Published in final edited form as:

J Allergy Clin Immunol. 2014 February ; 133(2): 350–356. doi:10.1016/j.jaci.2013.07.039.

Predictors of Asthma Control and Lung Function Responsiveness to STEP-3 Therapy in Children with Uncontrolled Asthma

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Abstract

Background—Predictors of improvement in asthma control and lung function to STEP-3 therapy in children with persistent asthma have not been identified despite reported heterogeneity in responsiveness.

Objective—To evaluate potential predictors of asthma control and lung function responsiveness to STEP-3 therapy.

Methods—A post-hoc analysis from the Best Add-On Giving Effective Response (BADGER) study tested the association between baseline biological, asthma control, pulmonary function and demographic markers and responsiveness to step-up to higher dose inhaled corticosteroids (ICS step-up), or addition of leukotriene receptor antagonist (LTRA step-up) or long acting beta-2 (LABA step-up) therapy.

Results—In multivariate analyses, higher impulse oscillometry (IOS) AX levels were associated ($p=0.047$) with a differential forced expiratory volume in 1-second (FEV_1) response favoring LABA over ICS step-up while higher levels of urinary leukotriene E_4 ($uLTE_4$) were marginally ($p=0.053$) related to a differential FEV_1 response favoring LTRA over LABA step-up therapy. Predictors of differential responses comparing ICS to LTRA step-up therapy were not apparent probably due to suppression of allergic markers with low-dose ICS treatment. Minimal overlap

was seen across FEV₁ and asthma control day (ACD) predictors suggesting distinct mechanisms related to lung function and ACD responses.

Conclusion—Levels of IOS AX indicating peripheral airway obstruction and uLTE₄ indicating cysteinyl leukotriene inflammation can differentiate LABA step-up responses from responses to LTRA or ICS step-up. Further studies with physiological, genetic and biological markers related to these phenotypes will be needed to predict individual responses to LABA step-up therapy.

Keywords

Asthma; BADGER; children; FENO; ICS; IOS; LABA; LTE₄; LTRA

INTRODUCTION

The Childhood Asthma Research and Education Network's (CARE) "Best Add-On Giving Effective Response" (BADGER) study evaluated the use of STEP-3 therapy (i.e. added controller therapies in children with uncontrolled asthma despite use of low-dose inhaled corticosteroids (ICS) ¹). Using a composite measure of exacerbations, asthma control days (ACDs) and forced expiratory volume in 1-second (FEV₁), approximately 44.1% of children responded best to the addition of the long acting beta agonist (LABA) salmeterol (LABA step-up), 26.7% responded best to medium dose ICS, fluticasone propionate (ICS step-up) and 29.2% responded best to the addition of the leukotriene receptor antagonist (LTRA) montelukast (LTRA step-up). The considerable response variability to STEP-3 therapy suggests that different phenotypes of poorly controlled asthma might respond differentially to one mode of STEP-3 therapy over another. Indeed, a series of STEP-2 pediatric studies ²⁻⁵ (i.e. initiation of controller therapy in children with persistent asthma receiving rescue medications only) had suggested phenotypic patterns that differentiated low-dose ICS responders from LTRA responders. Physiologically, children who responded best to low-dose ICS had worse asthma control, lower pulmonary function and lower PC20 levels indicating greater bronchial reactivity than LTRA responders. Biologically, ICS responders exhibited increased atopic markers including higher levels of fractional exhaled nitric oxide (FeNO), blood eosinophils, IgE and skin prick allergen sensitivity compared to LTRA responders while the ratio of urinary leukotriene E₄ (uLTE₄) to FeNO (uLTE₄:FeNO) was associated with a better response to LTRA monotherapy. These results suggested a predominantly eosinophilic inflammatory phenotype associated with better ICS response and a less atopic high cysteinyl leukotriene inflammatory milieu associated with a better LTRA response. The primary hypothesis of the present study was that pathophysiological profiles which distinguished groups responding best to LTRA compared to ICS monotherapy (i.e. STEP-2 therapy) would also distinguish groups that responded better to ICS compared to LTRA step-up therapy (i.e. STEP-3 therapy). We then explored other markers that might be associated with differential responses to all 3 therapies (i.e. ICS-LTRA step-up, ICS-LABA step-up, and LABA-LTRA step-up) studied in BADGER.

