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Effect of Dupilumab on Airway Inflammation in Patients With Persistent Asthma

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ABSTRACT

Background: Biologics targeting type 2 cytokines can inhibit airway inflammation and improve lung function in moderate-to-severe asthma; however, their impact on airway mucosal inflammatory cells is unclear. This study assessed the effects of dupilumab on airway mucosal and systemic inflammation, and related gene expression in patients with persistent asthma.

Methods: In the phase 2a EXPEDITION study (NCT02573233), patients aged 18–65 years were randomised to add-on dupilumab 300 mg ($n = 20$) or placebo ($n = 22$) every 2 weeks for 12 weeks. Pre- and post-treatment bronchial biopsies, bronchial brushings, bronchoalveolar lavage (BAL) fluid and blood samples were collected. Clinical and patient-reported outcomes, gene expression, type 2 biomarkers and safety outcomes were assessed.

Results: Dupilumab versus placebo improved lung function and asthma control. No significant changes in eosinophils, mast cells or type 2 helper cells were observed in bronchial biopsies. Downregulation of *M2 macrophage*- and *eosinophil*-associated gene sets was observed in BAL and brushing samples after dupilumab. Dupilumab decreased multiple circulating type 2 biomarkers in peripheral blood ($p_{unadj} < 0.001$, $p_{adj} < 0.01$), goblet cell numbers ($p_{unadj} = 0.0336$; $p_{adj} = 0.2554$) and mucus area ($p_{unadj} = 0.0426$; $p_{adj} = 0.2554$) in bronchial biopsies versus placebo. The safety profile was consistent with the known safety profile of dupilumab.

Conclusion: Dupilumab improved lung function and asthma control while reducing circulating type 2 biomarkers. No measurable impact was observed on type 2-associated inflammatory cell numbers in airway bronchial biopsies; however, dupilumab modulated the expression of inflammation-associated gene sets. These findings provide cellular and molecular data that may explain dupilumab-driven mechanisms of improved lung function in patients with type 2 asthma.

Emanuele de Rinaldis and Leda P. Mannent contributed equally to this work.

1 | Introduction

Asthma is a chronic inflammatory disease characterised by reversible and variable airway obstruction, airway hyperresponsiveness and episodes of bronchodilator-non-responsive airflow limitations associated with mucus plugging [1, 2]. Variability in the responsiveness of patients with asthma to standard-of-care treatment reflects disease heterogeneity [2]. Recent therapeutic approaches focus on targeting various immune mediators, including interleukins (IL)-4, −5 and −13, immunoglobulin E (IgE), prostaglandin D2 receptor 2 (CRTH2) and thymic stromal lymphopoietin (TSLP) [3]. IL-4 and IL-13–driven type 2 inflammation is critical in the pathogenesis of moderate-to-severe asthma [4, 5]. IL-4 regulates T-cell differentiation and B- tcell class switching to IgE, inducing the production of type 2-associated cytokines, chemokines and IgE, while IL-13 is involved in goblet cell hyperplasia, mucus hypersecretion, airway hyper-responsiveness and smooth muscle reactivity [4, 6]. Biomarker levels, like fractional exhaled nitric oxide (FeNO), blood/sputum eosinophils, serum total IgE and periostin, are also partially regulated by IL-4/IL-13–driven type 2 airway inflammation [4].

Dupilumab is a fully human monoclonal antibody [7, 8] targeting the IL-4 receptor α subunit (IL-4Rα), a component of Type I (IL-4 ligand only) and Type II (both IL-4 and IL-13 ligands) IL-4 heterodimeric receptors. Dupilumab binding to IL-4Rα blocks both receptors, limiting downstream pro-inflammatory pathway activation. Dupilumab is indicated for patients with diseases with underlying type 2 inflammation, including atopic dermatitis (AD), asthma, chronic rhinosinusitis with nasal polyps, eosinophilic esophagitis, chronic obstructive pulmonary disease and prurigo nodularis [9, 10]. It is efficacious in patients with type 2 asthma, and characterised by increased levels of blood eosinophils or FeNO [5, 11], but little is known about its impact on airway mucosal cells and modulation of transcriptional processes in various tissue and cellular compartments. In the EXPEDITION study (NCT02573233), we assessed the effects of dupilumab on airway inflammation in patients with persistent asthma using bronchoscopy and gene expression analysis.

2 | Methods

2.1 | Study Design and Patients

EXPEDITION was an exploratory, randomised, double-blind, placebo-controlled, phase 2 study conducted from January 27, 2016, to January 3, 2018, in Canada, Denmark, Germany, Sweden, the United Kingdom and the United States. Patients with persistent asthma completed a 4-week screening period, 12-week randomised treatment period and 12-week follow-up period, and underwent bronchoscopy before and after the treatment period (Figure S1). All patients provided written informed consent before participating in the trial.

Patients aged 18–65 years with physician-diagnosed persistent asthma for ≥12 months (Global Initiative for Asthma 2015 guidelines) [12] were eligible if they were receiving current treatment with a medium-to-high dose of inhaled corticosteroid (ICS) plus ≤2 additional controllers, had a pre-bronchodilator percent predicted forced expiratory volume in 1 s ($ppFEV_1$) of 55%–85% and an FEV_1 reversibility of ≥12% and 200 mL. Patients with FeNO <26 parts per billion (ppb) were excluded to enrich the population for patients with evidence of ongoing type 2 inflammation. No minimum threshold for blood or tissue eosinophil cell counts was required. Previous and current smokers and patients who had received systemic corticosteroids within 28 days were excluded. Full inclusion and exclusion criteria are provided in the Supporting Information.

Patients were randomised (1:1) to receive dupilumab 300 mg or placebo subcutaneously every 2 weeks (q2w) for 12 weeks, and stratified by ICS dose (Table S1) and region. Concomitant controller medications were continued at stable doses (Table S2). Further details of study design and methodology are provided in the Supporting Information.

2.2 | Sample Collection

Fibreoptic flexible bronchoscopy was performed in all patients before treatment initiation and at the end of the treatment period for endobronchial biopsy, bronchial brushing and bronchoalveolar lavage fluid (BALF) sample collection. Table S3 shows the locations where bronchoscopy sample collections were performed, which were randomly predetermined either from the right or left lower lobe for the first bronchoscopy (baseline). The second bronchoscopy (Week 12) samples were collected from the opposite side. At least four adequate tissue samples were collected during each bronchoscopy and stored in formalin for immunohistochemistry analysis. All tissue samples from the same bronchoscopy were paraffin embedded and stored until analysis. All analyses were done by a central, blinded reader. All patients received 3–5 days of oral corticosteroids (prednisone/prednisolone 40 mg daily) post bronchoscopy as a precaution to prevent bronchospasms. Blood samples were also collected at specified timepoints.

