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratio remained as a significant predictor of the differential montelukast response after controlling for atopy, serum IgE values, and percentage of blood eosinophils (see Table II in this article's Online Repository at www.jacionline.org).

DISCUSSION

The National Asthma Education and Prevention Program guidelines recommend ICSs as the preferred controller therapy

for persistent asthma in adults and children.¹ Despite this recommendation, the LTRA montelukast is the most frequently prescribed asthma controller monotherapy in the United States,⁹ although consistent findings in both population studies and clinical trials report greater efficacy and effectiveness of ICS than LTRA therapy. This prescribing trend probably reflects parents' and providers' preferences for an oral noncorticosteroid medication instead of an ICS inhaler because of the convenience of an oral medication and concern for potential growth effects associated with ICSs.¹⁰ Identification of variables predictive of greater responsiveness to LTRA than ICS therapy could help providers

TABLE IV. Baseline characteristics of children with high (≥ 75 th percentile) $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios

Baseline characteristic	Log2 ($\text{LTE}_4/\text{FE}_{\text{NO}}$; pg/mg/ppb)		P value*
	Lower ratio, <2.89	Higher ratio, ≥ 2.89	
Participants (no.)	205	68	
Age (y)	10.9 ± 2.89	9.2 ± 2.44	$<.0001$
Male sex, no. (%)	131 (63.9)	33 (48.5)	.03
White, no. (%)	161 (78.5)	48 (70.6)	.77
Hispanic or Latino, no. (%)	43 (21.0)	13 (19.1)	.74
BMI (kg/m^2)	20.3 ± 4.68	20.7 ± 5.68	.55
Age at onset of asthma symptoms (y)	4.2 ± 3.43	3.4 ± 3.15	.10
Mother smoked while pregnant, no. (%)	21 (10.2)	6 (8.8)	.89
Household member smokes indoors, no. (%)	26 (12.7)	9 (13.2)	.91
Parental asthma diagnosis, no. (%)	103 (51.8)	28 (45.2)	.42
Eczema, no. (%)	78 (38.1)	23 (33.8)	.53
Atopy, no. (%)	180 (87.8)	39 (57.3)	$<.0001$
Positive aeroallergen skin test responses (of 8)	3.0 ± 1.94	1.8 ± 2.21	$<.0001$
Log2 serum IgE (kU/L)	229.0 ± 365.89	58.4 ± 142.34	.0002
Blood eosinophils (%)	6.3 ± 3.77	3.2 ± 2.20	$<.0001$
Serum eosinophilic cationic protein ($\mu\text{g}/\text{L}$)	26.5 ± 32.07	15.0 ± 11.78	.004
Prebronchodilator FEV_1 (% predicted)	97.1 ± 12.27	99.4 ± 14.96	.20
Prebronchodilator FEV_1/FVC (%)	79.9 ± 7.83	81.6 ± 7.58	.13
Average ACDs/wk	1.0 ± 1.74	1.1 ± 1.59	.74
Average nighttime awakenings/wk	0.1 ± 0.35	0.1 ± 0.40	.83
Methacholine PC_{20} (mg/mL)	2.0 ± 2.76	2.9 ± 2.93	.02

*P values were calculated by using Cochran-Mantel-Haenszel tests for categorical variables and 1-way ANOVA for continuous variables.

recommend ICS therapy in the majority of patients, which is consistent with national guidelines, while selecting only the subset of children who might respond more effectively to LTRA therapy.

The aim of this secondary analysis was to test the hypothesis that the $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratio could predict preferential montelukast over FP responsiveness. As such, the study assessed consistency with both FEV_1 and ACD health outcomes, as well as reproducibility across different study groups by first analyzing data from the crossover CLIC study and then checking for reproducibility of these findings using data from the PACT parallel study. In this context a statistically significant association between $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios and preferential montelukast response was observed for both FEV_1 and ACD outcome measures with CLIC data. The association with a preferential FEV_1 response to montelukast was than reproduced by using data from PACT. Although significant associations with ACDs were not observed in PACT, this might have been due to inadequate power because PACT data produced relatively large SEs for this health outcome. In contrast to these findings, significant associations were not observed with individual ratio components (ie, LTE_4 and $1/\text{FE}_{\text{NO}}$) in CLIC or PACT, indicating that the $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratio had greater predictive value than either component alone. Significant associations with preferential FEV_1 and ACD responsiveness were also observed with the $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratio when data from the CLIC study and PACT were combined by using a meta-analytic approach. Although further work is needed to confirm its clinical value, these results support the proof of concept for measuring the $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratio as a predictor of preferential montelukast responsiveness in children with mild-to-moderate asthma.