METHODS

Study Protocol

Methods and primary outcome results for the BADGER study have been published¹. Briefly, BADGER was a randomized crossover study of 165 children (6 to 17 years of age) with uncontrolled asthma despite treatment with low-dose ICS therapy. After a 2 to 8 week run-in period to demonstrate at least 3 days per week of uncontrolled asthma while receiving low-dose fluticasone propionate (FP 100 micrograms twice daily), children were randomized to one of the 3 treatments; either FP (250 micrograms twice daily)(ICS step-up) or combination salmeterol 50 mcg twice daily and FP (100 mcg twice daily)(LABA step-up)

or montelukast (Mt) at the age appropriate dose (5 or 10 mg per day) and FP (100 mcg twice daily)(LTRA step-up). In a triple crossover design, study participants crossed over to a different drug regimen every 16 weeks until all 3-drug regimens were completed.

Peak expiratory flow maneuvers were performed daily and diaries were completed at baseline and throughout the study documenting symptoms and albuterol usage. Blood eosinophils, serum immunoglobulin E (IgE), fractional exhaled nitric oxide (FeNO) and urine were collected at randomization (one sample per child) while children were receiving low dose FP and measured as previously described ¹. Forced expiratory volume in 1-second (FEV₁), forced vital capacity (FVC), impulse oscillometry (IOS) resistance at 5 Hz (IOS R5) and reactance area (IOS AX) were collected at randomization as previously described ¹. Urine collected during the baseline period that had been frozen and stored was assayed for uLTE₄ by mass spectrometry ⁶ for the purposes of this analysis. Some of these tests including uLTE₄ and IOS are not commonly used but were available in select centers. Appropriate institutional review board approval was obtained for the study before recruitment. Written consent was provided by parent or guardian.

Statistical Analysis

This post-hoc analysis of predictors of ACD and FEV₁ responses from the BADGER study was similar to that previously employed for data obtained in a study of stepping-up to STEP-2 therapy ⁴. An ACD was defined as a day with no daytime or nighttime asthma symptoms, no rescue albuterol for asthma symptoms or peak flow less than 80% of personal best, no asthma health care use, and no asthma related absence from school or work. A placebo washout period between treatment sequences was not implemented for safety reasons. Instead the first 4 weeks of each treatment period served as “pseudo wash-out, wash-in period” so that only data collected during the final 12 weeks of each treatment period was used in the analysis. An ACD response was defined as a change from the worst (i.e. lowest number of ACDs) 2-week period during the baseline interval. This response was then converted to annualized asthma-control days (AACD) calculated as 365 times the change in proportion of ACDs during the final 12 weeks of the period and was adjusted for seasonal differences. Linear regression analysis was used to examine the potential predictive value of various baseline characteristics on the FEV₁ or AACD response to each treatment and also differences between treatments (differential responses). In order to determine which factors were independently associated with differential responses, a multivariable model analysis was performed which included significant predictors. Logarithmic transformation (base 2) was applied to the uLTE₄, FeNO, blood eosinophils and IgE predictors based on visual assessment of linearity. All analyses were carried out using the statistical software package SAS version 9.2 (SAS Inc. Cary, NC).

