

DR JENNIFER D. HAMILTON (Orcid ID : 0000-0003-1236-5245)

Article type : Original Article-Clinical Allergy

TITLE: Dupilumab suppresses type 2 inflammatory biomarkers across multiple atopic, allergic diseases

SHORT TITLE: Dupilumab suppresses type 2 biomarkers

AUTHORS: Hamilton J.D¹, PhD; Harel S¹, PhD; Swanson B.N², PhD; Brian W², PhD; Chen Z¹, PhD, MS, MA; Rice M.S³, ScD; Amin N¹, MD; Ardeleanu M¹, MD; Radin A¹, MD; Shumel B¹, MD; Ruddy M¹, MD; Patel N², MD; Pirozzi G², MD; Mannent L⁴, MD; and Graham N.M.H¹, MD

AFFILIATIONS: ¹Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA; ²Sanofi, Bridgewater, NJ, USA; ³Sanofi, Cambridge, MA, USA; ⁴Sanofi, Chilly-Mazarin, France

ACKNOWLEDGMENTS: We thank the participating investigators of the phase 3 LIBERTY AD SOLO-1 (NCT02277743), SOLO-2 (NCT02277769), CHRONOS (NCT02260986), LIBERTY ASTHMA QUEST (NCT02414854), and LIBERTY NP SINUS-52 (NCT02898454) studies and of the phase 2 proof-of-concept EoE study (NCT02379052). We would also like to thank Nora Crikelair and Linda Williams at Regeneron Pharmaceuticals, Inc. and Dianne Barry, Colin Mitchell, and El-Bdaoui Haddad at Sanofi. The authors received writing/editorial support in the preparation of this manuscript provided by Jennifer L. F. Port, PhD, of Excerpta Medica, funded by Sanofi Genzyme and Regeneron Pharmaceuticals, Inc.

CONFLICTS OF INTEREST: JDH, SH, ZC, NA, MA, AR, BS, and MR are employees and shareholders of Regeneron Pharmaceuticals, Inc. NMHG is a prior employee and shareholder of Regeneron Pharmaceuticals, Inc. WB, MSR, NP, and LM are employees and may hold stock and/or stock options in Sanofi. BG and GP are prior employees and may hold stock and/or stock options in Sanofi.

CONTRIBUTION STATEMENT: JDH, SH, BS, WB, NA, MA, AR, BS, MR, NP, GP, LM, and NMHG contributed to study design and data analysis or interpretation (ICMJE Criterion #1), drafting and providing critical feedback on the publication (ICMJE Criterion #2), gave final

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/CEA.13954](#)

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32 approval for submission (ICMJE Criterion #3), and agreed to be accountable for the accuracy and
33 integrity of this work (ICMJE Criterion #4). ZC and MSR provided statistical support and
34 contributed to data analysis or interpretation (#1), drafting and providing critical feedback on the
35 publication (#2), gave final approval for submission (#3), and agreed to be accountable for the
36 accuracy and integrity of this work (#4).

37 **ETHICAL STATEMENT:** All studies were conducted in accordance with the Declaration of
38 Helsinki and the International Conference on Harmonization's good clinical practice guideline. All
39 study documents and procedures were approved by institutional review board/ethics committees
40 at each study site. All patients provided written informed consent before participating in the study.

41 **DATA AVAILABILITY STATEMENT:** Qualified researchers may request access to patient level
42 data and related study documents including the clinical study report, study protocol with any
43 amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient
44 level data will be anonymized, and study documents will be redacted to protect the privacy of our
45 trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for
46 requesting access can be found at: <http://www.clinicalstudydatarequest.com/>

47

48 **Dupilumab suppresses type 2 inflammatory biomarkers across multiple atopic, allergic**
49 **diseases**

50 **Short title:** Dupilumab suppresses type 2 biomarkers

51 Abstract word count: 293/300

52 Text word count: 4341/5000

53 Tables + Figures: 7/8

54

55 Jennifer D. Hamilton¹ | Sivan Harel¹ | Brian N. Swanson² | William Brian² | Zhen Chen¹ | Megan S.
56 Rice³ | Nikhil Amin¹ | Marius Ardeleanu¹ | Allen Radin¹ | Brad Shumel¹ | Marcella Ruddy¹ |
57 Naimish Patel² | Gianluca Pirozzi² | Leda Mannent⁴ | Neil M.H. Graham¹

58 ¹Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA

59 ²Sanofi, Bridgewater, NJ, USA

60 ³Sanofi, Cambridge, MA, USA

61 ⁴Sanofi, Chilly-Mazarin, France

62 **Correspondence:** Jennifer Davidson Hamilton, Regeneron Pharmaceuticals, Inc., Tarrytown,
63 NY, USA

64 ORCID: 0000-0003-1236-5245

65 Telephone number: 001-914-847-1083

66 Email: Jennifer.Hamilton@regeneron.com

67 **Funding information:** This research was sponsored by Sanofi and Regeneron Pharmaceuticals,
68 Inc.

69 CONSORT check list and CONSORT diagram: CONSORT guidelines were adhered to for all
70 studies included in this analysis and flow diagrams were previously published in all primary
71 manuscripts, which have been cited throughout.

72 Abstract

73 **Background:** Type 2 inflammation is common in numerous atopic/allergic diseases and can be
74 identified by elevated biomarker levels. Dupilumab, a fully human monoclonal antibody, blocks
75 the shared receptor component for interleukin-4 and interleukin-13, key and central drivers of type
76 2 inflammation.