2.3 | Outcomes

2.3.1 | Main Pharmacodynamic Endpoints

The main pharmacodynamic endpoints included the change from baseline to Week 12 and/or the ratio of baseline to Week 12 values of cells in endobronchial biopsy samples stained for appropriate markers. Submucosal eosinophils (major basic protein$^+$ [MBP$^+$]), mast cells (chymase$^+$ or tryptase$^+$), total T-lymphocytes (cluster of differentiation [CD]3$^+$), T-helper lymphocytes (CD4$^+$), B cells (CD23$^+$) and goblet cells (mucin [MUC] 5 AC$^+$) were measured as cells/mm^2. Mucin-stained (MUC$^+$) area (%) was defined as positive area (mm^2)/area of tissue (mm^2)×100. A *post hoc* analysis of the submucosal eosinophil cell count was performed.

2.3.2 | Additional Pharmacodynamic Endpoints

Additional pharmacodynamic outcomes included the change and percent change from baseline at Week 12 in FeNO and the change from baseline in the averaged FeNO of Weeks 6–12.

2.3.3 | Exploratory Pharmacodynamic Endpoints

Exploratory pharmacodynamic endpoints included changes from baseline to Week 12 in subepithelial thickness in bronchial biopsy samples, differential cell counts in blood and BALF, cell types measured by immunocytochemistry, protein biomarkers (e.g., periostin, TARC, ECP, IL-4, IL-13, IL-5) and RNA expression markers in bronchial brushing, BAL-cell and biopsy samples, assessed through transcriptome (gene expression) analyses as described in the Supporting Appendix. RNA expression analyses and gene set enrichment analysis (GSEA) were performed using published meta-analyses of airway-epithelium transcriptional data from patients with asthma versus healthy controls [13, 14]. Full methods, including lower limits of quantification for protein biomarkers (Table S4), are provided in the Supporting Appendix.

2.3.4 | Clinical and Safety Endpoints

Clinical endpoints included change from baseline in pre-bronchodilator FEV_1 and 5-item Asthma Control Questionnaire (ACQ-5) score. Safety and tolerability were evaluated by the incidence of adverse events and findings from physical examination, laboratory testing and 12-lead electrocardiography.

A complete list of study objectives and endpoints is provided in the Supporting Appendix.

2.4 | Statistical Analysis

Details on the sample size determination and analyses of pharmacodynamic, efficacy and safety endpoints are provided in the Supporting Appendix.

For the transcriptome analysis, genes were tested for differences in pre- and post-treatment expression in dupilumab versus placebo. Transcriptome data from airway samples were evaluated by a linear mixed-effects model. No genes passed the significance threshold of 0.05 after correction for false discovery rate by the Benjamini–Hochberg procedure (Table S5). To gain statistical power, data were then analysed at the gene set level using GSEA. Thirty-four preselected gene sets relevant to inflammation and asthma were queried (Tables S6 and S7), and p-values were adjusted with the Benjamini–Hochberg procedure. Details of transcriptome data preprocessing, reference dataset construction and GSEA implementation are provided in the Supporting Appendix.

3 | Results

Forty-two patients were randomised to receive dupilumab ($n=20$) or placebo ($n=22$). All completed the treatment period (Figure S2). Overall, baseline characteristics were similar between the treatment arms (Table 1). The mean age was 41.0 years in the placebo group and 45.5 years in the dupilumab group; 65.0% and 36.4% of patients were female, and 45.5% (10 patients) and 55.0% (11 patients) had a BMI $\geq 30\,kg{\cdot}m^2$ in the dupilumab and placebo groups, respectively. Notably, median (IQR) MBP^+ tissue eosinophil counts were higher in the dupilumab group (30.0 [10.1–263.8] cells·mm^{-2}) than the placebo group (13.0 [9.9–21.2] cells·mm^{-2}) but more balanced using ECP^+ counts (26.1 ± 30.2 cells·mm^{-2} and 32.1 ± 32.8 cells·mm^{-2}). Median (IQR) blood eosinophil counts at baseline were 0.32 (0.18–0.48)$\times 10^9$ cells·L^{-1} in the dupilumab arm and 0.38 (0.25–0.57)$\times 10^9$ cells·L^{-1} in the placebo arm. Demographics of the patients with samples included in the transcriptome analysis were consistent (Table S8).

In bronchial biopsies obtained at baseline and end of treatment, 85.0% (17/20 patients) in the dupilumab group and 95.4% (21/22 patients) in the placebo group were of sufficient quality for analysis. Dupilumab versus placebo had no appreciable effect on tissue inflammatory cell counts in stained bronchial biopsy sections (Table 2). Notably, median baseline values of eosinophil MBP^+ cells were nearly 3-fold higher for dupilumab versus placebo (Table 1); however, post-treatment, change from baseline showed no significant between-group differences ($p_{unadj}=0.8400$; Table 2, Figure 1a–d). Normalised gene enrichment scores for eosinophil-associated gene expression signatures are shown in Figure 1e. After treatment, no significant changes from baseline were observed in ECP^+ eosinophil counts ($p=0.7048$), tissue mast cells (chymase$^+$ [$p_{unadj}=0.4795$], tryptase$^+$ [$p_{unadj}=0.4494$]), $CD3^+/CD4^+$ lymphocytes ($p_{unadj}=0.6865$ and $p_{unadj}=0.7588$, respectively) or B cells ($p_{unadj}=0.7481$) (Table 2; Table S5 and Figures S3–S5).

In contrast, goblet ($MUC5AC^+$) cell counts ($p_{unadj}=0.0336$, $p_{adj}=0.2554$) and the relative mucus (mucin)-stained area ($p_{unadj}=0.0426$, $p_{adj}=0.2554$) decreased from baseline to Week 12, although differences were not significant after adjusting for multiple testing (Table 2, Figure S3). The reticular basement membrane (RBM) thickness was comparable between treatments at baseline. Although a reduction in RBM thickness was seen in dupilumab-treated patients at Week 12, the difference between treatment arms was not statistically significant ($p_{unadj}=0.2629$; Table 2). Dupilumab versus placebo significantly improved pre-bronchodilator FEV_1 from baseline at Week 12 (LS mean difference [90% CI]: 0.27 L [0.10 to 0.44]; $p=0.0100$) (Table S9). ACQ-5 scores were significantly improved from baseline at Week 8 (LS mean difference [90% CI]: −0.53 [−0.93 to −0.13]; $p=0.0336$) and numerically improved at Week 12 (LS mean difference [90% CI]: −0.18 [−0.55 to 0.19]; $p=0.4059$) (Table S9 and Figure S6A). Dupilumab versus placebo significantly reduced FeNO levels from baseline at Week 12 ($p_{adj}=0.0013$) (Table S9 and Figure S6B). Exploratory outcomes included circulating type 2 biomarkers and inflammatory cells. In peripheral blood, no differences were observed in the Week 12 change from baseline in either mean eosinophil or neutrophil counts ($p_{unadj}>0.05$; Table S10). Dupilumab versus placebo was associated with significant reductions in circulating total IgE, periostin, eotaxin-3 and thymus and activation-regulated chemokine (TARC) at Week 12 ($p_{unadj}<0.001$ for all, $p_{adj}<0.01$) (Table S10 and Figure S7). Meanwhile, circulating IL-4 concentrations at Week 12 significantly increased (Table S10). Other circulating blood biomarkers did not significantly change in response to dupilumab after adjustment for false discovery rate (Table S10 and Figure S7).

