

Letter to the Editor

Peripheral blood gene expression predicts clinical benefit from anti-IL-13 in asthma

To the Editor:

Asthma is the most prevalent chronic disease of children in industrialized countries. However, the immunopathology of asthma is most commonly described in adults in whom the airways are characterized by heterogeneous eosinophilic airway inflammation. In moderate-to-severe asthma in adults, biologic therapies directed against type 2 inflammatory cytokines are currently under development; meaningful clinical benefit from these interventions is enriched in subjects selected on the basis of noninvasive biomarkers of type 2-“high” asthma, for example, serum periostin and blood or sputum eosinophils.¹

In a study of 277 pediatric subjects with asthma, serum periostin and peripheral blood eosinophils were significantly negatively correlated with age and serum periostin was substantially elevated in pediatric patients with asthma compared with adult patients with asthma.² These data suggest that factors other than type 2 inflammation may influence serum periostin and blood eosinophil levels in children with asthma. The ability of these biomarkers to predict clinical benefit from type 2 pathway-directed therapies in pediatric asthma is currently unknown. Therefore, alternative biomarkers of type 2 inflammation may need to be evaluated in children and adolescents with asthma.

We hypothesized that gene expression correlated with eosinophil count in asthma patients' peripheral blood (1) would be enriched for genes whose expression levels predict treatment

benefit from anti-IL-13 therapy, (2) would include genes whose expression was independent of age, and (3) whose cellular origin of expression is associated with type 2 inflammatory cells and/or type 2 cytokine signaling. To develop an eosinophil-related gene signature (ERGS), we evaluated genomewide transcript levels in stabilized RNA extracted from peripheral blood from multiple clinical studies of well-characterized patients with asthma. The clinical characteristics of subjects with asthma included in this analysis are summarized in Table E1 in this article's Online Repository at www.jacionline.org.

We assessed peripheral blood gene expression in 298 subjects (12-75 years old) from the EXTRA study by microarrays (see this article's Methods section in the Online Repository at www.jacionline.org). Although this study was not designed nor powered to detect an elevation in serum periostin or blood eosinophil count in adolescent (<18 years old, N = 15) versus adult (≥18 years old) patients with asthma, we observed median elevations of 37 ng/mL periostin ($P < .05$) and 20 eosinophils per μL (not statistically significant), respectively (see Fig E1 in this article's Online Repository at www.jacionline.org). Six hundred twenty-five genes were significantly correlated with blood eosinophil counts (adjusted P value $< .05$; 50 genes with the lowest adjusted P value are tabulated in Table E2 in this article's Online Repository at www.jacionline.org). Regression analysis (Fig 1, A) reveals a skewing toward genes that are positively correlated with absolute blood eosinophil count, suggesting that blood eosinophil count is an important source of expression variation. *CCL23*, *SIGLEC8*, *PTGDR2*, *CACNG6*, *IDO1*, and *HSD3B7* were among the most positively correlated genes with absolute blood eosinophil count (Fig 1, A; Table E2; see Fig E2 in this article's Online Repository at

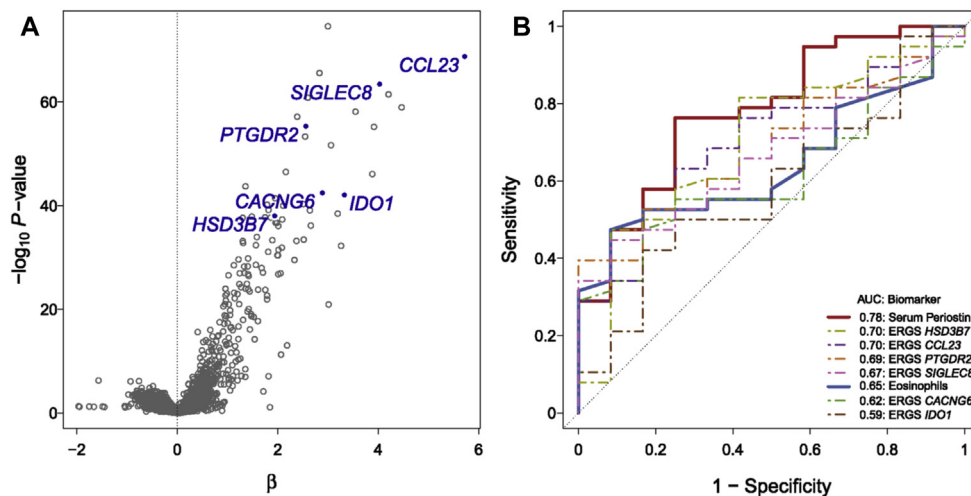


FIG 1. Discovery of an ERGS. **A**, Multiple regression of contemporaneously assessed absolute blood eosinophil count ($\sqrt{\#/\mu\text{L}}$), adjusted by subject age, with blood gene expression (PAXgene microarrays) was conducted for 298 subjects with moderate-to-severe asthma from the EXTRA study at baseline and is represented by volcano plot; coefficient estimate of blood eosinophil count (β) from multiple regression and $-\log_{10} P$ values are plotted on X and Y axes, respectively. Genes selected for ERGS evaluation are annotated. **B**, Predictive performance of ERGS genes for eosinophilic airway inflammation ($\geq 3\%$ sputum eosinophils or ≥ 22 tissue eosinophils/ mm^2) was assessed by receiver-operating characteristic analysis and compared with serum periostin, and blood eosinophils. Only subjects with complete biomarker data are included in this analysis ($n = 50$). AUC measures are tabulated in the legend. AUC, Area under the curve.