Previous studies have assessed the utility of individual biomarkers, such as urinary LTE_4 and FE_{NO} , to delineate heterogeneous patient profiles of inflammation that might respond differently to medications. These studies and the data presented here suggest that higher LTE_4 values might be related to either montelukast or FP therapy responsiveness (although these

patterns were not consistently observed), whereas FE_{NO} values were associated with FP response only. In contrast, $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios were associated with a better response to montelukast than FP therapy. As such, we speculate that urinary LTE_4 is a more general indicator of the inflammatory response to multiple triggers, whereas allergic-type triggers specifically increase FE_{NO} values. In this context high $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios were related to a lower degree of IgE sensitization, lower levels of eosinophilic markers, and less responsiveness to ICS treatment while retaining sensitivity to LTRA therapy.

This phenotype might be related to nonallergic asthma triggers, such as tobacco smoke exposure, that might increase urinary LTE_4 values¹¹ and decrease FE_{NO} concentrations.¹² In this context previous reports have suggested increased montelukast responsiveness in smokers¹³ or in children exposed to environmental tobacco smoke.⁶ Although associations were not observed with questionnaire data from the CLIC study and PACT, urinary cotinine measurements were not available to allow for a definitive assessment of the relationship between smoking exposure, higher $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios, and preferential montelukast responsiveness.

Additionally, FE_{NO} might be considered a marker of allergen-driven, local bronchial inflammation (readily targeted by an ICS), whereas urinary LTE_4 might originate in the systemic circulation, as well as in the airways. Systemic inflammation can be elicited by rhinovirus infections through the release of IFN-inducible protein 10 (and rhinovirus-induced effects are at least partially resistant to treatment with ICSs).¹⁴ Thus urinary LTE_4 might signal a virus-induced systemic component that is targeted by montelukast¹⁵ but not FP. Because viral specimens were not collected in the CLIC study and PACT, the relationship between viral exposure, higher $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios, and preferential montelukast responsiveness could not be assessed.

In addition to marking a phenotype with less IgE sensitization and lower levels of eosinophilic markers, high $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios were more likely to occur in girls. This observation is consistent

with some reports of increased likelihood of response to montelukast in female subjects^{6,15} and suggests that differential hormonal patterns might be related to inflammatory heterogeneity in asthma. In addition, younger age was related to high LTE_4/FE_{NO} ratios, probably reflecting the fact that urinary LTE_4 values decrease¹⁶ and FE_{NO} values increase¹⁷ with age because of the larger bronchial mucosa area available for FE_{NO} diffusion in older children. In the CLIC study² younger children were more likely to respond to montelukast, whereas older children participating in PACT⁵ tended to respond better to FP than montelukast therapy. These age-related trends support the need for future studies among preschool children, infants, and toddlers, who often manifest with frequent virus-induced exacerbations that are poorly responsive to ICS therapy.¹⁸ In this context Bisgaard et al¹⁹ reported that asthma exacerbations and symptoms of cough and wheeze after bronchiolitis in infants²⁰ were reduced significantly with montelukast therapy. As such, determining the urinary LTE_4/FE_{NO} ratio (or perhaps a related biomarker that can be measured in an effort-independent manner) could help identify a relatively large subset of younger patients who would derive greater benefit from LTRA therapy.

CLIC and PACT investigators have previously reported on potential demographic predictors of preferential montelukast response, but results were not consistent across asthma outcomes or across studies. For example, in the CLIC study younger age and female sex were significant predictors for FEV_1 response to montelukast, and higher PC_{20} values were significantly associated with a preferential FEV_1 response to montelukast, but these results were not reproducible in PACT. No significant demographic predictors of ACD response to montelukast treatment or preferential montelukast response were observed in either study. As such, although the present study suggests that these previously reported demographic associations might reflect higher LTE_4/FE_{NO} ratios, such demographic characteristics alone cannot be used as consistent and reproducible predictors of preferential montelukast response.