77 **Objective:** Assessment of dupilumab effect on type 2 inflammatory biomarkers in atopic
78 dermatitis (AD), asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), and eosinophilic
79 esophagitis (EoE).

80 **Methods:** Data were extracted from three randomized placebo-controlled trials of dupilumab in
81 AD (NCT02277743, N=671; NCT02277769, N=708; NCT02260986, N=740); and one each in
82 asthma (NCT02414854, N=1902); CRSwNP (NCT02898454, N=448); and EoE (NCT02379052,
83 N=47). Biomarkers assessed were serum thymus and activation-regulated chemokine (TARC),
84 plasma eotaxin-3, serum total immunoglobulin E (IgE), serum periostin, and blood eosinophil
85 count.

86 **Results:** Dupilumab versus placebo significantly suppressed most type 2 inflammatory biomarker
87 levels across all studies/indications where data were assessed. Reductions in serum TARC,
88 plasma eotaxin-3, and serum periostin occurred rapidly, whereas reductions in serum total IgE
89 were more gradual. Across diseases, at the end of treatment, median percentage change from
90 baseline in TARC levels ranged from -24.8% to -88.6% (placebo +2.6% to -53.6%); -38.2% to
91 -51.5% (placebo +8.3% to -0.16%) in eotaxin-3; -24.8% to -76.7% (placebo +8.3% to -4.4%) in
92 total IgE; and -13.6% to -41.1% (placebo +10.1% to -6.94%) in periostin levels. Blood eosinophil
93 responses to dupilumab varied by disease, with minimal changes in AD in the SOLO studies
94 (median percentage change from baseline to end of treatment: 0% [95% CI: -15.8, 0]); transient
95 increases followed by decreases to below-baseline levels in asthma (-14.6% [-20.0, -7.7]) and
96 CRSwNP (-29.4% [-40.0, -16.3]); and significant decreases in EoE (-50.0% [-50.0, -33.3]).

97 **Conclusion and clinical relevance:** Dupilumab reduced levels of type 2 biomarkers across
98 clinical studies in patients with AD, asthma, CRSwNP, and EoE.

99 **ClinicalTrials.gov Identifiers:** NCT02277743, NCT02277769, NCT02260986, NCT02414854,
100 NCT02898454, NCT02379052

101 **Funding:** Sanofi Genzyme and Regeneron Pharmaceuticals, Inc.

102

103 KEY MESSAGES

- 104 • This analysis assessed dupilumab effect on biomarkers of type 2 inflammation in AD,
105 asthma, CRSwNP, and EoE

- 106 • Dupilumab suppressed TARC, total IgE, periostin, and eotaxin-3 in all studies/indications;
107 blood eosinophil responses varied
108 • Dupilumab may affect a shared pathophysiological mechanism fundamental to type 2
109 inflammatory diseases
110

111 **KEYWORDS**

112 Atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyposis, eosinophilic esophagitis,
113 dupilumab

114 **Abbreviations**

115 *AD: atopic dermatitis*

116 *AR: allergic rhinitis*

117 *CRSwNP: chronic rhinosinusitis with nasal polyps*

118 *CCL17: CC chemokine ligand 17*

119 *CCL26: CC chemokine ligand 26*

120 *ECP: eosinophilic cationic protein*

121 *EoE: eosinophilic esophagitis*

122 *FeNO: fractional exhaled nitric oxide*

123 *IgE: immunoglobulin E*

124 *NO: nitric oxide*

125 *PoC: proof of concept*

126 *TARC: thymus and activation-regulated chemokine*

128 Type 2 inflammation underpins the pathophysiology of multiple atopic/allergic diseases that often
129 have diverse clinical manifestations involving various organ systems, including atopic dermatitis
130 (AD), severe asthma, chronic rhinosinusitis with nasal polyps (CRSwNP),¹ and eosinophilic
131 esophagitis (EoE).² Involving both innate and adaptive immune systems, type 2 inflammation is
132 characterized by the release of cytokines such as interleukin (IL)-4, IL-13, and IL-5, resulting in
133 tissue infiltration by eosinophils, mast cells, and basophils, excessive mucus production in the
134 airway, epithelial hyperplasia, tissue remodelling, and allergy association. These changes are
135 accompanied by debilitating symptoms, greater use of corticosteroids and other
136 immunosuppressants, suboptimal disease control, and poor quality of life.^{3,4}