In BAL samples, Week 12 changes in periostin, TNF-α and IL-6 did not differ between dupilumab and placebo ($p_{unadj}>0.05$).

TABLE 1 | Demographic and baseline characteristics (randomised population).

	Placebo ($n=22$)	Dupilumab ($n=20$)
Age, years	41.0 ± 10.3	45.5 ± 10.6
Sex		
Female	8 (36.4)	13 (65.0)
Male	14 (63.6)	7 (35.0)
Race		
Asian	0 (0)	1 (5.0)
Black	3 (13.6)	3 (15.0)
White	19 (86.4)	16 (80.0)
BMI, $kg \cdot m^{-2}$	29.4 ± 6.0	29.9 ± 6.6
Patients with $\geq 30\,kg \cdot m^{-2}$	10 (45.5)	11 (55.0)
Duration of asthma, years	26.8 ± 14.4	31.8 ± 10.8
Severe asthma exacerbations in the past year, *n*	1.00 ± 1.48	0.85 ± 1.90
ICS dose[a] at baseline		
High dose	10 (45.5)	11 (55.0)
Medium/low dose	12 (54.5)	9 (45.0)
Pre-bronchodilator FEV_1, L	2.70 ± 0.63	2.31 ± 0.52
Pre-bronchodilator $ppFEV_1$, %	73.5 ± 11.6	71.6 ± 9.5
FEV_1 reversibility, %	19.1 ± 7.4	14.0 ± 7.0
ACQ-5 score, points	1.73 ± 0.97	1.38 ± 0.63
Biomarkers		
Tissue eosinophil count (MBP^+), $cells \cdot mm^{-2}$	13.0 (9.9–21.2)	35.0 (10.1–263.8)
Tissue eosinophil count (ECP^+), $cells \cdot mm^{-2}$	32.1 ± 32.8	26.1 ± 30.2
Blood eosinophil count, $\times 10^9\ cells \cdot L^{-1}$	0.38 (0.25–0.57)	0.32 (0.18–0.48)
FeNO, ppb	28.0 (19.0–56.0)	30.0 (20.5–42.0)
Total IgE, $IU \cdot mL^{-1}$	177.0 (65.0–245.0)	137.0 (83.0–280.0)

Note: Data are presented as mean ± SD, *n* (%), or median (interquartile range).
Abbreviations: ACQ-5, 5-item Asthma Control Questionnaire; BMI, body mass index; ECP^+, eosinophil cationic protein$^+$; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroid(s); IgE, immunoglobulin E; IQR, interquartile range; IU, international unit; MBP^+, major basic protein$^+$; ppb, parts per billion; $ppFEV_1$, percent predicted FEV_1; SD, standard deviation.
[a]ICS dose as defined in GINA 2015 guidelines [12] (Table S1).

Mean reductions in TARC were greater in patients receiving dupilumab ($p_{unadj}=0.0344$, $p_{adj}=0.1374$) but not significant after adjustment (Table S11). Dupilumab had no effect on either MBP^+ eosinophil ($p_{unadj}=0.2513$) or $CD206^+$ macrophage count ($p_{unadj}=0.9249$). $CD68^+$ macrophages increased from baseline for dupilumab versus placebo ($p_{unadj}=0.0324$, $p_{adj}=0.2289$), although this was not significant after adjustment (Table S11).

In our study, dupilumab significantly modulated multiple gene sets in bronchial brushing samples relative to placebo, upregulating those previously reported to be downregulated in patients with asthma versus healthy controls (*Asthma-Down* gene set) and downregulating those upregulated in patients with asthma versus healthy controls (*Asthma-Up* gene set; Figure 2, Table 3 and Figures S8–S11). The largest effects were seen in the type 2-associated extracellular matrix protein periostin gene (*POSTN*), serine or cysteine protease inhibitor genes (e.g., *SERPINB2*, *SERPINB10*, *CST1* and *CST4*), mucus secretion and goblet cell hyperplasia-related genes (e.g., *CLCA1*, *MUC5AC*, *MUC5B*, *FOXA3* and *AGR2*) and chemokine genes *CXCL1*, *CXCL6* and *CX3CL1*. In all three airway matrices, dupilumab significantly reversed the upregulation of *type 2* genes previously reported in bronchial brushings of patients with asthma [13, 14] (Table 3, Figure 3a, Figure 3b). Individual sample-level analyses did not support the enrichment of other gene sets, including the type 1 immune response- and type 1 interferon response-associated gene sets (Figure 3c, Figure 3d). Further details are available in Tables S12–S14 and Figures S8–S11.

TABLE 2 | Differential cell counts in bronchial biopsy samples (bronchoscopy pharmacodynamic population).[a]

	Placebo ($n = 22$)		Dupilumab ($n = 20$)		Difference versus placebo at Week 12		
Endpoints[a]	**Baseline**	**Change from baseline at Week 12**[b]	**Baseline**	**Change from baseline at Week 12**[b]	**LS mean difference (90% CI)**	**Un-adjusted *p*-value**[b]	**Adjusted *p*-value**[c]
Tissue cell counts, cells/mm^2							
Eosinophils (MBP$^+$)	12.97 (9.91–21.24)	5.80 (−9.18 to 33.41)	34.96 (10.07–263.75)	−6.04 (−174.71 to 19.41)	NA	0.8400	0.9429
Eosinophils (ECP$^+$)	32.13 ± 32.76	9.78 ± 9.24	26.11 ± 30.24	4.61 ± 9.77	−5.17 (−28.09 to 17.75)	0.7048	NA[c]
Mast cells (chymase$^+$)	74.36 ± 58.63	−13.58 ± 13.08	79.17 ± 68.07	0.32 ± 14.20	13.89 (−19.00 to 46.78)	0.4795	0.8631
Mast cells (tryptase$^+$)	80.10 ± 64.92	0.45 ± 16.37	105.53 ± 104.68	−18.52 ± 18.24	−18.98 (−60.92 to 22.97)	0.4494	0.8631
Total T-lymphocytes (CD3$^+$)	301.09 (121.01–500.43)	−36.70 (−200.25 to 267.51)	153.33 (83.81–192.40)	34.21 (−95.08 to 232.99)	NA	0.6865	0.9106
T-helper lymphocytes (CD4$^+$)	237.10 (190.17–511.48)	7.26 (−179.43 to 224.96)	200.85 (102.49–398.08)	62.34 (−100.62 to 159.09)	NA	0.7588	0.9106
B cells (CD23$^+$)	14.90 (9.36–28.12)	−5.26 (−10.93 to 4.98)	12.66 (6.10–23.80)	0.05 (−7.79 to 9.96)	NA	0.7481	0.9106
Goblet cells (MUC5AC$^+$)	561.12 ± 289.00	76.61 ± 70.01	520.57 ± 511.69	−158.40 ± 78.37	−235.02 (−414.19 to −55.84)	0.0336	0.2554
Mucin-stained area, %	17.59 ± 8.21	1.68 ± 1.86	16.79 ± 11.87	−4.27 ± 2.09	−5.95 (−10.72 to −1.18)	0.0426	0.2554
Thickness of RBM, μm	6.36 ± 1.70	0.24 ± 0.34	5.98 ± 1.97	−0.35 ± 0.38	−0.59 (−1.47 to 0.29)	0.2629	0.2855

Note: Data are presented as mean ± SD or median (interquartile range), unless otherwise stated.