Enzyme immunoassays have been shown to be a sensitive method for measurement of LTE_4 values. The immunoassay used in this report has also been used in a number of other pediatric asthma reports.^{6,21-24} It uses enrichment methods to purify the urine and increase assay specificity. Despite these improvements, the LTE_4 immunoassay used in this study is labor intensive and sometimes produces higher readings, suggesting poorer specificity than obtained with solid-phase extraction and reverse-phase HPLC.⁷ Although multiple repeated biomarker measurements for each subject were not performed in the CLIC study and PACT, Rabinovitch et al^{6,23} have previously reported that daily changes in LTE_4 values were associated with individual changes in FEV_1 or rescue medication use. Unpublished observations by these authors using these repeated-measures data found moderate but not excellent intraclass correlations for LTE_4 values measured by means of immunoassay. These observations indicate the need for a more precise LTE_4 assay, repeated LTE_4 measurements, or both to maximize precision and minimize measurement error, which might produce estimates biased toward the null. Because the LTE_4/FE_{NO} ratio was consistently associated with differential montelukast response despite the immunoassay limitations, these findings should be seen as an important proof of concept supporting the need for further assay refinement and standardized collection methods. Similarly, when the use of exhaled nitric oxide levels in asthma was first studied, collection methods were

expensive and unstandardized. As the clinical relevance of exhaled nitric oxide levels became clearer, the technology advanced fairly quickly and became more user friendly. Fortunately, new high-throughput and less labor-intensive methods for measuring LTE_4 in urine based on automated sample enrichment and liquid chromatography/tandem mass spectrometry have now been developed that have lower coefficients of variation and almost 100% recovery using spiked samples, indicating high sensitivity and precision.^{16,25} One would expect even stronger predictive ability for the LTE_4/FE_{NO} ratio measured with these more precise and efficient assays. Further studies using these more refined assays are planned to identify cutoff points for guiding therapeutic decisions.

Because this study used a continuous instead of a categorical approach as a proof of concept, it did not select a cutoff point to determine the number of doublings of the ratio required to predict a clinically significant differential response nor is such a value for differential responses well defined in the literature. The report by Zeiger et al³ using the CLIC crossover data proposed 1 extra ACD per week as defining a clinically significant differential response. By using this definition, 29% of children in the CLIC study achieved a 1-ACD or greater differential FP response, 12% achieved a better montelukast response, and 59% of children responded similarly (within 1 ACD/wk) to either medication. Rather than basing treatment decisions solely on the average response, measuring the biomarker ratio allows for identification of children in the tails of the population distribution (eg, <10th percentile and >90th percentile for montelukast-FP responders). As such, the FE_{NO} variable alone could be used to identify the 29% of children with a clinically significant differential ACD response to FP therapy (FP-montelukast) and the LTE_4/FE_{NO} ratio to identify those 12% with a better montelukast response (montelukast-FP). Given the 0.3-ACD increase per week per doubling dose estimate for both the CLIC study alone and for the combined study population using the meta-analytic approach, this translates into 3.3 doubling doses or greater to achieve at least a 1-ACD per week difference in the montelukast-FP response.

In summary, LTE_4/FE_{NO} ratios were associated with a greater FEV_1 and ACD response to montelukast than FP therapy in analysis of 2 randomized studies of 318 schoolchildren with mild-to-moderate asthma. Children with LTE_4/FE_{NO} ratios at or greater than the 75th percentile were younger and more likely to be female and exhibited lower levels of atopic markers and bronchial hyperreactivity. Although clinical guidelines support the use of ICS therapy as first-line controller therapy in patients with mild-to-moderate persistent childhood asthma, measurement of LTE_4/FE_{NO} ratios might be useful in identifying individual children who achieve a greater improvement in FEV_1 and ACDs with an LTRA compared with an ICS.

Clinical implications: In children with mild-to-moderate asthma, the LTE_4/FE_{NO} ratio is associated with a better response to montelukast compared with fluticasone therapy.

REFERENCES

1. National Asthma Education and Prevention Program. Expert panel report 3: guidelines for the diagnosis and management of asthma—summary report 2007. *J Allergy Clin Immunol* 2007;120(suppl):S94-138.
2. Szeftler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;115:233-42.