137 Several biomarkers have been linked to type 2 inflammation (Figure 1), including serum
138 thymus and activation-regulated chemokine (TARC or CC chemokine ligand 17 [CCL17]), plasma
139 eotaxin-3 (CCL26), serum total immunoglobulin E (IgE), serum periostin, fractional exhaled nitric
140 oxide (FeNO), and blood/tissue eosinophil count. These markers were selected because they are
141 known to be regulated by IL-4 and/or IL-13 and are associated with various pathological features
142 of type 2 inflammatory diseases. TARC and eotaxin-3 are key chemokines for attracting
143 inflammatory cells into the target tissue, including Th2 lymphocytes and eosinophils.⁵⁻⁷ Periostin
144 is an extracellular matrix protein associated with activated fibroblasts, thought to play a role in the
145 tissue remodelling and fibrosis observed in atopic diseases.⁸ IL-4 and IL-13 signal via
146 transcription factors such as STAT6, inducing the downstream expression of TARC, eotaxin-3,
147 and periostin. In the STAT6 pathway, IL-4 binds to IL-4 α , and the IL-4/IL-4Ra complex recruits
148 and pairs with either IL-2R γ or IL13Ra1 in heterodimeric receptors. Binding of the complex to
149 Type I chain forms Type I receptors, common in lymphocytes, whereas both IL-4 and IL-13 bind
150 the shared Type II receptor complex, containing IL13Ra1.⁹ Activation of Janus kinases results in
151 generation of phosphorylated STAT6 which in turn upregulates genes that govern production of
152 mediators of inflammation. Since the distribution of Type I and II receptor expression varies
153 among cells types, IL-4/IL-13 have differential effects in tissues. IL-4 is also required for
154 immunoglobulin isotype switching to IgE, a key downstream mediator in the type 2 adaptive
155 immune response.¹⁰ Nitric oxide, a ubiquitous messenger molecule, is also a key inflammatory
156 mediator in the respiratory tract whose production is partly regulated by IL-13. Nitric oxide is
157 produced by several cell types, including epithelial cells, mast cells, macrophages, neutrophils
158 and vascular endothelial cells.¹¹ Eosinophils are pro-inflammatory leukocytes recruited from the
159 bloodstream to inflammation sites by IL-5 and the eotaxin family of chemokines.⁵ Eosinophilia and

160 IgE production are hallmarks of type 2 inflammation in asthma, AD, and CRSwNP,^{1,10} and type 2
161 activation results in eosinophilia and other features of EoE.¹²
162 Dupilumab, a fully human monoclonal antibody,^{13,14} blocks the shared IL-4R α component of
163 IL-4 and IL-13 receptors, thus inhibiting signalling of both cytokines, key and central drivers of
164 type 2 inflammation in multiple diseases.¹⁵ Preclinical evidence indicates that dupilumab blocks
165 IL-4/IL-13-induced B cell IgE isotype class switching and eosinophil infiltration at sites of
166 inflammation, and expression of IL-5, *CCL11*, *CCL17*, *CCL24*, *CCL2*, *CXCL1*, and inducible nitric
167 oxide synthase, the latter resulting in reduction in airway nitric oxide.¹⁵ Assessment of biomarkers
168 of type 2 inflammation has the potential to provide an insight into the impact of therapy on the
169 underlying disease pathogenesis and complements existing severity measures, thus aiding in
170 identification of patients who may be more appropriate for the targeted biologic therapy within
171 heterogenous disease populations.¹⁶ In previous clinical studies in asthma, dupilumab has shown
172 greater efficacy in patients with higher levels of blood eosinophils and FeNO – biomarkers of type
173 2 inflammation.^{17,18} We hypothesized that levels of circulating biomarkers in type 2 inflammatory
174 diseases where IL-4 and/or IL-13 play critical role would be modulated by dupilumab. To
175 investigate this, we evaluated the effect of dupilumab on type 2 inflammatory biomarkers, with the
176 objective of demonstrating a similar suppression of type 2 inflammation across several diseases
177 shown to respond to dupilumab therapy.

178 2 | METHODS

179 2.1 | Studies and patients

180 Data were extracted from six randomized, double-blind, placebo-controlled dupilumab studies.
181 Three were phase 3 studies in adults with moderate-to-severe AD: 16-week LIBERTY AD SOLO-
182 1 (N = 671, NCT02277743) and SOLO-2 (N = 708, NCT02277769) studies,^{19,20} and the 52-week
183 LIBERTY AD CHRONOS (N = 740, NCT02260986).²¹ The 52-week, phase 3 LIBERTY ASTHMA
184 QUEST study (N = 1902, NCT02414854)²² evaluated patients (aged ≥ 12 years) with
185 uncontrolled, moderate-to-severe asthma; whereas the 52-week, phase 3 LIBERTY NP
186 SINUS-52 study (N = 448, NCT02898454)²³ was conducted in adults with severe CRSwNP. The
187 12-week, phase 2, proof-of-concept (PoC) EoE study (NCT02379052)²⁴ was conducted in 47
188 adults with active EoE.

189 Patients were inadequately controlled on medium-to-high-dose inhaled steroids (asthma);¹⁹
190 topical steroids or topical therapy (AD);²⁰⁻²² intranasal steroids (CRSwNP),²³ or refractory to
191 proton pump inhibitors (EoE).²⁴

192 Dupilumab 300 mg every 2 weeks was administered subcutaneously in the phase 3 studies¹⁹⁻
193 ²³ and 300 mg weekly in the phase 2 EoE study.²⁴ The asthma²² and AD studies¹⁹⁻²¹ also had
194 regimens of 200 mg every 2 weeks and 300 mg weekly, respectively; the CRSwNP study
195 included a regimen of 300 mg every 2 weeks followed by 300 mg every 4 weeks. For the purpose
196 of this analysis and to facilitate a fair comparison, only patients receiving 300 mg every 2 weeks
197 are included.

198 All studies were conducted in accordance with the Declaration of Helsinki and the
199 International Conference on Harmonization's good clinical practice guideline. All study documents
200 and procedures were approved by institutional review board/ethics committees at each study site.
201 All patients provided written informed consent before participating in the study.