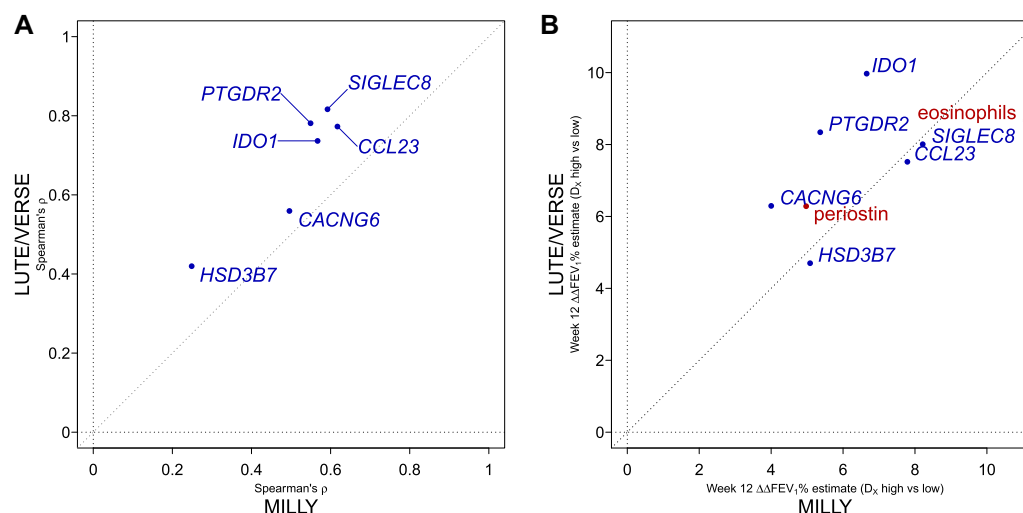


FIG 2. Predictive performance of ERGS genes in MILLY and LUTE/VERSE studies of anti-IL-13. Measures of ERGS predictive performance were assessed in MILLY and LUTE/VERSE studies. **A**, Positive correlations (Spearman ρ) of continuous values of ERGS expression and blood eosinophil count were observed. **B**, Predictive performance of baseline ERGS expression for enriched clinical benefit from lebrikizumab was assessed. Treatment effect difference ($\Delta\Delta\text{FEV}_1\%$) as week 12 between dichotomous diagnostic classification of baseline ERGS expression was estimated and compared with reference biomarkers blood eosinophil count and serum periostin.

www.jacionline.org) and selected as candidate genes for subsequent assessment due to (1) minimal evidence of age dependency across a wide range of age (12-75 years old; Fig E2), (2) biological plausibility, and (3) technical robustness of quantitative PCR assay development on a clinically amenable assay platform, the COBAS system (data not shown). Using this assay platform, we then characterized the expression of these genes on the basis of their ability to predict eosinophilic airway inflammation, blood eosinophil count, and clinical response to anti-IL-13 treatment in patients with moderate-to-severe asthma according to a prespecified analysis plan in 3 independent clinical studies.

CCL23 is a CCR1 agonist whose expression is upregulated in PBMCs upon IL-13 or IL-4 stimulation.³ SIGLEC8 has an extracellular binding domain, which, on binding of specific glycans, induces apoptosis in eosinophils and inhibits FcεRI-dependent mediator release in mast cells.^{4,5} Single-nucleotide variants in the SIGLEC8 locus are associated with asthma and eosinophilic esophagitis susceptibility.⁶ PTGDR2 encodes CRTh2, a receptor for prostaglandin receptor D₂, which is expressed on multiple type 2 inflammatory cells such as eosinophils, basophils, T_H2 cells, group 2 innate lymphoid cells, and mast cells,⁷ and is under investigation as a therapeutic target in asthma. Taken together, these data suggest that the expression of these genes may not be exclusive to blood eosinophils and may also represent the activity of secreted effector molecules, for example, IL-13, on multiple type 2 inflammatory cells in peripheral blood.

The link between type 2 inflammation and CACNG6, IDO1, and HSD3B7 is less clear from the literature, and may represent novel associations *in vivo* with respect to asthmatic type 2 inflammation. Polymorphisms of CACNG6, a gene encoding a gamma subunit protein of the L-type calcium channel, have been associated with aspirin-intolerant asthma.⁸ IDO1 encodes the enzyme indoleamine 2,3-dioxygenase 1, which mediates

oxidative cleavage of tryptophan. Its role as an important regulator of immunosuppression has led to interest in pharmacological inhibition of this enzyme as a therapeutic strategy to subvert tumor immune tolerance.⁹ HSD3B7 metabolizes 7 α -hydroxylated sterols and is abundantly expressed in the liver though it is also detectable in lymphoid tissues and may be involved in regulating the behavior of immune cells.¹⁰

We assessed ERGS performance to predict asthmatic eosinophilic airway inflammation (see this article's Methods section) in the BOBCAT study of severe asthma by receiver-operating characteristic analysis (Fig 1, B). With the exception of IDO1 and CACNG6, ERGS performance exceeded that of absolute blood eosinophil count (area under the curve = 0.65) but not that of serum periostin (area under the curve = 0.78).

The predictive performance of baseline ERGS expression for blood eosinophil count and differential treatment response is summarized in Fig 2. The correlations between baseline ERGS expression and blood eosinophil count are plotted for the MILLY and LUTE/VERSE phase 2 studies of lebrikizumab (Fig 2, A). All correlations were statistically significant (P value < .001). Notably, the relative rankings of genes with correlation to blood eosinophil count were consistent between MILLY and LUTE/VERSE studies, highlighting technical and biological consistency of the assay and genes, respectively.