3. Zeiger RS, Szefer SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, et al. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. *J Allergy Clin Immunol* 2006;117:45-52.
4. Sorkness CA, Lemanske RF Jr, Mauger DT, Boehmer SJ, Chinchilli VM, Martinez FD, et al. Long-term comparison of 3 controller regimens for mild-moderate persistent childhood asthma: the Pediatric Asthma Controller Trial. *J Allergy Clin Immunol* 2007;119:64-72.
5. Knuffman JE, Sorkness CA, Lemanske RF Jr, Mauger DT, Boehmer SJ, Martinez FD, et al. Phenotypic predictors of long-term response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma. *J Allergy Clin Immunol* 2009;123:411-6.
6. Rabinovitch N, Strand M, Stuhlman K, Gelfand EW. Exposure to tobacco smoke increases leukotriene E4-related albuterol usage and response to montelukast. *J Allergy Clin Immunol* 2008;121:1365-71.
7. Westcott JY, Maxey KM, Macdonald J, Wenzel SE. Immunoaffinity resin for purification of urinary leukotriene E4. *Prostaglandins Other Lipid Mediat* 1998;55:301-21.
8. American Thoracic Society. ATS/ERS recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
9. Available at: http://www.wikinvest.com/concept/Asthma_drug_market.
10. Maspero JF, Leff JA. Oral montelukast versus inhaled beclomethasone in 6- to 11-year-old children with asthma: results of an open-label extension study evaluating long-term safety, satisfaction, and adherence with therapy. *Curr Med Res Opin* 2001;17:96-104.
11. Fauler J, Frolich JC. Cigarette smoking stimulates cysteinyl leukotriene production in man. *Eur J Clin Invest* 1997;27:43-7.
12. Warke TJ, Mairs V, Fitch PS, Ennis M, Shields MD. Possible association between passive smoking and lower exhaled nitric oxide in asthmatic children. *Arch Environ Health* 2003;58:613-6.
13. Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. *Am J Respir Crit Care Med* 2007;175:783-90.
14. Kelly JT, Busse WW. Host immune responses to rhinovirus: mechanisms in asthma. *J Allergy Clin Immunol* 2008;122:671-82.
15. Johnston NW, Mandhane PJ, Dai J, Duncan JM, Greene JM, Lambert K, et al. Attenuation of the September epidemic of asthma exacerbations in children: a randomized, controlled trial of montelukast added to usual therapy. *Pediatrics* 2007;120:702-12.
16. Armstrong M, Liu AH, Harbeck R, Reisdorph R, Rabinovitch N, Reisdorph N. Leukotriene-E4 in human urine: comparison of on-line purification and liquid chromatography-tandem mass spectrometry to affinity purification followed by enzyme immunoassay. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877:3169-74.
17. Linn WS, Rappaport EB, Berhane KT, Bastain TM, Avol EL, Gilliland FD. Exhaled nitric oxide in a population-based study of southern California schoolchildren. *Respir Res* 2009;10:28.
18. Pao CS, McKenzie SA. Randomized controlled trial of fluticasone in preschool children with intermittent wheeze. *Am J Respir Crit Care Med* 2002;166:945-9.
19. Bisgaard H, Zielen S, Garcia-Garcia ML, Johnston SL, Gilles L, Menten J, et al. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. *Am J Respir Crit Care Med* 2005;171:315-22.
20. Bisgaard H. Study Group on Montelukast and Respiratory Syncytial Virus. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Crit Care Med* 2003;167:379-83.
21. Covar RA, Szefer SJ, Martin RJ, Sundstrom DA, Silkoff PE, Murphy J, et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild-to-moderate asthma. *J Pediatr* 2003;142:469-75.
22. Strunk RC, Szefer SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003;112:883-92.
23. Rabinovitch N, Zhang L, Gelfand EW. Urine leukotriene E4 levels are associated with decreased pulmonary function in children with persistent airway obstruction. *J Allergy Clin Immunol* 2006;118:635-40.
24. Rabinovitch N, Strand M, Gelfand EW. Particulate levels are associated with early asthma worsening in children with persistent disease. *Am J Respir Crit Care Med* 2006;173:1098-105.
25. Kishi N, Mano N, Asakawa N. Direct injection method for quantitation of endogenous leukotriene E4 in human urine by liquid chromatography/electrospray ionization tandem mass spectrometry with a column-switching technique. *Anal Sci* 2001;17:709-13.