RESULTS

Differential Predictors of AACD Responses (Table 1)

Urinary LTE₄—Urinary LTE₄ levels were significantly associated (p=0.009) with AACD responses to ICS step-up therapy and marginally (p=0.057) to LTRA step-up but not to LABA step-up therapy (Table 1). For each doubling of uLTE₄ at baseline, the response to ICS step-up was increased by 29 AACDs (Table 1). The difference between ICS and LABA therapy (ICS-LABA) was marginally associated with uLTE₄ levels (favors ICS over LABA step-up, 13.2 AACD difference per uLTE₄ doubling, p=0.092)(Table 1). This differential association did not remain in multivariable analyses with all significant AACD predictors summarized in Table 3.

ACDs—The number of asthma control days during the worst 2 baseline weeks was significantly ($p<0.0001$) associated with responses to all 3 therapies (i.e. AACD responses were less when asthma control days were higher at baseline)(Table 1). The difference between ICS and LTRA therapy (ICS-LABA) was marginally associated with ACD levels (favors ICS over LTRA step-up, 3.8 AACD difference per 1 day increase in asthma control, $p=0.093$)(Table 1). This differential association did not remain in multivariable analyses containing significant AACD predictors summarized in Table 3.

Differential Predictors of FEV₁ Responses (Table 2)

Urinary LTE₄—Urinary LTE₄ levels were significantly ($p=0.01$) associated with FEV₁ responses to LTRA step-up therapy and marginally ($p=0.093$) associated with ICS step-up therapy but not to LABA step-up therapy (Table 2). Each doubling of the uLTE₄ level at baseline was associated with a 3.3% increase in the FEV₁ response to LTRA step-up and a 2.2% increase with ICS step-up therapy (Table 2). Higher uLTE₄ levels were marginally ($p=0.099$) associated with a differential (LABA-LTRA) response (favors LTRA over LABA step-up)(Table 2). Each doubling of uLTE₄ at baseline was related to a 1.8% FEV₁ difference favoring LTRA over LABA step-up responses. This association remained marginally significant after control for significant FEV₁ predictors summarized in Table 3. In multivariate analysis controlling for baseline FEV₁, FEV₁/FVC, uLTE₄, Age, IOS R5 and IOS AX, uLTE₄ was associated ($p=0.053$) with a differential (LABA-LTRA) response of 2.2% per doubling of uLTE₄ (favors LTRA over LABA step-up)(Table 2).

FEV₁/FVC—Pre-BD FEV₁/FVC was significantly associated ($p=0.001$) with FEV₁ responses to LTRA and LABA step-up and marginally ($p=0.085$) to ICS step-up therapy (i.e. FEV₁ responses were greater when baseline FEV₁/FVC was lower – i.e. more airway obstruction)(Table 2). For each 10% decrease in pre-BD FEV₁/FVC (i.e. more airway obstruction) at baseline, the FEV₁ response was increased by 4.7% with LTRA or LABA step-up therapy (Table 2). Lower pre- bronchodilator (BD) FEV₁/FVC levels were marginally related to differential (ICS-LTRA) or (ICS-LABA) responses (Table 2). Each 10% decrease in the pre-BD FEV₁/FVC (i.e. more airway obstruction) was marginally related to a 2.1% difference in response favoring LABA over ICS step-up therapy ($p=0.059$) and to a 2.2% response difference favoring LTRA over ICS step-up therapy ($p=0.078$)(Table 2). This differential association did not remain in multivariable analyses containing significant FEV₁ predictors summarized in Table 3.

Impulse Oscillometry—Higher pre-BD IOS R5 (i.e. greater airway obstruction) was significantly associated ($p<0.05$) with a favorable FEV₁ response to LABA or ICS step-up therapy but not to LTRA step-up therapy (Table 2). For each kilopascal per liter per second (KPa/L/s) increase in pre-BD IOS R5, the FEV₁ response was increased by 16.1% with LABA and by 11.6% with ICS step-up therapy (Table 2). Higher pre-BD IOS R5 levels (i.e. more airway obstruction) was associated with a differential (LABA-LTRA) response (Table 2). For each KPa/L/s increase in pre-BD IOS R5, the difference (LABA-LTRA) in response was increased by 9.5% ($p=0.025$)(favors LABA over LTRA step-up)(Table 2). These differential associations did not remain in multivariable analyses containing significant FEV₁ predictors summarized in Table 3.