We assessed the predictive performance of baseline ERGS expression to enrich for clinical benefit in change in FEV₁ from baseline to week 12 with anti-IL-13 treatment. For each ERGS gene, subjects were dichotomized into biomarker low/high to assess treatment effect (see this article's Methods section). The treatment effect differences between biomarker high versus low ($\Delta\Delta\text{FEV}_1\%$) were assessed and are plotted in Fig 2, B, for MILLY and LUTE/VERSE studies. Although all biomarkers positively enriched for treatment benefit, SIGLEC8, CCL23, PTGDR2, IDO1, and CACNG6 consistently yielded the largest $\Delta\Delta\text{FEV}_1\%$ improvement, comparable to or exceeding the performance of

blood eosinophil count or serum periostin. The estimates at week 12 were also supported by consistent groupwise changes observed at weeks 4 and 8 (see Figs E3 and E4 in this article's Online Repository at www.jacionline.org).

Although these data are promising and establish that the expression levels of these genes individually in whole blood may be alternative biomarkers of type 2 asthma in adults, the therapeutic and diagnostic hypotheses remain to be tested formally in children. Although our observations in the EXTRA study are consistent with previous observations² that serum periostin is elevated in pediatric patients, the observation of elevated blood eosinophils was not replicated in this study, which may suggest that the EXTRA population, which had only 15 patients younger than 18 years, did not sufficiently represent the pediatric population and/or the relationship is weaker than concluded previously. Additional independent prospective studies may identify combinations of transcripts that perform better than individual genes for which the current studies are insufficiently powered, particularly considering the strong degree of intercorrelation between ERGS genes. In conclusion, we describe that peripheral blood expression of *SIGLEC8*, *CCL23*, *PTGDR2*, *IDO1*, *CACNG6*, and *HSD3B7* appear independent of age, correlate with peripheral blood eosinophil count, and enrich for treatment benefit from anti-IL-13 treatment; therefore, ERGS expression levels may be clinically useful biomarkers of type 2 "high" asthma.

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Disclosure of potential conflict of interest: D. F. Choy is employed by, has patents with, and has stock/stock options in Genentech. G. Jia, A. R. Abbas, K. B. Morshead, N. Lewin-Koh, J. M. Ray, C. R. Cabanski, and M. S. Bradley are employed by and have stock/stock options in Genentech. R. Dua, P. Rivera, P. Moonsamy, M. Fontecha, A. Balasubramanyam, and C. Santini are employed by Roche Molecular Solutions. E. Bassett and R. Maciucă are employed by and have stock/stock options in Roche/Genentech. H. Scheerens is employed by Genentech and has stock/stock options in Roche. J. R. Arron is employed by and has a patent through Genentech and has stock/stock options in Roche. S. Mosesova declares no relevant conflicts of interest.

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METHODS

Clinical cohorts

EXTRA. EXTRA (ClinicalTrials.gov identifier: NCT00314574) was a randomized, double-blind, placebo-controlled study of omalizumab (anti-IgE) in subjects 12 to 75 years old with moderate-to-severe persistent asthma. Full details of the study design have been published previously.^{E1,E2} In brief, after a 2- to 4-week run-in period, eligible patients were randomized in a 1:1 ratio to receive omalizumab or placebo (in addition to high-dose inhaled corticosteroid and long-acting β_2 -adrenergic agonist [LABA], with or without additional controller medications) for 48 weeks.

BOBCAT. BOBCAT^{E3,E4} was a multicenter observational study conducted in the United States, Canada, and the United Kingdom of 67 adult patients with moderate-to-severe asthma. Inclusion criteria required a diagnosis of moderate-to-severe asthma (confirmed by an FEV₁ between 40% and 80% of predicted value, as well as evidence within the past 5 years of >12% reversibility of airway obstruction with a short-acting bronchodilator or methacholine sensitivity [PC₂₀] <8 mg/mL) that was uncontrolled (as defined by at least 2 exacerbations in the previous year or a score of >1.50 on the Asthma Control Questionnaire (ACQ)^{E5} while receiving a stable dose regimen [>6 weeks] of a high-dose ICS [>1000 mg fluticasone or equivalent per day]) with or without a LABA. Permitted concomitant medications also included leukotriene receptor antagonists and oral corticosteroids. Peripheral blood was collected across 3 visits spanning 18 to 42 days, with the third visit having matched bronchial biopsy and blood samples.

MILLY. MILLY (ClinicalTrials.gov identifier: NCT00930163) was a randomized, double-blind, placebo-controlled study of lebrikizumab (anti-IL-13) in adults who had asthma that was inadequately controlled despite inhaled glucocorticoid therapy.^{E6}

Last observation carried forward imputation of FEV₁ was used for these analyses as previously described.^{E6}

LUTE/VERSE. LUTE and VERSE (ClinicalTrials.gov identifiers: NCT01545440 and NCT01545453) were randomized, double-blind, placebo-controlled studies of lebrikizumab (anti-IL-13) in adults who had asthma that was inadequately controlled despite inhaled glucocorticoid therapy.^{E7}

For all analyses, LUTE and VERSE studies were analyzed together, that is, pooled. The multiple doses of lebrikizumab were not considered separately and treatment effect analyses compared all lebrikizumab treatment arms combined versus placebo.

No imputation was used for FEV₁ measurements.