202 Clinical data, study design, patient population details, and Consolidated Standards of
203 Reporting Trials diagrams were previously published for all studies.¹⁹⁻²⁵

204 2.2 | Assessments

205 Change from baseline over time in the following type 2 biomarkers were assessed and reported
206 in the QUEST and SINUS-52 studies as pre-specified endpoints:^{22,23} serum TARC (human TARC

207 Quantikine ELISA kit; R&D Systems, Minneapolis, MN); plasma eotaxin-3 (human eotaxin-3
208 Quantikine ELISA kit; R&D Systems); serum total IgE (ImmunoCAP® fluorescence enzyme
209 immunoassay method; Phadia AB, Uppsala, Sweden); serum periostin (ELISA kit; Shino-Test,
210 Kanagawa, Japan); and blood eosinophil count (measured as part of the standard 5-part white
211 blood count differential cell count on a haematology autoanalyzer). QUEST also assessed FeNO
212 (NIOX instrument; Aerocrine AB, Solna, Sweden) and eosinophil cationic protein (ECP);
213 (ImmunoCAP® fluorescence enzyme immunoassay method; Phadia AB, Uppsala, Sweden).
214 CHRONOS, SOLO-1, and SOLO-2 also assessed the effect of treatment on serum TARC, serum
215 total IgE as exploratory endpoints, and blood eosinophils as pre-specified laboratory testing.¹⁹⁻²¹
216 The EoE PoC study assessed the effect of treatment on serum TARC, serum total IgE, serum
217 periostin, and blood eosinophils from samples collected for exploratory research.²⁴ Allergen-
218 specific IgE levels were also measured in the assessed studies. However, these data have not
219 been included in the current analysis, as kinetics are dependent on allergen exposure (eg,
220 season, region, etc.)²⁰

221 2.3 | Statistical analysis

222 Consistent with previous analyses, biomarker levels were analysed using the observed values
223 with censoring after rescue treatment use for CHRONOS, SOLO-1, SOLO-2, and PoC safety
224 populations; observed on treatment values without censoring in the SINUS-52 exposed
225 population; and observed values without censoring in QUEST exposed population. The rationale
226 behind censoring post-rescue treatment values in the AD and EoE studies was to remove any
227 effect that could not be attributed to dupilumab, whereas in the asthma and CRSwNP studies, the
228 preference was to evaluate dupilumab in a real-world-like setting.

229 For this analysis, median absolute values, median change from baseline, median percentage
230 change from baseline, and corresponding mean values were analysed in dupilumab and matched
231 placebo arms for all available biomarkers at each visit. Changes from baseline were summarized
232 by descriptive statistics. Differences between dupilumab and placebo were analysed using a rank
233 analysis of covariance model. Covariates included the corresponding baseline value and the
234 stratification variables used in each of the studies. Given the distribution of biomarkers, mean
235 changes from baseline were reported as descriptive statistics, without *P*-values. A *P*-value of less
236 than .05 for the comparison between dupilumab and placebo (within each study) was considered
237 statistically significant.

238 3 | RESULTS

239 3.1 | Patients

240 Appendix S1 summarizes baseline demographics and comorbid type 2 inflammatory diseases in
241 all studies. Individual study treatment groups had similar baseline demographics and disease
242 characteristics. Type 2/Th2 immune-mediated comorbidities were frequent, particularly allergic
243 rhinitis (AR; 42.5%-70.1%) in patients across all indications, and asthma (33.3%-59.5%) in AD
244 and CRSwNP patients. Most EoE patients had comorbid AR or food allergy. At baseline, higher
245 median concentrations of serum TARC (1936.5-4030.1 pg/mL) and total IgE (2396.0-4925.5
246 IU/mL) were observed in AD patients compared with asthma, CRSwNP, and EoE patients (293.0-
247 333.5 pg/mL [TARC]; 67.00-178.5 IU/mL [IgE]) (Table 1). Median baseline levels of eosinophils
248 were also relatively higher in AD (0.400-0.45 Giga/L) than in asthma (0.25-0.27 Giga/L), CRSwNP
249 (0.34-0.36 Giga/L), or EoE (0.30-0.40 Giga/L) (Table 1). Median levels of eotaxin-3 were only
250 assessed in CRSwNP and asthma patients, with numerically higher baseline concentrations
251 observed in CRSwNP (60.50-61.55 and 37.45-38.30 pg/mL, respectively). Periostin (not
252 measured in the AD studies presented here) ranged from 56.20-56.95 ng/mL in EoE patients to
253 69.70-71.00 ng/mL and 99.60-104.00 ng/mL in asthma and CRSwNP patients, respectively
254 (Table 1).

255 3.2 | Effect of dupilumab on type 2 inflammatory biomarkers

256 Compared with placebo, dupilumab significantly suppressed circulating levels of most type 2
257 inflammatory biomarkers across all studies/disease types where a particular biomarker was
258 assessed (Table 1). Specifically, dupilumab versus placebo significantly suppressed serum TARC
259 and total IgE levels in patients with asthma, AD, CRSwNP, and EoE. Plasma eotaxin-3 and
260 serum periostin were significantly suppressed in asthma and CRSwNP; periostin was also
261 significantly suppressed in EoE patients. Blood eosinophil responses to dupilumab varied by
262 disease, with minimal changes observed in median values in AD, transient increases followed by
263 decreases to below-baseline levels in asthma and CRSwNP, and significant suppression
264 observed in EoE. Mean absolute and percentage changes generally followed a similar course to
265 median values, although in AD, transient mean increases in eosinophils from baseline were
266 observed (Appendix S2).