Higher pre-BD IOS AX at baseline (i.e. greater peripheral airway obstruction) was significantly associated ($p=0.0001$) with a favorable FEV₁ response to LABA step-up and marginally ($p=0.073$) to ICS but not to LTRA step-up therapy (Table 2). For each KPa/L increase in pre-BD IOS AX, the FEV₁ response was increased by 2.8% with LABA step-up treatment and by 1.4% with ICS step-up therapy (Table 2). Higher IOS AX (i.e. more peripheral airway obstruction) was also significantly associated with a differential (LABA-

LTRA) FEV₁ response (favors LABA over LTRA step-up, 1.6% per KPa/L increase, $p=0.021$) and marginally to a differential (ICS-LABA) response (favors LABA over ICS step-up, 1.2% per KPa/L increase, $p=0.073$) (Table 2). This latter association remained significant with control for significant FEV₁ predictors summarized in Table 3. In multivariable analysis controlling for baseline FEV₁, FEV₁/FVC, uLTE₄, age, IOS R5 and IOS AX, for each KPa/L increase in IOS AX at baseline (i.e. more peripheral airway obstruction), the difference (LABA-ICS) in step-up response was increased by 2.5% ($p=0.048$) (favors LABA over ICS step-up)(Table 2)..

Significant Predictors of AACD and FEV₁ Responses (Table 3)

Except for uLTE₄, there was no overlap between predictors of asthma control and lung function outcomes (Table 3). In general, the magnitude of AACD response from baseline to all therapies was primarily related to markers of baseline asthma control while FEV₁ responses were associated with markers of baseline lung function (Table 3).

DISCUSSION

As it has become clearer that chronic diseases such as asthma consist of multiple phenotypes leading to common health endpoints, there has been increased interest in relating phenotypic differences to clinical responses. Using data from the BADGER study, this analysis sought to identify biological, asthma control, pulmonary function and demographic markers that were associated with differential medication responses to STEP-3 therapy.. We concluded that predictors of STEP-2 ICS and LTRA monotherapy were not predictive of differential STEP-3 responses to ICS and LTRA step-up and in fact, no significant differential associations with any predictors were seen for these 2 medication options in multivariable analyses. We did, however, observe differential associations with LABA step-up therapy. In multivariable analyses, higher IOS AX levels were associated ($p=0.047$) with a differential response favoring LABA over ICS step-up while uLTE₄ was marginally associated ($p=0.053$) with a differential response favoring LTRA over LABA step-up therapy.

The lack of consistency for predictors of STEP-2 and STEP-3 therapy might relate to features that distinguished the BADGER study from these STEP-2 studies. These include the selection of non-responders to low-dose ICS therapy and the presence of concurrent low-dose ICS therapy when predictor information was collected. In this context, the response to ICS step-up therapy may be quite different than response to initiation of ICS therapy due to flattening of the ICS dose-response curve⁷ or inclusion of participants who failed to achieve control with low dose ICS therapy alone. This may explain why indicators of asthma severity such as low lung function, PC20, ACDs or higher albuterol usage at baseline were not associated with a better response to ICS step-up than to LTRA step-up therapy in BADGER. In addition, the response to ICS step-up therapy in BADGER was not associated with eosinophil levels (for FEV₁), FeNO and IgE as it had been in STEP-2 studies presumably due to modulation of these atopic markers by existing ICS treatment. FeNO production, for example is very sensitive to ICS therapy both because it is easily targeted by ICS deposition in the lungs and because ICS therapy can directly inhibit FeNO production by modulation of inducible nitric oxide synthase^{8,9}.