Abbreviations: ANCOVA, analysis of covariance; CD, cluster of differentiation; CI, confidence interval; ECP$^+$, eosinophil cationic protein$^+$; EOT, end of treatment; FDR, false discovery rate; ICS, inhaled corticosteroid(s); IQR, interquartile range; LS, least squares; MBP$^+$, major basic protein$^+$; MUC5AC$^+$, mucin 5 AC$^+$; NA, not applicable (to rank ANCOVA tests); RBM, reticular basement membrane; SE, standard error; SEM, standard error of the mean.

[a]Bronchoscopy pharmacodynamic population (all randomised and treated patients with an evaluable bronchoscopy performed at baseline and at Week 12/EOT). For each endpoint, results are reported for the patients with assessment at both baseline and Week 12/EOT for that specific endpoint.

[b]Parameters were analysed preferentially using a linear fixed-effect model with treatment, region and ICS dose level as fixed effects and baseline as a covariate. Baseline values are presented as mean (SD), the change from baseline values as LS mean (SE) and the difference versus placebo at Week 12 as LS mean difference (90% CI). In case of deviations from the linear model assumptions, rank ANCOVA was performed. In these cases, baseline and changes from baseline values are presented as a median (Q1 to Q3).

[c]*p*-values were adjusted to control the FDR using the Benjamini–Hochberg procedure at a 10% threshold, and separately for each group of biomarkers. This was a *post hoc* exploratory analysis, and not included in the multiplicity adjustment procedure.

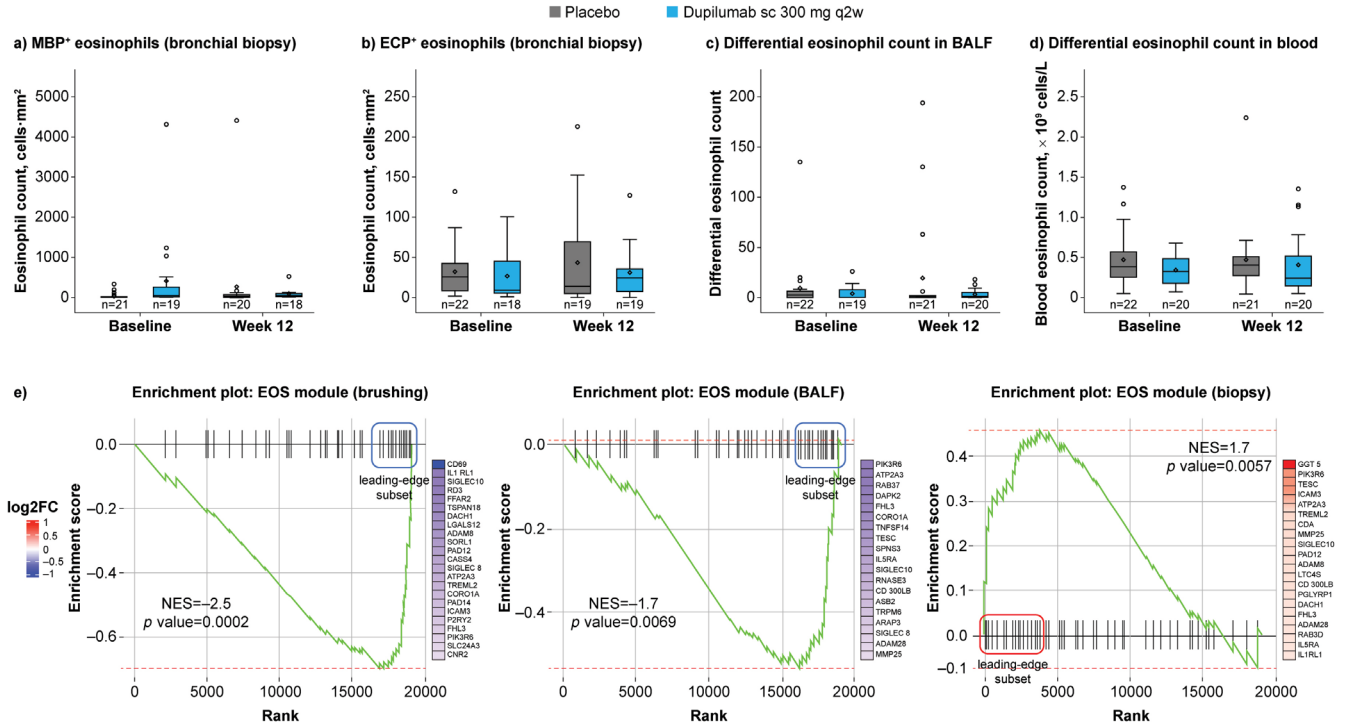

FIGURE 1 | Tissue, airway and blood eosinophil counts and eosinophil-associated gene expression signature (a) MBP⁺ and (b) ECP⁺ tissue eosinophil counts (staining by IHC). (c) Differential eosinophil counts in BALF. (d) Blood eosinophil counts over the 12-week treatment period. (e) NES for an eosinophil-associated gene expression signature. For the (e) panel, the ES of a gene set is the maximum deviation from zero, represented by red dotted lines; leading-edge genes are the subset of genes that contribute the most or reach the maximum ES; and the preranked list of genes is used instead of the statistical significance of an individual gene. NES is defined as the ratio between the ES and the mean score obtained by random permutations of the dataset. BALF, bronchioalveolar lavage fluid; ECP⁺, eosinophil cationic protein⁺; IHC, immunohistochemistry; MBP⁺, major basic protein⁺; NES, normalised enrichment score; q2w, every 2 weeks; sc, subcutaneous.

The proportion of patients with treatment-emergent adverse events (TEAEs) was similar between treatment groups (Table S15). The most common TEAEs were headache, nasopharyngitis and injection-site erythema. One patient in the placebo group reported eosinophilia as a TEAE. No bronchoscopy-related events were reported. There was one serious TEAE reported in the dupilumab group (neutropenia related to a virus, not to dupilumab, according to the investigator). No deaths were reported, and no patient had a TEAE leading to permanent treatment discontinuation.