267 3.3 | Serum TARC

268 Treatment with dupilumab was associated with sustained reductions in median serum levels of
269 TARC (Table 1 and Figure 2) in all four diseases. In AD patients, rapid, significant reductions
270 versus placebo were evident from week 2, with maximum effects observed at week 8, and
271 maintained until the last assessment at week 52 or 16. Significant reductions, sustained
272 throughout the treatment period, were also observed by the first assessment in patients with EoE
273 (at week 2) and in patients with asthma and CRSwNP (week 12 and 24, respectively). At the end
274 of the 52-week treatment period in the AD, asthma, and CRSwNP studies, median percentage
275 changes from baseline in serum TARC levels were between -88.6% and -33.5% for dupilumab
276 groups, compared with -53.6% and 2.6% for placebo groups (all $P < .0001$). In the pooled SOLO
277 studies at week 16, the median percentage change in TARC was -78.9% in the dupilumab group
278 versus -16.4% in the placebo group ($P < .0001$), and at the end of the EoE study at week 12,
279 -24.8% in the dupilumab group versus -8.2% in the placebo group ($P = .006$).

280 3.4 | Plasma eotaxin-3

281 In asthma and CRSwNP patients, dupilumab significantly reduced plasma eotaxin-3 versus
282 placebo by the first assessment (week 12 and 24, respectively; both $P < .0001$) (Table 1 and
283 Figure 3). This reduction was sustained to the end of treatment in both studies: at week 52, the
284 median percentage change from baseline in plasma eotaxin-3 in dupilumab- versus placebo-
285 treated patients was -38.24% versus -0.16% in asthma patients and -51.47% versus 8.30% in
286 CRSwNP patients (both $P < .0001$ versus placebo). Eotaxin-3 was not assessed in the AD or
287 EoE studies.

288 3.5 | Serum total IgE

289 Dupilumab versus placebo significantly reduced concentrations of serum total IgE in AD, asthma,
290 CRSwNP, and EoE during treatment; however, across all type 2 diseases, the effect was more
291 gradual than with the other type 2 biomarkers (Table 1 and Figure 4). In the 52-week studies,
292 maximum reductions in median percentage change in serum total IgE from -70.0% to -76.7%
293 were observed in AD (CHRONOS), asthma, and CRSwNP patients in the dupilumab-treated
294 groups by the end of treatment (all $P < .0001$ versus placebo). In the shorter studies, maximum
295 reductions of -46.5% were observed in AD patients (SOLO-1 & SOLO-2) at week 16, and
296 -24.8% in EoE patients at week 12 (both $P < .0001$ versus placebo). Serum total IgE levels in the
297 placebo groups remained relatively constant during treatment across all studies.

298 3.6 | Serum periostin

299 In patients with asthma, CRSwNP, and EoE, dupilumab treatment was associated with rapid,
300 sustained reductions in serum levels of periostin versus placebo. Significant reductions versus
301 placebo were evident from the earliest assessment (week 2 or 4), with the maximum effect
302 observed at week 52 (Table 1 and Figure 5). At week 52, median percentage changes from
303 baseline in periostin in asthma and CRSwNP patients were -18.93% and -41.08% for dupilumab
304 groups, versus -6.94% and -3.52% for placebo groups (both $P < .0001$ versus placebo). In EoE
305 patients, median percentage change in periostin by week 12 was -13.6% with dupilumab versus
306 10.1% with placebo ($P = .0005$).

307 **3.7 | Blood eosinophils**

308 In dupilumab-treated patients with asthma and CRSwNP, transient elevations in blood
309 eosinophils were observed in a subset of patients (median percentage change from baseline),
310 followed by a decrease to near-baseline levels by the end of treatment (Table 1 and Figure 6A-B),
311 with mean eosinophil values following a similar course (Appendix S2 and S3). In patients with
312 EoE, dupilumab reduced blood eosinophil counts at all timepoints, with a maximum reduction at
313 week 12 of -50.0% compared with -12.5% in the placebo-treated group ($P = .0282$) (Table 1 and
314 Figure 6C-D). In the 16-week AD studies, the effect of dupilumab treatment on blood eosinophils
315 did not differ significantly from that of placebo at any timepoint despite a transient increase in
316 median blood eosinophil levels at week 4 (Figure 6C-D). In the 52-week AD study, median
317 absolute values of eosinophils initially fell from baseline to week 4 in both dupilumab- and
318 placebo-treated groups, then fluctuated thereafter but remained below baseline levels; no
319 statistically significant differences were observed between treatment groups at any timepoint
320 (Figure 6A-B). The corresponding mean eosinophil levels also fell from baseline to week 4, before
321 rising to slightly above baseline at week 8, falling below baseline at week 12, and generally
322 declining thereafter (Appendix S2 and S3).

323 4 | DISCUSSION

324 Atopic and allergic diseases share common underlying type 2 inflammatory mechanisms
325 manifesting as disease in diverse organs. This analysis evaluated whether the effects of
326 dupilumab on systemic type 2 inflammatory biomarkers were analogous in patients with AD
327 (SOLO-1 and SOLO-2, 16 weeks; and CHRONOS, 52 weeks), asthma (QUEST, 52 weeks),
328 CRSwNP (SINUS-52, 52 weeks), and EoE (PoC, 12 weeks).

329 The type 2 inflammatory biomarkers assessed (serum TARC, plasma eotaxin-3, serum total
330 IgE, serum periostin, and peripheral blood eosinophils) reflect a range of processes involved in
331 type 2 inflammation, though their baseline levels vary according to the underlying type 2 disease.
332 The reductions observed in most circulating biomarkers with dupilumab treatment were consistent
333 across indications. Consistent with the mechanism of action of dupilumab,¹⁵ these findings
334 demonstrate the key and central roles of IL-4 and IL-13 in type 2 inflammation and further support
335 the hypothesis that dual blockade of these cytokine pathways has important anti-inflammatory
336 effects in multiple type 2 inflammatory diseases. However, while IL-4 and IL-13 play central roles,
337 varying effects of these cytokines resulted in differences in the effector cells involved in the
338 pathogenesis of each disease, as evidenced by differences in biomarkers outlined below.