In contrast to the other biological markers related to atopy, uLTE₄ levels are not significantly different in atopic and non-atopic asthmatics suggesting that it is a non-specific marker of both atopic and non-atopic inflammation. Unlike atopic biomarkers, which were associated solely with ICS responses in STEP-2 studies^{2,3,4,5}, uLTE₄ was associated with favorable ACD and FEV₁ responses to both ICS and LTRA monotherapy⁴. As such, patterns of association for uLTE₄ with ICS or LTRA step-up therapy in BADGER were similar to that seen for ICS and LTRA STEP-2 therapy. This may partially be due to relative

insensitivity to ICS treatment for uLTE₄ compared to eosinophilic markers. Although most studies suggest some inhibition of cysteinyl leukotriene formation with ICS treatment^{10,11}, this inhibition appears to be substantially less than for FeNO and does not modulate the ability of LTE₄ to discriminate between patients with and without asthma-related airway inflammation¹². In BADGER, the response to anti-inflammatory treatment with LTRA and ICS step-up therapy increased with higher uLTE₄ levels, presumably indicating greater airway inflammation that responds better to treatment with anti-inflammatory step-up therapy than to LABA step-up treatment^{13,14}.

Aside from uLTE₄, there was little overlap between AACD and FEV₁ predictors. Baseline albuterol usage, peak flow and number of ACDs were all related to AACD but not FEV₁ responsiveness while pre-BD FEV₁, FEV₁/FVC and IOS resistance and reactance were associated only with FEV₁ and not AACD responses. Taken together, these findings highlight the dissociation between phenotypes related to poor asthma control and low FEV₁¹⁵. These 2 outcome measures provide different information about disease severity. Asthma control days, by definition, provide direct information about asthma control while FEV₁ levels provide a measurement of risk for asthma worsening. FEV₁ is probably a more reliable outcome as an objective single measurement instead of a composite of subjective indices that make up the asthma control day outcome. This may explain why we found more differential associations with FEV₁ than AACD responder groups. An interesting illustration of the dichotomy between asthma control and lung function outcomes is the strong relationship between higher blood eosinophils and AACD but not FEV₁ responsiveness in this study. Assuming that peripheral eosinophils at least partially reflect eosinophilic airway inflammation¹⁶, these data suggest that factors other than persistent allergic inflammation are related to persistent lung function deficits in a proportion of children with uncontrolled asthma receiving low-dose ICS therapy. Based on the results from this study, it would appear that changes in lung function might be a more sensitive differentiator of medication responses to specific STEP-3 treatment.

In multivariable analyses, each doubling of uLTE₄ was related to a 2.2% FEV₁ differential response favoring LTRA over LABA step-up while each KPa/L increase in IOS AX was associated with a 2.5% FEV₁ increase favoring LABA over ICS step-up therapy. Previous studies have defined a 5% FEV₁ difference as a clinically significant differential response⁵. This difference would occur with 2.3 uLTE₄ doublings or a 2 KPa/L increase in IOS AX - well within the range of levels measured in the study. On a group level, these associations suggest that children with low lung function related primarily to small airway obstruction will respond better to LABA step-up while those with low lung function related to persistent inflammation will respond best to enhanced anti-inflammatory treatment with ICS or LTRA step-up¹⁷. The IOS response pattern is similar to that reported by Larsen et al. in children participating in the Pediatric Asthma Control Trial (PACT)¹⁸. The PACT study compared once daily low-dose ICS plus LABA (combination) with twice daily low-dose ICS or LTRA monotherapy and found that only the combination group demonstrated a significant IOS response over the 1st twelve weeks of the study; at this time point, the combination response was significantly greater than the LTRA response with an intermediate response to ICS therapy. Although not directly comparable, these findings suggest that children in BADGER with high IOS levels likely respond best to LABA step-up therapy because it more effectively reverses peripheral airways obstruction thereby improving overall lung function. The fact that IOS AX was differentially related to a better response to LABA step-up compared to enhanced anti-inflammatory therapy with ICS or LTRA step-up treatment suggests that compromised small airway function is related more to increased smooth muscle tone than to small airway inflammation which reportedly responds to LTRA step-up therapy^{19,20}.