4 | Discussion

The phase 2a EXPEDITION study demonstrated no measurable effect of dupilumab versus placebo on cellular measures (immunohistochemical) of type 2 airway inflammation, eosinophils, mast cells or type 2 helper cells in bronchial biopsy samples of patients with type 2 persistent asthma, despite significant improvements in lung function at Week 12 and asthma control at Week 8 in patients with type 2 persistent asthma. Lung function and asthma control improvements were consistent with those in other dupilumab asthma studies [5, 11]. While goblet cell counts and mucus coverage of the epithelium numerically decreased following dupilumab treatment, differences were not significant after multiplicity adjustment.

While the cellular findings of this study were unexpected, dupilumab significantly reduced type 2 inflammation biomarkers, including FeNO, circulating total IgE, periostin, eotaxin-3 and TARC/CCL17 versus placebo. TARC/CCL17 is a ligand for CCR4, which is predominantly expressed on T-helper type 2 cells and regulatory T cells. High levels of both IgE and TARC have been detected in samples, including BAL samples in patients with asthma [15, 16]. A reduction in TARC levels by dupilumab supports its role in blocking T-helper type 2 cell infiltration and airway inflammation. Reductions in TARC/CCL17 and eotaxin-3 may, over time, result in tissue reductions in T-lymphocytes and eosinophils, respectively, but these were not detected here. Gene expression analysis demonstrated that dupilumab shifted the transcriptome towards the molecular phenotype of healthy controls, consistent with analyses in AD [17] and eosinophilic oesophagitis [18], by increasing genes downregulated in asthma (e.g., *type 1 interferon [IFN] response*, *M1 macrophage*, *type 1 immune response*) and decreasing genes upregulated in asthma (e.g., *type 2 immune response* and *M2 macrophage*). Our findings are generally consistent with previous studies evaluating the effect of anti-IL-13 monoclonal antibodies on airway eosinophils. Neither tralokinumab nor lebrikizumab treatment reduced eosinophil counts in bronchial biopsy samples, although, like dupilumab, tralokinumab reduced FeNO and total IgE levels [19, 20]. Also consistent with previous studies [21], dupilumab versus placebo significantly increased circulating IL-4 levels, an expected indicator of IL-4Ra receptor inhibition. However, dupilumab treatment has been observed to reduce airway eosinophils when measured in sputum samples, suggesting that different compartments may yield different results [22]. Overall,

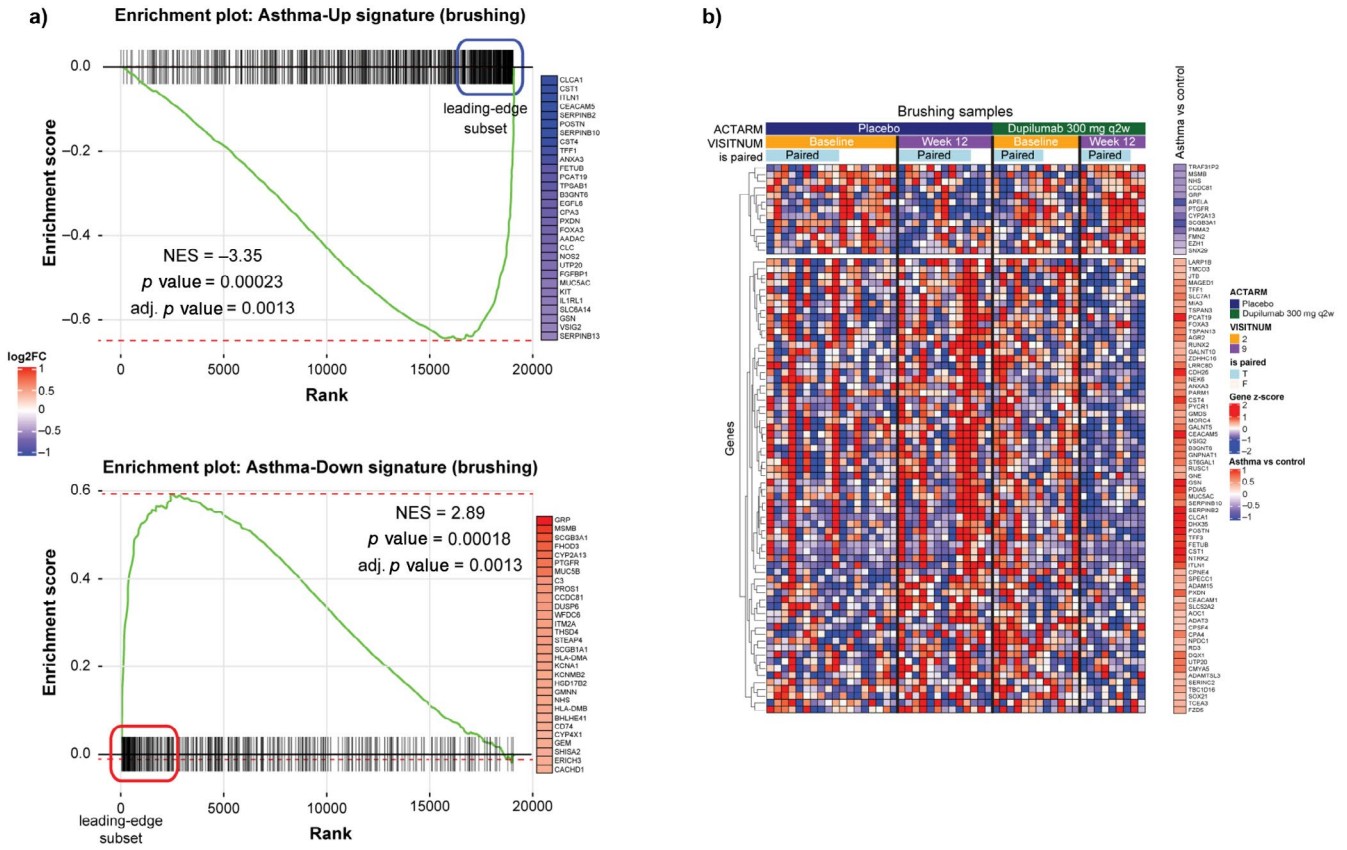

FIGURE 2 | Dupilumab effects on asthma-up and asthma-down gene sets and leading-edge genes (a) GSEA results of dupilumab effects on the *Asthma-Up* and *Asthma-Down* gene sets in the top 30 leading-edge genes. (b) Heatmap of the top 79 leading-edge genes, respectively, up- and down-regulated in the *Asthma-Down* and *Asthma-Up* gene sets (nominal $p < 0.05$). The asthma versus healthy control column indicates up- or downregulation of each of these genes in patients with asthma. For the (a) panel, the ES of a gene set is the maximum deviation from zero, represented by red dotted lines; leading-edge genes are the subset of genes that contribute the most or reach the maximum ES, and the preranked list of genes is used instead of the statistical significance of an individual gene. NES is defined as the ratio between the ES and the mean score obtained by random permutations of the dataset. ACTARM, treatment arm; ES, enrichment score; GSEA, gene set enrichment analysis; NES, normalised enrichment score; q2w, every 2 weeks; VISITNUM, visit number.

these data suggest that neutralising IL-13 and IL-4 signalling may be insufficient to elicit changes in airway eosinophil counts, but that this may be affected by the compartment investigated.