339 All of the patient populations included in these studies had moderate-to-severe disease. As
340 previously reported,²⁶ AD is characterized by higher baseline levels of type 2 biomarkers (eg.
341 TARC and total IgE) than asthma, CRSwNP, and EoE. This may be related to the more severe
342 barrier dysfunction that occurs in AD, with open skin lesions allowing deeper allergen penetration
343 and presentation to submucosal lymphoid elements. In AD, TARC is primarily secreted from
344 keratinocytes, and serum TARC levels are known to be correlated with the severity of disease,²⁷
345 although not predictive of clinical response to dupilumab.²⁸ In the airways, TARC is secreted by
346 respiratory epithelial cells, and serum concentrations in patients with asthma and CRSwNP, while
347 overall elevated, substantially overlap with concentrations in healthy subjects,²⁹ and do not
348 correlate with changes in FEV₁ during treatment with dupilumab.¹⁷ Across diseases, dupilumab
349 treatment resulted in reductions in TARC, eotaxin-3, and periostin concentrations, with maximum
350 effect achieved as early as first assessment and suppression maintained throughout treatment.
351 This was particularly evident for TARC concentrations in AD patients, which reflects these
352 patients' markedly higher baseline levels. Eosinophil chemotaxis is regulated by the local
353 production of eotaxins. Although circulating eotaxin-3 was not measured in the AD or EoE studies
354 of this analysis, suppression of eotaxin-3 messenger RNA was observed in the skin of AD
355 patients and oesophageal biopsies of EoE patients treated with dupilumab in other studies.³⁰⁻³²

356 Similarly, significant suppression of periostin has been observed in dupilumab-treated AD
357 patients though it was not assessed here.³⁰ Further, previous phase 2 asthma and CRSwNP trials
358 with earlier sampling showed marked decreases in TARC and eotaxin-3 by week 2 of
359 treatment.^{33,34} These data collectively confirm that upregulation of TARC, eotaxin-3 and periostin
360 during type 2 inflammation is substantially dependent on IL-4 and IL-13 signalling, consistent with
361 known regulation of their expression by IL-4R-mediated activation of the gene transcription factor
362 STAT6.³⁵⁻³⁷

363 In contrast, reductions in serum total IgE progressed more gradually in all disease settings,
364 with greater effects observed during longer treatment durations. In AD, for example, the exact
365 role of IgE in pathogenesis remains unclear, although adults with AD have a higher incidence of
366 serum IgE autoantibodies that are autoreactive to proteins in skin. In principle, mast cells and
367 other cells with IgE receptors (eg. eosinophils) in the skin can be sensitized and degranulate in
368 response to allergens entering the skin either due to an intrinsic barrier defect or excoriation.
369 Furthermore, expression of Type I IgE receptor (FcεRI) is by Langerhans cells, and inflammatory
370 dendritic epidermal cells are upregulated by IgE. Through receptor-bound IgE molecules specific
371 for certain allergens, these cells can uptake and process allergens, and present the processed
372 allergens to T cells, resulting in local T cell activation.³⁸ Similarly, in asthma, IgE-FcεRI binding in
373 the bronchial mucosa results in mast cell activation and degranulation, driving a hypersensitivity
374 reaction in the airways, including smooth muscle contraction, oedema, and hypersecretion of
375 mucus.³⁹ Dupilumab has the potential to modulate IgE-related mechanisms by diminishing
376 infiltrates of cells expressing FcεRI,³⁰ including mast cells, eosinophils, and Langerhans cells.
377 Suppression of mast cell infiltration and activation may also be one of the mechanisms by which
378 dupilumab diminishes itch in patients with AD for example, as dupilumab treatment has been
379 associated with reduced monocyte chemotactic protein-4, which is substantially upregulated in
380 AD skin.³⁰ The slower effect of dupilumab on IgE production compared with the other type 2
381 biomarkers suggests that IL-4 and IL-13 blockade of immunoglobulin class switching in B cells may
382 not immediately affect plasmablasts and long-lived plasma cells pre-programmed to produce IgE.
383 Thus, a gradual decline in serum IgE progressing as much as a year after initiating dupilumab
384 treatment may be a function of both diminished production of new IgE-secreting plasmablasts as
385 well as gradual turnover of pre-existing IgE-secreting cells.^{40,41}

386 Blood eosinophil responses to dupilumab varied by disease type. Transient increases in a
387 subset of patients were followed by decreases to below-baseline levels in asthma and CRSwNP.
388 As previously published for QUEST, transient increases were also observed in levels of ECP, a