What are the clinical implications of these findings? The disparate results in BADGER compared to STEP-2 studies for FeNO as a predictor of ICS responsiveness imply that concurrent low-dose ICS therapy may confound any correlation between FeNO and levels of allergic airway inflammation. This finding suggests that clinicians should be cautious in their interpretation of low FeNO values in children receiving ICS therapy and should not necessarily assume that their inflammation is well controlled. This might explain why randomized studies using FeNO levels to titrate STEP-3 ICS therapy in children or adults do not observe any added benefit over guideline-based management^{21,22}. The present study also highlights the importance of separating asthma control responses from FEV₁ responses to STEP-3 therapy. After treatment, clinicians should not necessarily assume that children with improved FEV₁ are well controlled or that lung function has been normalized in children with few symptoms.

Because this analysis utilized largely exploratory statistical methodology, the predictors of differential responsiveness reported here will require validation in other datasets using a hypothesis-driven approach. Further analyses, preferably, with larger datasets would also be needed to determine how well the marginally significant group associations for uLTE₄ and IOS AX might predict responses to therapy in individual children. Given the heterogeneity of asthma phenotypes, it seems probable that combinations of predictors will be needed to capture the considerable variability within and across medication responder groups. Until such studies are performed, clinical decisions should continue to be based on the most likely best response to LABA step-up therapy as reported in the initial BADGER paper with appropriate weighing of risks for each of the STEP-3 options²³. Future studies of STEP-3 responsiveness might include panels of relatively steroid insensitive physiologic, genetic^{24–26} and biological markers related to peripheral airway obstruction and/or cysteinyl leukotriene inflammation to help identify the over 50% of children who respond better to step-up ICS or LTRA compared to LABA therapy.

Acknowledgments

Supported by grants from the National Heart, Lung, and Blood Institute (HL064307, HL064288, HL064295, HL064287, HL064305, and HL064313), the National Institute of Allergy and Infectious Diseases (T32AI007635), and the Clinical Translational Science Award program of the National Center for Research Resources (UL1-RR025011 [Wisconsin] and UL1-RR024992 [St. Louis]). This study was performed in part by the General Clinical Research Centers at Washington University School of Medicine (M01-RR00036), and the University of Wisconsin (M01-RR03186). This study was also supported by Colorado CTSA Grant UL1 RR025780 from NCRR/NIH and UL1 TR000154 from NIH/NCATS.

Abbreviations

AACD	Annualized asthma control day
BADGER	Best Add-On Giving Effective Response
BMI	body mass index
CARE	Childhood Asthma Research and Education Network
FP	fluticasone propionate
FEV₁	forced expiratory volume in 1-second
FVC	forced vital capacity
FeNO	fractional exhaled nitric oxide
ICS	inhaled corticosteroids
LTRA	leukotriene receptor antagonist

LABA	long acting beta-2 agonist
Mt	montelukast
uLTE₄	urinary LTE ₄
IOS	impulse oscillometry
IOS R5	resistance at 5 Hz
IOS AX	reactance area
IgE	serum immunoglobulin E

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Clinical Implications

Although responses to STEP-3 therapy may be related at the group level to peripheral airway obstruction and cysteinyl leukotriene inflammation, further study is needed to identify clinically effective predictors of individual responses related to these phenotypes.