Gene expression microarrays

Peripheral blood gene expression was assessed by transcript microarray (Human Genome U133 Plus 2.0 Array, Affymetrix, Santa Clara, Calif) of mRNA isolated from PAXgene blood collections performed at baseline in EXTRA.

Biomarker measurements

ERGS. Blood RNA was isolated from PAXgene whole blood collection tubes. *GPR44*, *SIGLEC8*, *CCL23*, *IDO1*, *CACNG6*, and *HSD3B7* target gene mRNA expression was assessed by multiplexed quantitative PCR (using the COBAS system), normalized by *TFRC* as a housekeeping gene. Relative expression was assessed using the comparative C_T method^{E8,E9} and is expressed as herein in units of $-\Delta C_T$.

Biomarkers of type 2 inflammation. Peripheral blood eosinophil counts were obtained from standard complete blood cell counts. Fractional exhaled nitric oxide was measured using the NIOX MINO (Aerocrine, Solna, Sweden). Serum periostin, a systemic biomarker of eosinophilic inflammation in patients with asthma,^{E4} was measured using the clinical trial version of the Elecsys Periostin immunoassay (Roche Diagnostics, Penzberg, Germany) intended for use on the cobas e 601 analyzer. The assay is an automated electrochemiluminescence immunoassay, based on the sandwich principle.^{E10}

Statistical analysis

All statistical calculations were performed using the R Project software package (<http://www.R-project.org>).

Subject filtering. Subjects were filtered on the basis of having complete sets of available gene expression (microarray or ERGS) and absolute blood eosinophil data (baseline visits for interventional studies and at the bronchoscopy visit for the BOBCAT study) and only these subjects are represented herein for all analyses.

Microarrays. Array quality control was assessed using the arrayQualityMetrics package for R. Microarrays were normalized and probe intensities transformed using the RMA algorithm. Independent filtering^{E11} of microarray data was conducted using the genefilter package to increase statistical power to detect true positives while maintaining type I error control. Because array features that are invariant or do not correspond to an Entrez gene transcript are uninformative for differential gene expression analysis, filtering on the basis of annotation and expression variability was conducted. Briefly, filtering was conducted as follows: (1) Only array features representing Entrez genes were retained and (2) multiple array features corresponding to a single Entrez gene were reduced to a single array feature on the basis of highest interquartile range. Multiple regression of square root-transformed absolute blood eosinophil values ($\sqrt{\#/\mu L}$), adjusted by subject age (years), was conducted with the limma package of Bioconductor. Adjusted *P* values to account for multiple hypothesis testing was addressed using the method of Benjamini and Hochberg.^{E12}

Biomarkers. Correlation between continuous values of biomarkers was measured using Spearman correlation, and significance was assessed using a rank order test.

The predictive performance of baseline biomarker levels to enrich for clinical benefit from anti-IL-13 treatment was assessed as the treatment effect difference between lebrikizumab and placebo at week 12 ($\Delta\Delta FEV_1\%$). Dichotomous diagnostic classifications of biomarkers at baseline were based on respective study median (closed left, ie, <median and $\geq$ median) with the exception of serum periostin, which was prespecified at 50 ng/mL (closed left, ie, <50 ng/mL and $\geq$ 50 ng/mL). ANOVA of $\Delta FEV_1\%$ with terms for treatment (placebo or lebrikizumab), dichotomous diagnostic classification, and the interaction of these terms were conducted. $\Delta\Delta FEV_1\%$ was estimated by examination of the interaction term.

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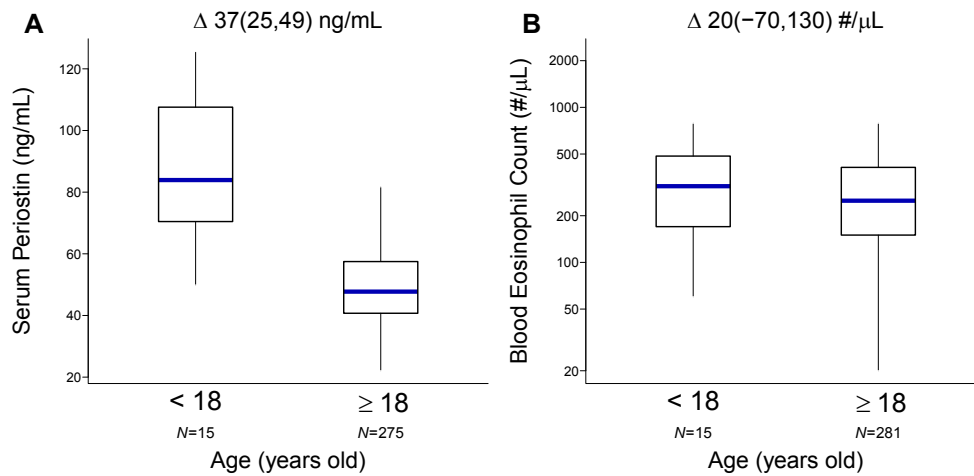


FIG E1. Boxplots of blood biomarkers in pediatric and adult patients with asthma in the EXTRA study. **A**, Serum periostin. **B**, Blood eosinophil count. Difference in medians (95% CI, Hodges-Lehmann estimator) between less than 18-year-old versus more than 18-year-old subjects is annotated in the plots.