389 ribonuclease released during eosinophil degranulation.²² No meaningful change was observed in
390 AD patients treated with concomitant topical corticosteroids, but transient increases were seen in
391 the monotherapy studies. In contrast, significant decreases in circulating eosinophils were
392 observed in dupilumab-treated EoE patients, although this finding needs to be corroborated in a
393 larger sample of patients. Based on preclinical work, the transient increase in eosinophils
394 observed in asthma and CRSwNP patients may be related to blockade of IL-4R α with dupilumab
395 reducing eotaxin production, thus inhibiting eosinophil chemotaxis and tissue infiltration.¹⁵ This
396 hypothesis is further supported by the suppression of eotaxin-3 messenger RNA expression
397 observed in AD lesional skin^{30,31} and EoE oesophageal biopsies³¹ following dupilumab treatment.
398 In contrast to anti-IL-5 therapeutic antibodies, dupilumab does not appear to have direct effects
399 on bone marrow or circulating eosinophil counts,^{5,42} but it does lead to a marked decrease of
400 eosinophils in tissue by different mechanisms, including eotaxin and *vascular cell adhesion*
401 *molecule 1* (VCAM1) downregulation.^{15,22,24} The trafficking of eosinophils into tissues is
402 dependent on the adhesion protein VCAM-1,⁴³ the expression of which is regulated by STAT-6
403 and IL-4. The transient increase in blood eosinophils observed in some patients with asthma and
404 CRSwNP during dupilumab treatment^{22,23} tends to occur in patients with higher baseline
405 eosinophil counts, which may suggest these individuals have higher baseline eosinophil
406 production rates. If this is the case, blocking eosinophil recruitment into tissue, eg. by reduced
407 expression of eotaxin-3 or VCAM1 by endothelial cells,⁴⁴ without affecting eosinophil production
408 may explain the transient increase in peripheral blood eosinophil counts observed in a subset of
409 patients in some indications. It should be noted that most increases were not associated with
410 clinical symptoms.

411 There is increasing evidence that nitric oxide plays a key role in modulating type 2
412 inflammation and in regulating type 2 immune responses, as an endogenous modulator of airway
413 function and as a proinflammatory and immunomodulatory mediator.^{11,45} In the context of asthma,
414 the inflammatory response results in increased symptoms and airway obstruction.^{11,46} Increased
415 levels of exhaled nitric oxide in asthma are often associated with airway eosinophilic inflammation
416 and may also be associated with exacerbations and disease severity.¹¹ As previously published,
417 dupilumab was associated with greater reductions in FeNO levels over the course of the
418 treatment period in QUEST, compared with placebo.²² Further, we have also previously reported
419 a significant correlation between suppression of FeNO and improvement in FEV₁ in dupilumab
420 asthma trials.⁴⁷

421 The data on type 2 inflammation suppression and efficacy of dupilumab observed in the
422 indications tested to date demonstrate that the common underlying pathological mechanism in
423 these diseases is mediated by IL-4 and IL-13, and that suppression of inflammatory mediators by
424 dual blockade of these cytokines and their downstream pathways, consequently reduces disease
425 activity. Significant improvements have been observed in lung function and exacerbation rates in
426 asthma;^{22,42} signs and symptoms of AD;¹⁹⁻²¹ sinonasal outcomes in CRSwNP;²³ and dysphagia,
427 oesophageal eosinophil counts and other histopathologic features in EoE.²⁴ Type 2 inflammatory
428 diseases often coexist in the same patient^{2,48-51}, and several studies have shown that dupilumab
429 can significantly improve outcome measures in patients with comorbid type 2 diseases.⁵²⁻⁵⁴ These
430 improvements have been associated with consistent suppression of type 2 biomarkers across
431 type 2 inflammatory diseases.^{52,55} Type 2 inflammatory diseases are defined not simply by their
432 anatomic location but also by their common underlying pathogenesis, which is effectively the
433 “treatable trait” against which treatment can be targeted. A treatment that is effective in multiple
434 type 2 inflammatory diseases would therefore be expected to offer important benefits to patients
435 with several comorbidities.

436 One of the limitations of the current analysis was that only blood biomarkers were included.
437 Peripheral blood levels may not be as representative of physiology within the specific target
438 tissue and therefore, of changes in type 2 inflammation within the affected tissues underlying the
439 disease. Analysis of biomarkers in tissue gives greater insights into local effects, including target
440 tissues and effects on clinical disease. More invasive tissue sampling in other studies has shown
441 that dupilumab can suppress type 2 biomarker levels in target organs, such as suppression of
442 ECP, eotaxin-1, eotaxin-2, eotaxin-3, PARC, IgE, and IL-13 in nasal polyp tissue of CRSwNP
443 patients in a phase 2 study;⁷ and suppression of ECP, total IgE, eotaxin-3, and IL-5 in nasal
444 secretions in SINUS-52.²³ Decreased eosinophil infiltration in the oesophageal tissue has also
445 been observed in EoE patients treated with dupilumab.²⁴ Similarly, skin biopsies in AD patients
446 have shown marked local reductions in expression of type 2 inflammatory pathway genes,
447 including the STAT-6 regulated genes eotaxin-3, TARC, and PARC (CCL-18).^{30,31}

448 Caution should be taken when comparing the absolute values of biomarkers measured in our
449 clinical trials versus those reported in other clinical studies. Of the various biomarkers, only the
450 assay for serum total IgE is well standardized and subject to proficiency testing across clinical
451 laboratories. Hence, it is preferable when making cross-study comparisons to reference percent
452 changes from baseline (relative to placebo as appropriate). In particular, the assay results for the

453 periostin ELISA used in our studies is highly correlated with the assay developed for the
454 ELECSYS platform but systematically reports higher values.⁵⁶

455 Control of type 2 inflammation, as evidenced by reduced biomarkers observed in this study
456 together with the observed clinical efficacy of dupilumab across the diseases studied and
457 additional transcriptome analysis^{25,57} indicate that dupilumab is acting on the underlying disease
458 pathogenesis and by reducing inflammation. The reduction in the type 2 mediators measured
459 and described in this study also contribute to the improvement of barrier mucosal and epithelial
460 function to a healthier phenotype, including direct effects suppressing epithelial or epidermal
461 hyperproliferation seen in these diseases.^{30,32} For example, reducing IgE in patients with asthma
462 may restore the antiviral functions of plasmacytoid dendritic cells, thus reducing the risk of
463 exacerbations.³ By disrupting the inflammatory cycle, barrier function can be restored, reducing
464 the exposure to causative agents and thus further reducing inflammation and release of pro-
465 inflammatory cytokines.⁵⁸ The consistent modulation of type 2 biomarkers with dupilumab
466 treatment seen in these studies highlights the shared etiology of type 2 inflammation in these
467 diseases driven by IL-4 and IL-13.