Several study limitations may have contributed to the inability to detect a significant treatment effect on cellular measures of type 2 inflammation in bronchial biopsies. As eosinophilia was not an inclusion criterion, patients may have lacked sufficient baseline tissue eosinophil counts to demonstrate a significant difference. The inclusion of patients with more severe disease who did have an indication for biological treatment may have resulted in clearer differences between the treatment groups. As observed in a lebrikizumab study [20], a significant imbalance in MBP^+ cells between placebo and dupilumab bronchial biopsy samples at baseline may have led to variability in treatment response. While unlikely to impact biomarker levels 12 weeks after administration, the receipt of oral corticosteroids by all patients to prevent bronchospasms after the biopsy may have impacted the results. The study duration may have been limiting, as the reduction in chemokine levels observed here and in other dupilumab studies [21, 23] may take longer to translate to reductions in submucosal cell numbers. Microarray analysis showed differing expression of eosinophil-associated genes in brushing and BAL samples versus biopsies. These results are consistent with those from a bronchial allergen challenge study, in which dupilumab suppressed an eosinophil-associated gene signature [24]. Limitations for this technique included difficulty in discriminating mucosal from intravascular eosinophils in bronchial biopsy samples and observed imbalances in eosinophil levels at baseline between treatment groups. Additionally, tissue sampling is limited in endoscopic biopsies; matched pre- and post-treatment samples were not available for all patients; thus, mean measurements were evaluated. These factors likely increased variability in the data. Larger bronchial biopsy and sputum studies are needed. Finally, the high number of pharmacodynamic variables analysed, requiring statistical adjustment for multiplicity, may have reduced significance.

By complementing our study with transcriptional changes driven by dupilumab, we contextualised the observed trends at the cellular level and can hypothesise potential treatment effects that may otherwise not be detectable via conventional cellular and pathological markers alone (particularly in a study where those measures did not have a required threshold at the time of enrolment). For example, while no significant differences were observed in MBP^+ eosinophil or macrophage counts between dupilumab and placebo, GSEA revealed significant differences in gene expression associated with both

TABLE 3 | Summary of GSEA results (normalised enrichment score and statistical significance).

	Asthma versus healthy control brushing [15]			Bronchial brushing			BAL cell			Biopsy		
Gene set	**NES**	***p***	**Adjusted *p*-value**	**NES**	***p***	**Adjusted *p*-value**	**NES**	***p*-value**	**Adjusted *p*-value**	**NES**	***p*-value**	**Adjusted *p*-value**
Down in AD upon dupilumab treatment	1.61	0.0002	0.0013	−1.09	0.2091	0.3116	−1.54	0.0003	0.0014	−1.14	0.1210	0.2337
Asthma-down	−4.28	0.0002	0.0013	2.89	0.0002	0.0013	−1.00	0.4814	0.8184	−1.80	0.0002	0.0023
Eosinophil	1.62	0.0065	0.0275	−2.54	0.0002	0.0013	−1.68	0.0069	0.0213	1.65	0.0057	0.0274
M1 macrophage	−0.85	0.8789	1	1.32	0.0221	0.0536	2.07	0.0006	0.0024	1.38	0.0085	0.0290
M2 macrophage	1.70	0.0012	0.006	−1.69	0.0036	0.0123	−2.32	0.0001	0.0013	−1.24	0.1201	0.2337
M3.4 IFN response	−0.47	0.9992	1	2.18	0.0002	0.0013	2.46	0.0003	0.0014	−1.02	0.4179	0.5075
M4.1 T cells	−1.28	0.1117	0.2532	1.63	0.0091	0.0283	−1.10	0.3224	0.6089	−1.26	0.1340	0.2397
M4.10 B cells	−1.52	0.0309	0.105	−0.65	0.9337	0.9620	−1.36	0.1168	0.2837	1.91	0.0008	0.0055
M4.14 monocytes	−1.56	0.0140	0.0527	1.14	0.2589	0.3386	−0.80	0.7676	0.9535	1.28	0.1130	0.2337
M4.2 inflammation	1.30	0.1066	0.2532	−1.37	0.0821	0.1470	−1.02	0.4224	0.7559	1.07	0.3410	0.4489
M4.7 lymphoid lineage	−1.26	0.0959	0.2508	1.80	0.0006	0.0024	1.17	0.1730	0.3499	−2.05	0.0002	0.0023
M4.9 granulocytes	1.00	0.4602	0.6258	0.57	0.9985	0.9985	−0.66	0.9615	0.9615	−1.58	0.0075	0.0284
M5.1 inflammation	1.57	0.0002	0.0013	−0.94	0.6258	0.6863	−0.76	0.9255	0.9535	−0.78	0.9664	0.9664
M5.11 lymphoid lineage	−1.41	0.0465	0.1436	1.72	0.0033	0.0123	−0.69	0.9038	0.9535	−1.04	0.3864	0.4866
M5.12 IFN response	0.62	0.9848	1	1.16	0.2311	0.3274	1.20	0.1750	0.3499	0.87	0.6907	0.7117
M5.15 neutrophils	1.02	0.4167	0.5904	−1.48	0.0643	0.1215	−0.88	0.6191	0.9096	0.93	0.5609	0.6357
M6.12 lymphoid lineage	1.06	0.3444	0.5576	0.97	0.5034	0.5901	1.25	0.1332	0.3019	−1.45	0.0359	0.0939
M6.13 inflammation	0.60	0.98574	1	−1.15	0.2429	0.3304	1.70	0.0054	0.0183	1.18	0.2131	0.3293
M6.15 T cells	−1.24	0.1747	0.3494	1.71	0.0100	0.0284	0.77	0.7934	0.9535	−1.08	0.3433	0.4489
M6.19 T cells	1.03	0.4033	0.5904	−0.88	0.6258	0.6863	−0.94	0.5281	0.855	−0.85	0.6774	0.7117
M6.20 neutrophils	−0.59	0.9872	1	1.51	0.0296	0.0672	0.80	0.7922	0.9525	−1.17	0.2238	0.3309
M6.7 lymphoid lineage	1.15	0.2122	0.3797	0.79	0.8507	0.9039	−0.9	0.6280	0.9096	−1.71	0.0029	0.0165
M6.9 lymphoid lineage	−1.14	0.2669	0.4537	1.65	0.0135	0.0354	−0.69	0.8695	0.9535	1.08	0.3349	0.4489