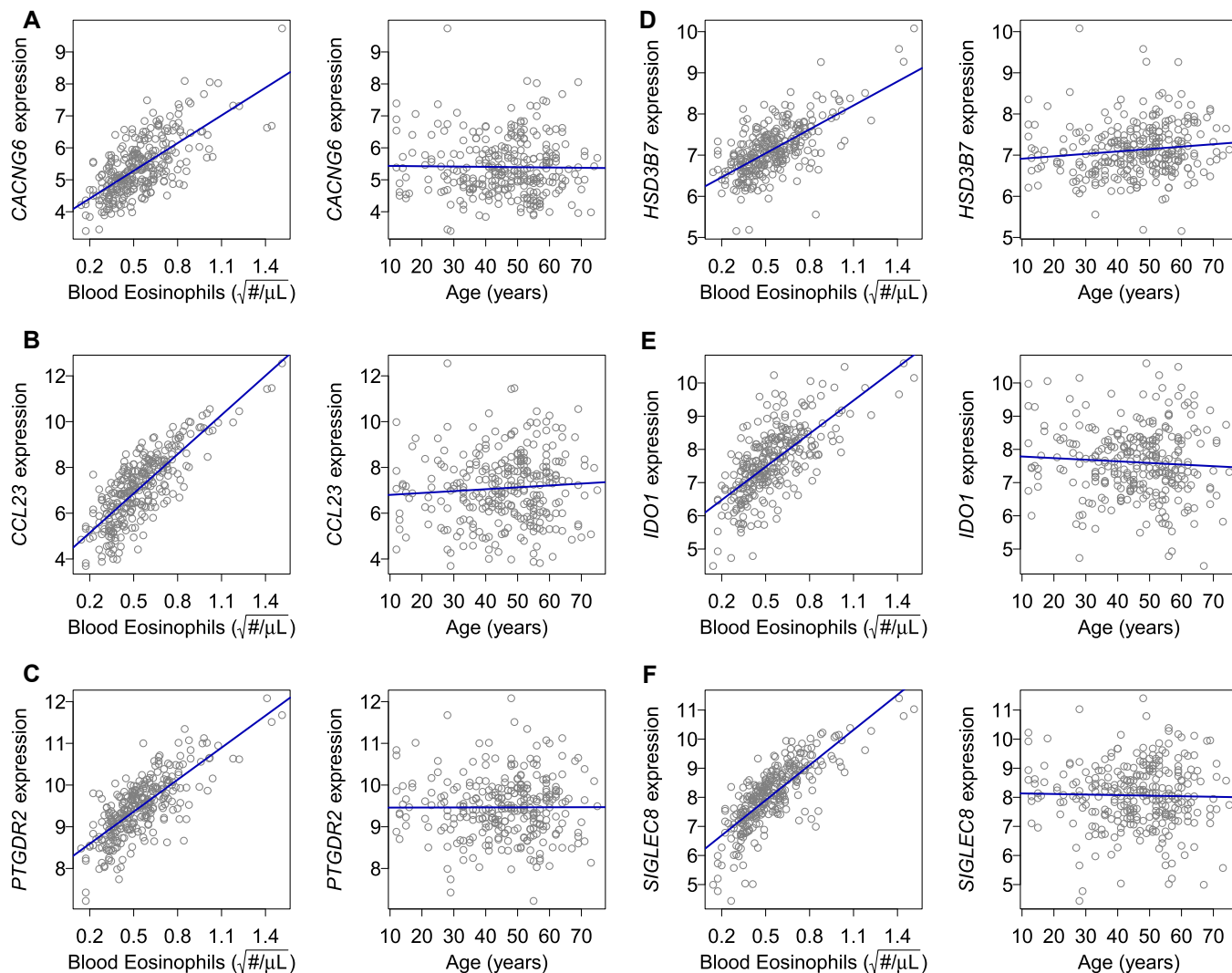


FIG E2. Scatterplots of ERGS gene expression with blood eosinophils and age from the EXTRA study. ERGS gene expression (Y axes) from PAXgene is plotted versus blood eosinophils count ($\sqrt{\#/\mu\text{L}}$, X-axes) and age (years) from the EXTRA study for *CACNG6* (A), *CCL23* (B), *GPR44* (C), *HSD3B7* (D), *IDO1* (E), and *SIGLEC8* (F). Linear regression lines are plotted in blue.