APPENDIX E1**MEMBERS OF THE CHILDHOOD ASTHMA
RESEARCH AND EDUCATION NETWORK FOR THE
CLIC AND PACT STUDIES****Clinical Centers**

National Jewish Medical and Research Center, Denver, Colorado: Stanley J. Szeffler, MD (Principal Investigator); Gary Larsen, MD (Co-Investigator); Joseph Spahn, MD (Co-Investigator); Ronina Covar, MD (Co-Investigator); Andrew Liu, MD (Co-Investigator); D. Sundström (Coordinator); Amy Grumann, RN (Coordinator); Melanie Phillips (Coordinator); Katie Patterson (Coordinator); Michael White (Research Assistant); Keith Breese (Research Assistant); Kate Morgan (Research Assistant).

University of Wisconsin, Clinical Science Center, Madison, Wisconsin: Robert F. Lemanske, Jr, MD (Principal Investigator); Christine A. Sorkness, PharmD (Co-Investigator); Mark H. Moss, MD (Co-Investigator); Marzena E. Krawiec, MD (Co-Investigator); James E. Gern, MD (Consultant); David B. Allen, MD (Consultant); Kristen Blotz, RN (Coordinator); Sarah Garibay, RN (Coordinator); Kelly Miller (Coordinator); Holly Eversoll, RN (Coordinator); Alana Clark (Coordinator); Kathleen Kelly Shonovich, NP; Rick Kelley (Pulmonary Function Manager); Luke Weasler (Pulmonary Function Technician).

University of California—San Diego Medical Center and Kaiser Permanente Allergy Center, San Diego, California: Robert S. Zeiger, MD, PhD (Principal Investigator); Gregory Heldt, MD (Co-Investigator); Michael H. Mellon, MD (Co-Investigator); Noah J. Friedman, MD (Co-Investigator); Alfredo A. Jalowayski, PhD (Co-Investigator); Michael Schatz, MD, MS (Co-Investigator); Sandra C. Christiansen, MD (Co-Investigator); Michael S. Kaplan, MD (Co-Investigator); Hal Hoffman, MD (Co-Investigator); Kathleen Harden, RN (Coordinator); Catherine Nelle, RN (Coordinator); Eva Rodriguez, RRT; Elaine Jenson (Research Assistant); Linda Galbreath (Research Assistant).

Washington University School of Medicine, St Louis, Mo: Robert C. Strunk, MD (Principal Investigator); Leonard B. Bacharier, MD (Co-Investigator); Gordon R. Bloomberg, MD (Co-Investigator); James M. Corry, MD (Co-Investigator); Tina Oliver-Welker CRTT, CAE (Coordinator); Valerie Morgan RRT (Coordinator); Kevin Hodgdon, RRT, CPFT (Coordinator); Wanda Caldwell, RRT, MBA (Coordinator); Cindy Moseid (Secretary).

Respiratory Sciences Center, University of Arizona, College of Medicine, Tucson, Arizona: Fernando D. Martinez, MD (Co-Investigator); Wayne Morgan, MD (Co-Investigator); Theresa W. Guilbert, MD (Co-Investigator); John D. Mark, MD (Co-Investigator); Mark Brown, MD (Co-Investigator); James Goodwin PhD (Coordinator); Melisa Celaya (Coordinator); Anna Valencia (Coordinator); Janet Lawless, RN (Coordinator); Rosemary Weese, RN (Coordinator); Shelley Radford, RT; William Hall, RT.

Resource Centers

Chair's Office, National Jewish Medical and Research Center, Denver, Colorado: Lynn M. Taussig, MD (Study Chair).

Project Office, National Heart, Lung and Blood Institute, Bethesda, Maryland: James Kiley, PhD, (Director of the NHLBI Division of Lung Diseases); Virginia Taggart, MPH (NHLBI Project Scientist); Gail Weinmann MD; Gang Zheng, PhD.