(Continues)

TABLE 3 | (Continued)

Gene set	Asthma versus healthy control brushing [15]			Bronchial brushing			BAL cell			Biopsy		
	NES	*p*	Adjusted *p*-value	NES	*p*	Adjusted *p*-value	NES	*p*-value	Adjusted *p*-value	NES	*p*-value	Adjusted *p*-value
M7.15 granulocytes	−1.19	0.1659	0.3494	1.16	0.2094	0.3116	1.29	0.0888	0.2322	−1.18	0.1858	0.3008
M7.22 monocytes	0.76	0.8859	1	1.18	0.2108	0.3116	−1.56	0.0229	0.0648	0.99	0.4776	0.5599
M7.24 lymphoid lineage	−0.65	0.9844	1	1.03	0.3970	0.4821	0.74	0.9185	0.9535	−1.57	0.0107	0.0332
M7.25 lymphoid lineage	−0.50	1	1	1.39	0.0400	0.0799	−0.79	0.8133	0.9535	−1.48	0.0179	0.0507
Mast cell activation	1.21	0.2077	0.3797	−1.6	0.0316	0.0672	−0.86	0.6421	0.9096	0.90	0.5985	0.6564
Type 1 IFN response M1.2	−0.58	0.9754	1	2.25	0.0002	0.0013	2.26	0.0003	0.0014	1.74	0.0064	0.0274
Type 1 immune response	−1.03	0.3992	0.5904	1.26	0.1686	0.2866	2.26	0.0003	0.0014	2.17	0.0002	0.0023
Type 2 immune response (internal)	2.09	0.0002	0.0013	−2.14	0.0004	0.0020	−2.16	0.0002	0.0013	−1.26	0.1436	0.2441
Type 2 immune response	2.99	0.0002	0.0013	−2.95	0.0002	0.0013	−2.09	0.0002	0.0013	−1.47	0.0411	0.0998
Up in AD upon dupilumab treatment	−1.24	0.0556	0.1574	−1.06	0.3034	0.3820	−0.80	0.8771	0.9535	1.17	0.1237	0.2337
Asthma-up	4.56	0.0002	0.0013	−3.35	0.0002	0.0013	−1.81	0.0001	0.0013	−1.35	0.0007	0.0055

Abbreviations: AD, atopic dermatitis; BAL, bronchoalveolar lavage; GSEA, gene set enrichment analysis; IFN, interferon; NES, normalised enrichment score.

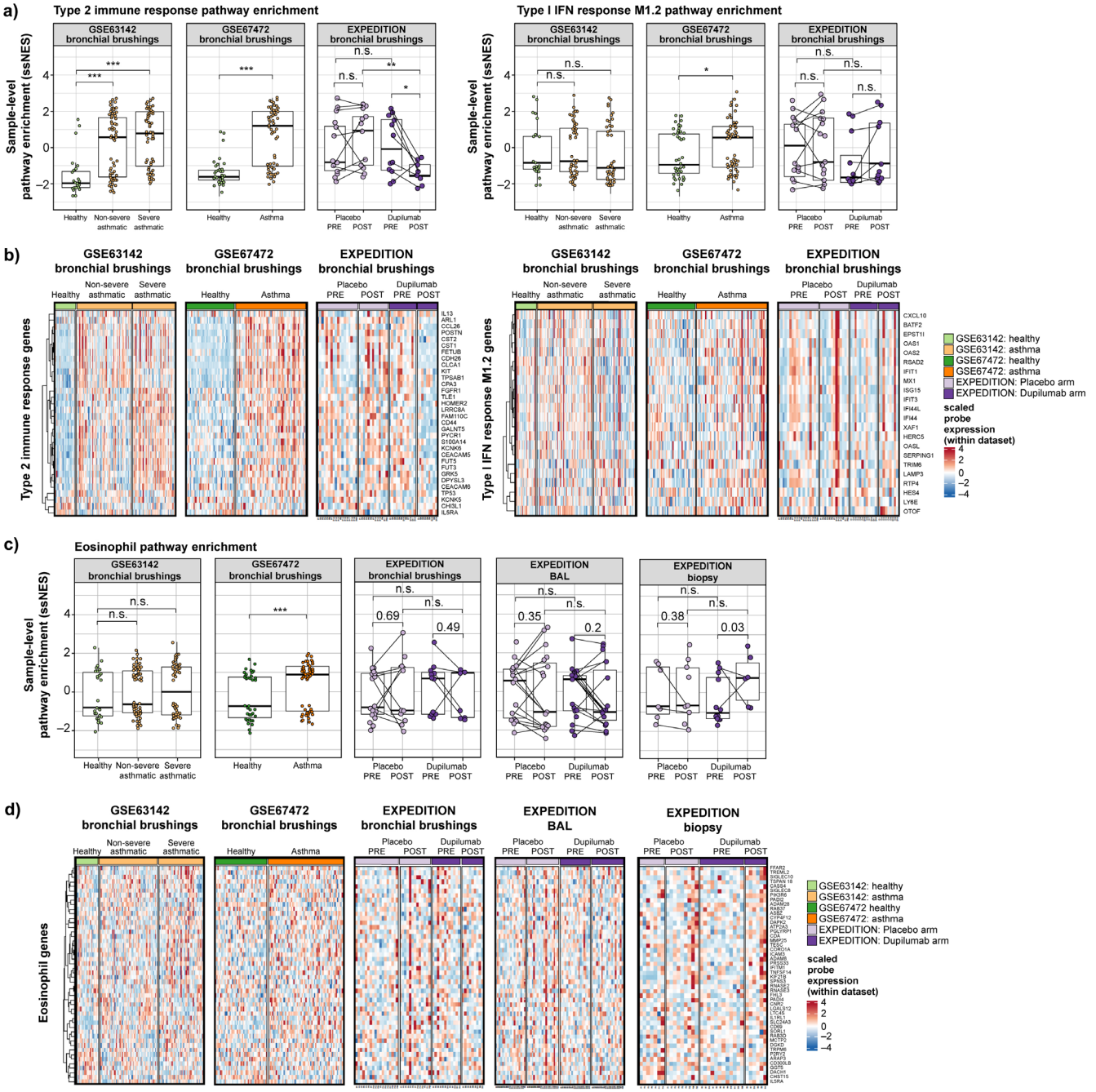

FIGURE 3 | Type 2- and type 1-associated gene expression changes in bronchial brushing samples (a) GSEA NESs for the type 2-associated gene sets, up- or downregulated, in bronchial brushing samples from patients with asthma versus healthy controls from public datasets, as well as pre- and post-treatment NESs in the EXPEDITION study. (b) Heatmap showing the expression of each gene included in the GSEA NES associated with type 2 inflammation. (c) GSEA NES for the type 1-associated gene set, up- or downregulated, in bronchial brushing samples from patients with asthma versus healthy controls from public datasets and pre- versus post-treatment samples in the EXPEDITION study. (d) Heatmap showing the expression of each gene included in the GSEA NES associated with type 1 inflammation. GSEA, gene set enrichment analysis; NES, normalised enrichment score.