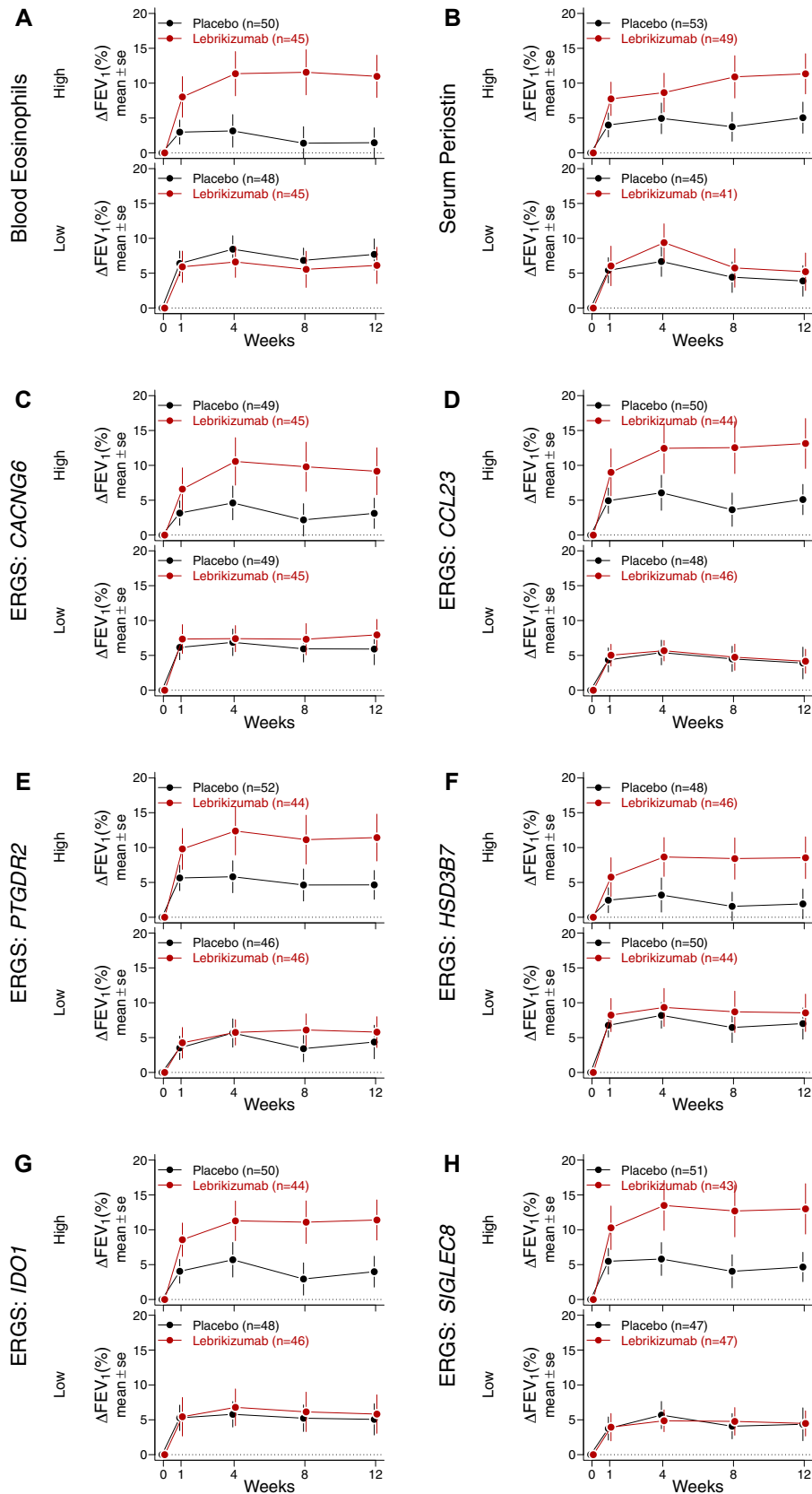


FIG E3. Relative change in FEV₁ (Δ FEV₁) by biomarker subgroups in the MILLY study. Mean $\pm$ standard error of Δ FEV₁ are plotted by dichotomous diagnostic classification of blood eosinophils (A), serum periostin (B), and ERGS genes (C-H) for placebo and lebrikizumab treatment arms. Diagnostic population is divided at the respective study median (closed left) for all biomarkers except for serum periostin (50 ng/mL).