Data Coordinating Center, Penn State University College of Medicine, Hershey, Pennsylvania: Vernon M. Chinchilli, PhD

(Principal Investigator); Elizabeth Mauger, PhD (Co-Investigator); David Mauger, PhD (Co-Investigator); Timothy Craig, DO (Co-Investigator); Ian Paul, MD (Co-Investigator); Gavin Graff, MD (Co-Investigator); Susan Boehmer, (Scientific Coordinator); Brenda Phillips, (Scientific Coordinator); Jessica Beiler, (Scientific Coordinator); Loretta Doty (Project Coordinator); Anne-Marie Dyer; Lindsay Texter; Jim Schmidt; Patsy Rawa; Jill Kuennen; Jill Hofsass; Sherrie Whisler; Linda Ferrari; Pamela Arminavage; Brenda Beers; Linda Miller; Judy Potteiger; Lori Schell; Venus Mintz-Grella, Liza Say; Margaret Sheely; Trina Armstrong; Holly Hess; Vanessa Simmons; Sherrie Whisler; Thuy Tran; Lincoln Milner; Brian Moore; Andrew Sutton; Stephanie Olexovitch.

Committees

Data and Safety Monitoring Board: Thomas F. Boat, MD (Chair), Children's Hospital Medical Center, Cincinnati, Ohio; William C. Bailey, MD, the University of Alabama at Birmingham, Birmingham, Alabama; Mary Kay Garcia, PhD, RN, CPNP; Carolyn M. Kercsmar, MD, Case Western Reserve University, Cleveland, Ohio; H. William Kelly, PharmD, University of New Mexico Health Sciences Center, Albuquerque, New Mexico; Lester Lyndon Key, Jr, MD, Medical University of South Carolina, Charleston, South Carolina; James Tonascia, PhD, Johns Hopkins University, Baltimore, Maryland; Benjamin Wilfond, MD, National Human Genome Research Institute, Bethesda, Maryland.

Protocol Review Committee: Philip Ballard, MD, PhD (Chair), Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; Clarence E. Davis, PhD, University of North Carolina, Chapel Hill, North Carolina; Diane E. McLean, MD, PhD, MPH, New York State Psychiatric Institute, New York, New York; Gail Shapiro, MD, ASTHMA, Inc, Seattle, Washington; Paul O'Byrne, MD, St Joseph's Hospital, Hamilton, Ontario, Canada; Mark Liu, MD, Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland.

Executive Committee: Lynn M. Taussig, MD; Virginia S. Taggart, MPH; Stanley J. Szeffler, MD; Robert F. Lemanske, Jr, MD; Robert S. Zeiger, MD, PhD; Robert C. Strunk, MD; Fernando D. Martinez, MD; Vernon M. Chinchilli, PhD.

Publication and Presentation Committee: Robert F. Lemanske, Jr, MD (Chair); Stanley J. Szeffler, MD; Fernando D. Martinez, MD.

Quality Control Committee: Robert S. Zeiger, MD, PhD, (Chair); Vernon M. Chinchilli, PhD; A. Sorkness, PharmD; Robert C. Strunk, MD; Theresa W. Guilbert, MD; Stanley Szeffler, MD; David Mauger, PhD; Timothy J. Craig, DO; Susan Boehmer; Jill T. Kuennen; James Goodwin PhD; James R. Schmidt; Brenda Phillips; D. Sundström; James Schmidt; Kristen Blotz, RN; Sarah Garibay, RN; Kelly Miller.

Equipment Committee: Wayne Morgan, MD (Chair); Gregory Heldt, MD; Gary Larsen, MD; Christine A. Sorkness, PharmD; Joseph D. Spahn, MD; Kevin Hodgdon; Rick Kelley; Shelley Radford; Eva Rodriguez; Melanie Phillips; Brenda Phillips; Loretta Doty; Richard Evans; Jason Lennon; Lori Sanders; Jeff Davis.

Genetics Committee: Fernando D. Martinez, MD (Chair); Stanley J. Szeffler, MD; Robert F. Lemanske, Jr, MD; Vernon M. Chinchilli, PhD; David Mauger, PhD, Brenda Phillips.

Pharmaceutical Suppliers

CLIC: GlaxoSmithKline, Inc, Research Triangle Park, North Carolina; Merck & Co, Inc, West Point, Pennsylvania.

PACT: GlaxoSmithKline, Inc, Research Triangle Park, North Carolina.