Table 1
Predictors of AACD Differential Responsiveness to ICS, LTRA or LABA Step-up Therapy

Predictor	Outcome	Estimate	SE	P value	Relationship between Predictor and Outcome
Biological Markers					
uLTE₄ on Low-dose ICS (Log₂ Scale) (pg/mg)	ICS response	29.08	10.95	0.009	1 doubling of baseline uLTE ₄ predicts 29 AACD increase
	LTRA response	22.86	11.93	0.057	1 doubling of baseline uLTE ₄ marginally predicts 23 AACD increase
	LABA response	9.25	9.72	0.34	
	Differential response (ICS-LABA)	13.18	7.76	0.092	1 doubling of baseline uLTE ₄ marginally predicts 13 day AACD difference favoring ICS over LABA
Blood Eosinophils (Log₂ Scale) (absolute) (/cu.mm)	ICS response	20.23	6.94	0.004	Doubling of baseline eosinophils predicts 20 AACD increase
	LTRA response	24.76	7.46	0.001	Doubling of baseline eosinophils predicts 25 AACD increase
	LABA response	16.55	6.37	0.010	Doubling of baseline eosinophils predicts 17 AACD increase
Markers of Asthma Control					
Days Alb Use - No Exercise: Worst 2 Weeks	ICS response	7.22	2.26	0.002	1 day increase in baseline albuterol usage predicts 7 AACD increase
	LTRA response	9.63	2.41	<0.0001	1 day increase in baseline albuterol usage predicts 10 AACD increase
	LABA response	8.35	2.02	<0.0001	1 day increase in baseline albuterol usage predicts 8 AACD increase
AACD: Worst 2 Weeks	ICS response	-18.48	2.29	<0.0001	1 day decrease in baseline AACD predicts 18 AACD increase
	LTRA response	-22.78	2.33	<0.0001	1 day decrease in baseline AACD predicts 23 AACD increase
	LABA response	-18.58	2.00	<0.0001	1 day decrease in baseline AACD predicts 19 AACD increase
	Differential response (ICS-LTRA)	3.76	2.23	0.093	1 day decrease in baseline AACD marginally predicts 4 day AACD difference favoring LTRA over ICS
Average AM Peak Flow % Personal Best (L/min)	ICS response	-39.99	1.11	0.0004	10% decrease in baseline AM peak flow % personal best predicts 40 AACD response to ICS step-up therapy
	LTRA response	-41.11	1.21	0.0009	10% decrease in baseline AM peak flow % personal best predicts 41 AACD response to LTRA step-up therapy
	LABA response	-34.20	1.01	0.0009	10% decrease in baseline AM peak flow % personal best predicts 34 AACD response to LABA step-up therapy

The following potential predictors were not significantly associated with differential AACD responses: Maximum bronchodilator (BD) response, pre-BD FEV₁/FVC, log₂ PC20, log₂ IgE, FeNO, uLTE₄: FeNO, number of positive aeroallergen skin tests, age at randomization, duration of asthma, body mass index, pre bronchodilator IOS R5, pre-bronchodilator IOS AX.

Table 2
Predictors of FEV₁ Differential Responsiveness to ICS, LTRA or LABA Step-up Therapy