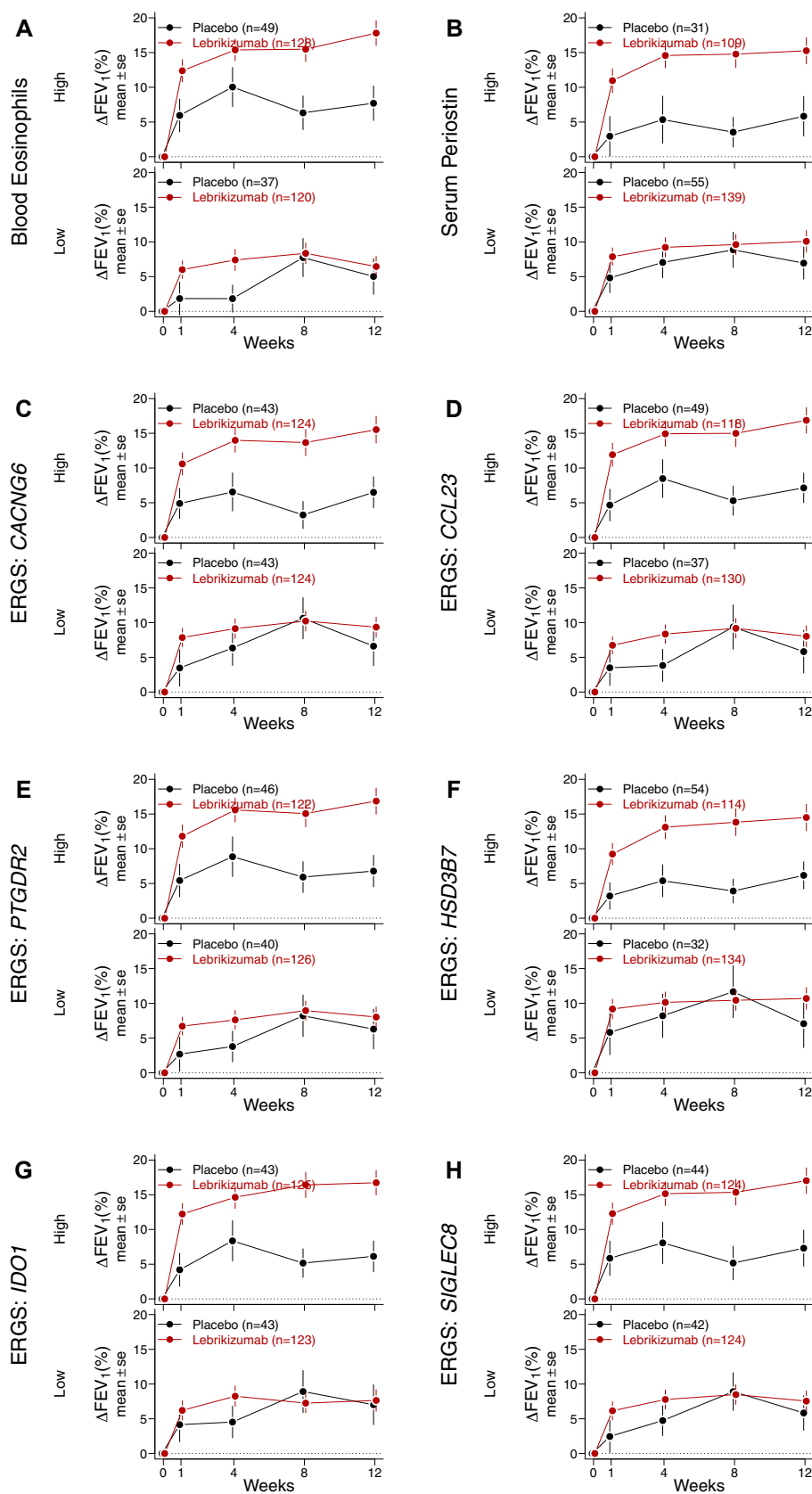


FIG E4. Relative change in FEV₁ (Δ FEV₁) by biomarker subgroups in LUTE/VERSE studies. Mean $\pm$ standard error of Δ FEV₁% are plotted by dichotomous diagnostic classification of blood eosinophils (A), serum periostin (B), and ERGS genes (C-H) for placebo and lebrikizumab treatment arms. Diagnostic population is divided at the respective study median (closed left) for all biomarkers except for serum periostin (50 ng/mL).

TABLE E1. Baseline clinical characteristics of cohorts

Characteristic	EXTRA	BOBCAT	MILLY	LUTE/VERSE
Biomarker subpopulation	298 of 848 (35.1%)	59 of 67 (88.1%)	188 of 218 (86.2%)	334 of 463 (72.1%)
Age (y)	48 (12, 38, 56, 75)	47 (20, 38, 54, 67)	45 (18, 36, 53, 65)	49 (18, 39, 58, 75)
Sex: F/M, n (%)	203 (68.1)/95 (31.9)	32 (54.2)/27 (45.8)	124 (66)/64 (34)	199 (59.6)/135 (40.4)
Oral corticosteroid: N/Y, (n/%)	271 (90.9)/27 (9.1)	50 (84.7)/9 (15.3)	188 (100)/0 (0)	334 (100)/0 (0)
FEV ₁ % predicted	67 (25, 55, 75, 98)	63 (33, 53, 71, 83)	67 (40, 58, 74, 87)	61 (31, 55, 71, 86)
FENO (ppb)	18 (5, 12, 30, 54)	27 (8.3, 19, 40, 64)	21 (4.8, 15, 39, 70)	21 (2.5, 13, 34, 65)
Blood eosinophils ($\times 10^9/L$)	255 (20, 150, 420, 790)	210 (40, 140, 310, 490)	210 (50, 130, 330, 610)	250 (10, 160, 370, 670)
Serum periostin (ng/mL)	48 (22, 41, 60, 88)	49 (28, 40, 59, 83)	51 (28, 44, 59, 79)	48 (23, 41, 56, 79)

Subjects represented in these analyses are expressed as counts and percentage of subjects who completed the respective study in the top row (biomarker subpopulation). Continuous data are summarized as median (minimum, first quartile, third quartile, maximum). Categorical data are summarized as the counts and percent of total for each level (indicated in table margins).

F, Female; FENO, fraction of exhaled nitric oxide; M, male; N, no; Y, yes.

TABLE E2. Top 50 most correlated peripheral blood gene expression with absolute blood eosinophil count