eosinophils and macrophage polarisation. Additionally, while not statistically significant after adjustment for multiplicity testing, the numerical reduction in goblet cell hyperplasia and mucus coverage in bronchial tissue is consistent with the observed decrease in gene expression of *ALOX15* and its downstream genes, *MUC5AC* and *POSTN* (periostin), which are among the genes driving the dupilumab-mediated dampening of the *type 2 immune response*-associated gene set [25, 26]. Periostin secretion, induced by IL-4 and IL-13 in airway epithelial cells and lung fibroblasts [27], is associated with subepithelial fibrosis in asthma, airway hyper-responsiveness [27] and type 2 inflammation in severe asthma [28]. Additionally, the upregulation of *MUC5AC* and downregulation of *MUC5B* production ultimately culminate in a heterogeneous airway mucus gel that can compromise mucociliary clearance, leading to airway obstruction [26, 29]. Our findings therefore suggest a potential mechanism of action of dupilumab in improving lung function, although the conflicting

results between the biopsy cell counts and brushing gene expression evaluations of the present study hinder interpretation. Our gene expression findings complement findings from the recent phase 4 VESTIGE study, which showed clear effects of dupilumab on reducing mucus volume and plugging [30], indicating that the limitations discussed above may have had a substantial impact on the unexpected negative immunohistochemical findings of this study.

In conclusion, although the phase 2a EXPEDITION study demonstrated no significant changes in cellular measures of type 2 airway inflammation in dupilumab versus placebo bronchial biopsy samples, significant effects were observed on clinical outcomes and pharmacodynamic biomarkers of type 2 inflammation in the airway and circulation. The study also provided insight into changes in gene expression induced by dupilumab in the lung microenvironment, including modulating the expression of inflammation-related gene sets. In adult patients with asthma, these clinical improvements and gene expression profiles demonstrate both systemic and local reduction in type 2 inflammation with dupilumab treatment.

Author Contributions

Michael E. Wechsler, Sally E. Wenzel, Steve D. Groshong, Mario Castro, Ian D. Pavord and Klaus F. Rabe acquired data. Elizabeth Laws, Alexandre Jagerschmidt, Sivan Harel, Jennifer D. Hamilton, Nikhil Amin, Heming Xing, Bema Coulibaly, Souâd Naimi, Sara Hamon, Paul J. Rowe, Frank Nestle, Danen M. Cunoosamy, Emanuele de Rinaldis and Leda P. Mannent contributed to the conception and design of the study. Kaitlyn Gayvert, Lu Zhang, E. Gerard and Anissa Elfakir accessed and verified the data and did statistical analyses. All authors had full access to all of the data. All authors participated in the interpretation of the data, provided critical feedback and took responsibility for the accuracy, completeness and protocol adherence of data and analyses; all authors took final responsibility to submit for publication. All investigators had confidentiality agreements with the sponsors.

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Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice guideline and applicable regulatory requirements. An independent data and safety monitoring committee conducted blinded monitoring of patient safety data on a periodic basis throughout the course of the study. The local institutional review board or ethics committee at each study centre oversaw study conduct and documentation. All patients provided written informed consent before participation.

Conflicts of Interest

Michael E. Wechsler has received personal fees from Amgen, AstraZeneca, Boehringer Ingelheim, Cohero Health, Equillium, Gala Therapeutics, Genentech, Genzyme, Mylan, Novartis, Regeneron Pharmaceuticals Inc., resTORbio, Sentien Biotechnologies and Teva; and grants and personal fees from GSK Sanofi. Sally E. Wenzel has received personal fees from AstraZeneca, GSK, Novartis and Sanofi; participated in industry trials for AstraZeneca, GSK, Novartis and Sanofi; and has been involved in broad support of the Severe Asthma Research Programme of Boehringer Ingelheim and Teva. Steve D. Groshong has been a consultant for Veracyte. Mario Castro has received research support from the American Lung Association, AstraZeneca, GSK, NIH, Novartis, PCORI, Pulmatrix, sanofi-aventis and Shionogi; speaker fees from AstraZeneca, Genentech, GSK, Regeneron Pharmaceuticals Inc., Sanofi and Teva; royalties from Elsevier; and is a consultant to Genentech, Novartis, sanofi-aventis and Teva. Ian D. Pavord has received speaker fees from Aerocrine, Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GSK, Novartis, Regeneron Pharmaceuticals Inc., Sanofi and Teva; payments for organising educational events from AstraZeneca, GSK, Regeneron Pharmaceuticals Inc., Sanofi and Teva; consultant fees from AstraZeneca, Boehringer Ingelheim, Chiesi, Circassia, Genentech, GSK, Knopp Biosciences, Merck, Novartis, Regeneron Pharmaceuticals Inc., Sanofi and Teva; international scientific meeting sponsorship from AstraZeneca, Boehringer Ingelheim, Chiesi, GSK and Teva; a research grant from Chiesi; payments to support FDA approval meetings from GSK; payments for use of the Leicester Cough Questionnaire (of which he is a co-patent holder) in clinical trials from Bayer, Insmed and Merck; and has been an expert witness for a patent dispute involving AstraZeneca and Teva. Klaus F. Rabe has been a consultant and received speaker fees from AstraZeneca, Boehringer Ingelheim, Chiesi, Novartis, Regeneron Pharmaceuticals Inc. and Sanofi. Elizabeth Laws, Alexandre Jagerschmidt, Lu Zhang, Heming Xing, Bema Coulibaly, Souâd Naimi, Paul J. Rowe, Danen M. Cunoosamy, Emanuele de Rinaldis and Leda P. Mannent are employees of Sanofi and may hold stock and/or stock options in the company. Kaitlyn Gayvert, Jennifer D. Hamilton and Nikhil Amin are employees and shareholders of Regeneron Pharmaceuticals Inc. Anissa Elfakir is an employee of Ividata Life Sciences and an external contractor to Sanofi. Frank Nestle is a former employee of Sanofi and may hold stock and/or stock options in the company. Sivan Harel is a former employee and shareholder of Regeneron Pharmaceuticals Inc.

Data Availability Statement

All model results and details of input gene sets in this study are available in the Supporting Information. Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form and statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymised patient data will be considered for sharing once the product and indication have been approved by major health authorities (e.g., FDA, EMA, PMDA), if there is legal authority to share the data and there is not a reasonable likelihood of patient reidentification. Submit requests to https://vivli.org/.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** all70391-sup-0001-Supinfo.docx. **Table S3:** all70391-sup-0002-TableS3.xlsx. **Table S5:** all70391-sup-0003-TableS5.xlsx. **Table S10:** all70391-sup-0004-TableS10.xlsx. **Table S11:** all70391-sup-0005-TableS11.xlsx.