Equipment Support

CLIC: Lincoln Diagnostics (Multi-Test II kits, donated), Decatur, Illinois; Monaghan Medical (Aerochamber and masks), Plattsburgh, NY; MEMS, Medication Event

Monitoring Systems, AARDEX, Zug, Switzerland; Aerocrine, Inc, Chicago, Illinois; VIASYS Healthcare GmbH, Hoechberg, Germany.

PACT: Lincoln Diagnostics (Multi-Test II kits, donated), Decatur, Illinois; MEMS, Medication Event Monitoring Systems, AARDEX, Zug, Switzerland; Aerocrine, Inc, Chicago, Ill; VIASYS Healthcare GmbH, Hoechberg, Germany, Methapharm.

TABLE E1. Distribution of biomarker variables

Biomarker variable	No.	Minimum (log2)	5th Percentile (log2)	10th Percentile (log2)	25th Percentile (log2)	Median (log2)	75th Percentile (log2)	90th Percentile (log2)	95th Percentile (log2)	Maximum (log2)
LTE ₄ (pg/mg)	296	25.0 (4.6)	45.0 (5.5)	52.0 (5.7)	69.0 (6.1)	100.0 (6.6)	133.0 (7.0)	193 (7.6)	221 (7.8)	363.0 (8.5)
FE _{NO} (ppb)	294	1.9 (0.9)	5.4 (2.4)	7.1 (2.9)	11.9 (3.6)	26.4 (4.7)	53.9 (5.8)	90.4 (6.5)	120.8 (6.9)	206.8 (7.7)
1/FE _{NO} (ppb)	294	0.004 (0.1)	0.01 (0.1)	0.01 (0.2)	0.02 (0.2)	0.04 (0.2)	0.1 (0.3)	0.1 (0.4)	0.2 (0.4)	0.5 (1.1)
LTE ₄ /FE _{NO} (pg/mg/ppb)	273	0.4 (−1.5)	0.8 (−0.4)	1.1 (0.2)	1.9 (0.9)	3.9 (2.0)	7.4 (2.9)	13.0 (3.7)	19.0 (4.2)	53.8 (5.8)

TABLE E2. Relationship between $\text{LTE}_4/\text{FE}_{\text{NO}}$ ratios and percentage change in FEV_1^*

	CLIC (n = 127)			PACT (n = 191)			CLIC + PACT (meta-analytic results; n = 318)		
	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)	MT	FP	Difference (MT – FP)
Log2 ($\text{LTE}_4/\text{FE}_{\text{NO}}$)									
Estimate	2.5	0.4	+2.1	−0.1	−0.6	+0.5	0.1	−0.6	+0.7
SE	0.76	2.76	0.63	0.21	0.21	0.26	0.20	0.21	0.24
P value†	.001	.60	.001	.70	.003	.04	.68	.005	.002
Log2 LTE_4									
Estimate	1.3	1.7	−0.4	1.9	2.4	−0.5	1.6	2.1	−0.4
SE	1.43	1.43	1.27	1.37	1.34	1.86	0.99	0.98	1.05
P value†	.38	.23	.72	.16	.07	.79	.10	.03	.68
Log2 FE_{NO}									
Estimate	−2.1	−0.3	−1.8	0.1	0.5	−0.4	−0.1	0.4	−0.6
SE	0.85	0.85	0.61	0.25	0.23	0.27	0.24	0.22	0.25
P value†	.01	.71	.004	.62	.04	.19	.75	.04	.01
1/(Log2 FE_{NO})									
Estimate	11.9	5.5	+6.4	−2.1	−8.9	+6.8	−0.2	−6.7	+6.7
SE	9.90	9.90	7.86	3.87	4.19	4.53	3.60	3.86	3.92
P value†	.23	.58	.42	.59	.04	.13	.95	.08	.09

MT, Montelukast.

*CLIC = Second 4 weeks of data in each treatment phase; PACT = first 6 weeks of data after randomization to a treatment. Estimates are adjusted for atopy, serum IgE level, and percentage of blood eosinophils.

†P values computed by using a regression model to see whether the estimate (slope) is significantly different from zero. Comparison of CLIC data and CLIC plus PACT data uses a mixed linear model to account for repeated measures among CLIC subjects.