Gene symbol	Gene name	β	t Statistic	BH-adjusted P value
<i>SLC29A1</i>	Solute carrier family 29 (equilibrative nucleoside transporter), member 1	3.000439721	25.01354907	4.86×10^{-71}
<i>CCL23</i>	Chemokine (C-C motif) ligand 23	5.721662194	23.38130733	1.59×10^{-65}
<i>SMPD3</i>	Sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)	2.831928884	22.49830163	1.67×10^{-62}
<i>SIGLEC8</i>	Sialic acid binding Ig-like lectin 8	4.029314453	21.91607271	1.67×10^{-60}
<i>OLIG2</i>	Oligodendrocyte lineage transcription factor 2	4.205011922	21.37403627	1.31×10^{-58}
<i>HRASLS5</i>	HRAS-like suppressor family, member 5	2.599951764	21.20212651	4.72×10^{-58}
<i>PRSS33</i>	Protease, serine, 33	4.466619363	20.69818641	2.96×10^{-56}
<i>IL5RA</i>	Interleukin 5 receptor, alpha	3.549321393	20.47325808	1.77×10^{-55}
<i>PIK3R6</i>	Phosphoinositide-3-kinase, regulatory subunit 6	2.387788094	20.2115415	1.48×10^{-54}
<i>PTGDR2</i>	Prostaglandin D2 receptor 2	2.559997899	19.71989515	9.08×10^{-53}
<i>PMP22</i>	Peripheral myelin protein 22	3.916741638	19.68614513	1.10×10^{-52}
<i>CEBPE</i>	CCAAT/enhancer binding protein (C/EBP), epsilon	2.546329265	19.18607636	7.53×10^{-51}
<i>ADORA3</i>	Adenosine A3 receptor	3.059719579	18.74168943	3.24×10^{-49}
<i>LRRC17</i>	Leucine rich repeat containing 17	2.161503595	17.37167495	4.34×10^{-44}
<i>ALOX15</i>	Arachidonate 15-lipoxygenase	3.882886153	17.26215613	1.05×10^{-43}
<i>ACOT11</i>	Acyl-CoA thioesterase 11	1.358281941	16.63595096	2.26×10^{-41}
<i>CACNG6</i>	Calcium channel, voltage-dependent, gamma subunit 6	2.889514482	16.30318772	3.81×10^{-40}
<i>IDO1</i>	Indoleamine 2,3-dioxygenase 1	3.327094023	16.19231011	9.42×10^{-40}
<i>MEIS2</i>	Meis homeobox 2	1.950023798	16.02226237	3.89×10^{-39}
<i>HRH4</i>	Histamine receptor H4	2.512104136	15.72228102	4.96×10^{-38}
<i>PYROXD2</i>	Pyridine nucleotide-disulphide oxidoreductase domain 2	1.788790804	15.68978533	6.26×10^{-38}
<i>OLIG1</i>	Oligodendrocyte transcription factor 1	2.109174306	15.64111811	9.10×10^{-38}
<i>SPNS3</i>	Spinster homolog 3 (Drosophila)	1.816567067	15.42155925	5.80×10^{-37}
<i>STAC</i>	SH3 and cysteine-rich domain	2.640873766	15.40285397	6.54×10^{-37}
<i>SLC16A14</i>	Solute carrier family 16, member 14	3.194358366	15.23395401	2.69×10^{-36}
<i>HSD3B7</i>	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	1.939685477	15.11742257	7.07×10^{-36}
<i>THBS4</i>	Thrombospondin 4	1.48335329	15.08021631	9.38×10^{-36}
<i>CLC</i>	Charcot-Leyden crystal galectin	1.747763787	15.04487155	1.23×10^{-35}
<i>ASB2</i>	Ankyrin repeat and SOCS box containing 2	1.861110467	15.0310616	1.33×10^{-35}
<i>SEMA7A</i>	Semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)	1.301624717	15.0158034	1.47×10^{-35}
<i>SORD</i>	Sorbitol dehydrogenase	2.089315653	14.93228568	2.92×10^{-35}
<i>LGALS12</i>	Lectin, galactoside-binding, soluble, 12	1.957389121	14.77001118	1.14×10^{-34}
<i>PDE4D</i>	Phosphodiesterase 4D, cAMP-specific	1.986447945	14.76342462	1.17×10^{-34}
<i>GPR82</i>	G protein-coupled receptor 82	2.659646884	14.61843744	3.94×10^{-34}
<i>UGT2B28</i>	UDP glucuronosyltransferase 2 family, polypeptide B28	2.011650339	14.59608739	4.63×10^{-34}
<i>RPS6KA2</i>	Ribosomal protein S6 kinase, 90kDa, polypeptide 2	1.583495273	14.0202488	6.16×10^{-32}
<i>IL1RL1</i>	Interleukin 1 receptor-like 1	2.517527966	13.8879985	1.84×10^{-31}
<i>SVOP</i>	SVOP-like	1.893487419	13.85681577	2.34×10^{-31}
<i>LINC00323</i>	Long intergenic nonprotein coding RNA 323	2.373802292	13.82079105	3.09×10^{-31}
<i>ABTB2</i>	Ankyrin repeat and BTB (POZ) domain containing 2	1.304545363	13.81407741	3.19×10^{-31}
<i>CACNG8</i>	Calcium channel, voltage-dependent, gamma subunit 8	1.31111396	13.71152129	7.43×10^{-31}
<i>CAMK1</i>	Calcium/calmodulin-dependent protein kinase I	1.566106368	13.59341275	1.97×10^{-30}
<i>GLOD5</i>	Glyoxalase domain containing 5	3.261115277	13.56055898	2.54×10^{-30}
<i>CYSLTR2</i>	Cysteinyl leukotriene receptor 2	2.082036968	13.46211506	5.70×10^{-30}
<i>CAT</i>	Catalase	2.015964542	13.34744238	1.46×10^{-29}
<i>BACE2</i>	Beta-site APP-cleaving enzyme 2	1.811492185	13.24183731	3.48×10^{-29}
<i>CYP4F12</i>	Cytochrome P450, family 4, subfamily F, polypeptide 12	1.993404559	13.05849273	1.59×10^{-28}
<i>CACNA1D</i>	Calcium channel, voltage-dependent, L type, alpha 1D subunit	2.006190808	13.05039837	1.66×10^{-28}
<i>CSF1</i>	Colony stimulating factor 1 (macrophage)	1.409012675	12.89224139	6.10×10^{-28}
<i>KHDRBS3</i>	KH domain containing, RNA binding, signal transduction associated 3	1.220727754	12.84037803	9.21×10^{-28}

Regression of contemporaneously assessed absolute blood eosinophil count ($\sqrt{\#/\mu L}$), adjusted by subject age (y), with blood gene expression (PAXgene microarrays) was conducted from 298 subjects with moderate-to-severe asthma from the EXTRA study at baseline. The top 50 genes (by Benjamini-Hochberg adjusted P value) are tabulated with gene annotation and test statistics.