Predictor	Outcome	Estimate	SE	P value	Relationship between Predictor and Outcome
Biological Markers					
uLTE ₄ on Low-dose ICS (Log ₂ Scale) (pg/mg)	ICS response	2.17	1.28	0.093	1 doubling of baseline uLTE ₄ marginally predicts 1.3% FEV ₁ increase
	LTRA response	3.33	1.32	0.012	1 doubling of baseline uLTE ₄ predicts 3.3% FEV ₁ increase
	LABA response	1.63	1.16	0.161	
	Differential response (LTRA-LABA)	1.85	1.11	0.099	1 doubling of baseline uLTE ₄ marginally predicts 1.8% FEV ₁ difference favoring LTRA over LABA
	Multivariate analysis for differential response (LTRA-LABA) controlling for baseline FEV ₁ , FEV ₁ /FVC, uLTE ₄ , Age, IOS R5 and IOS AX (p=0.053)				
Pulmonary Function Markers					
Pre-BD FEV ₁ /FVC (%)	ICS response	-2.44	1.41	0.085	10% decrease in baseline pre-BD FEV ₁ /FVC marginally predicts 2.4% FEV ₁ increase
	LTRA response	-4.68	1.40	0.001	10% decrease in baseline pre-BD FEV ₁ /FVC predicts 4.7% FEV ₁ increase
	LABA response	-4.72	1.21	0.0001	10% decrease in baseline pre-BD FEV ₁ /FVC predicts 4.7% FEV ₁ increase
Pre-BD IOS R5 (kPa/L/s)	Differential response (LTRA-ICS)	-2.18	1.22	0.078	10% decrease in baseline pre-BD FEV ₁ /FVC marginally predicts 2.2% FEV ₁ difference favoring LTRA over ICS
	Differential response (LABA-ICS)	-2.10	1.11	0.059	10% decrease in baseline pre-BD FEV ₁ /FVC marginally predicts 2.1% FEV ₁ difference favoring LABA over ICS
	ICS response	11.61	4.90	0.019	1 kPa/L/s increase in baseline pre-BD IOS R5 predicts 11.6% FEV ₁ increase
	LTRA response	6.42	5.07	0.207	
	LABA response	16.12	4.39	0.0003	1 kPa/L/s increase in baseline pre-BD IOS R5 predicts 16% FEV ₁ increase

Predictor	Outcome	Estimate	SE	P value	Relationship between Predictor and Outcome
Pre-BD IOS AX (kPa/L)	Differential response (LABA-LTRA)	9.52	4.20	0.025	1 kPa/L increase in baseline pre-BD IOS R5 predicts 9.5% FEV ₁ difference favoring LABA over LTRA
	ICS response	1.38	0.80	0.086	1 kPa/L increase in baseline pre-BD IOS AX marginally predicts 1.4% FEV ₁ increase
	LTRA response	1.09	0.8	0.182	
	LABA response	2.79	0.70	0.0001	1 kPa/L increase in baseline pre-BD IOS AX predicts 2.8% FEV ₁ increase
	Differential response (LABA-LTRA)	1.62	0.68	0.019	1 kPa/L increase in baseline pre-BD IOS AX predicts 1.6% FEV ₁ difference favoring LABA over LTRA
	Differential response (ICS- LABA)	-1.15	0.64	0.073	1 kPa/L increase in baseline pre-BD IOS AX marginally predicts 1.2% FEV ₁ difference favoring LABA over ICS
	Multivariate analysis for differential response (LABA- ICS) controlling for baseline FEV ₁ , FEV ₁ /FVC, uLTE ₄ , Age, IOS R5 and IOS AX				1 kPa/L increase in baseline pre- BD IOS AX predicts 2.5% FEV ₁ difference favoring LABA over ICS (p=0.048)

The following potential predictors were not significantly associated with differential FEV₁ responses: average daily albuterol use, proportion ACDs, maximum bronchodilator response, Pre-BD FEV₁ % predicted, Pre-BD PEF/Personal Best PEF, log₂ PC20, log₂ eosinophils, log₂ IgE, FeNO, uLTE₄: FeNO, number of positive aeroallergen skin tests, duration of asthma and body mass index.

Table 3

Significant Predictors of AACD and FEV₁ Responses

	AACD ICS Response	AACD LABA Response	AACD LTRA Response	FEV ₁ ICS Response	FEV ₁ LABA Response	FEV ₁ LTRA Response
Biological Markers						
Urinary LTE ₄	***		*	*		**
Blood Eosinophils	***	**	***			
Asthma Control Markers						
Days Alb Use	***	****	****			
Asthma Control Days	****	****	****			
AM PEF /Personal Best PEF	****	****	****			
Pulmonary Function Markers						
Pre-BD FEV ₁ /FVC				*	***	***
Pre-BD FEV ₁ % predicted				****	****	****
Pre-BD IOS R5				**	****	
Pre-BD IOS AX				*	****	
Demographic Markers						
Age				**		

* p<0.1

** p<0.05

*** p<0.01

**** p<0.001