468

469 CONCLUSIONS

470 Dupilumab consistently and significantly reduced most type 2 inflammatory biomarkers in patients
471 with AD, asthma, CRSwNP, and EoE. The data support the hypothesis that IL-4 and IL-13 are
472 key and central drivers of type 2 inflammation across numerous type 2 inflammatory diseases.
473 Added to other clinical and preclinical evidence, the findings demonstrate dupilumab inhibits a
474 common pathophysiological mechanism fundamental to type 2 inflammatory diseases.

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Figures and Tables

TABLE 1 Concentrations of circulating type 2 inflammatory biomarkers at baseline and effect of dupilumab on concentrations at the end of treatment for patients with AD, asthma, CRSwNP, and EoE

Type 2 disease	Atopic dermatitis		Asthma		Chronic rhinosinusitis with nasal polyps		Atopic dermatitis		Eosinophilic esophagitis	
Study name	CHRONOS		QUEST		SINUS-52		SOLO-1 and SOLO-2		EoE PoC	
Study duration	52 weeks		52 weeks		52 weeks		16 weeks		12 weeks	
Treatment group	Placebo qw + TCS	Dupilumab 300 mg q2w + TCS	Placebo 2 mL q2w + ICS/LABA	Dupilumab 300 mg q2w + ICS/LABA	Placebo q2w + INCS	Dupilumab 300 mg q2w + INCS	Placebo qw	Dupilumab 300 mg q2w	Placebo qw	Dupilumab 300 mg qw
Serum TARC (pg/mL)										
n	313	110	319	622	149	147	454	464	24	22
Baseline median value (95% CI)	2868.20 (2099.10 to 3665.10)	4030.10 (2989.00 to 5137.10)	300.00 (277.00 to 334.00)	295.00 (277.00 to 315.00)	304.00 (267.00 to 341.00)	303.00 (275.00 to 347.00)	2195.00 (1840.00 to 2636.00)	1936.50 (1637.00 to 2573.00)	333.50 (225.00 to 396.00)	293.00 (196.00 to 377.00)
n	120	84	248	479	127	139	194	361	19	22
Median value	965.10	359.95	303.50	201.00	246.00	187.00	1025.00	385.00	292.00	197.50 (133.00 to

(95% CI) at end of treatment	(729.10 to 1227.80)	(313.40 to 454.50)	(281.00 to 337.00)	(189.00 to 214.00)	(227.00 to 296.00)	(165.00 to 210.00)	(755.00 to 1231.00)	(355.00 to 417.00)	(196.00 to 398.00)	292.00)
n	119	84	246	473	126	137	193	360	19	21
Median change from baseline	-987.40	-4238.55	8.50	-85.00	-9.50	-111.00	-108.00	-1297.00	-13.00	-90.00
(95% CI) at end of treatment	(-1218.0 to 0 to -552.20)	(-6103.5 to 0 to -2795.60)	(-13.00 to 19.00)	(-95.00 to -73.00)	(-26.00 to 2.00)	(-130.00 to -94.00)	(-257.00 to -40.00)	(-1602.0 to -1002.0)	(-51.00 to 28.00)	(-111.00 to -15.00)
<i>P</i> -value dupilumab vs placebo		< .0001		< .0001		< .0001		< .0001		.0143
n	119	84	246	473	126	137	193	360	19	21
Median % change from baseline	-53.6	-88.6	2.60	-33.51	-2.75	-39.44	-16.4	-78.9	-8.2	-24.8
(95% CI) at end of treatment	(-61.7 to -43.8)	(-93.6 to -82.0)	(-4.03 to 7.11)	(-36.00 to -29.84)	(-8.02 to 0.85)	(-43.17 to -35.08)	(-26.0 to -6.2)	(-81.5 to -76.6)	(-12.8 to 13.5)	(-38.7 to -11.7)
<i>P</i> -value dupilumab vs placebo		< .0001		< .0001		< .0001		< .0001		.006
Plasma eotaxin-3 (pg/mL)										
n			318	625	150	146				
Baseline median value (95% CI)			37.45 (34.60 to 40.10)	38.30 (36.00 to 40.10)	61.55 (51.80 to 71.00)	60.50 (55.10 to 72.40)				

n		246	473	127	139					
Median (95% CI) at end of treatment		38.45 (35.50 to 42.60)	23.50 (22.40 to 25.70)	62.90 (53.70 to 68.80)	28.50 (25.10 to 31.20)					
n		243	469	127	136					
Median change from baseline (95% CI) at end of treatment		-0.10 (-1.70 to 2.00)	-12.90 (-15.80 to -11.40)	3.60 (-0.70 to 9.10)	-26.70 (-33.30 to -21.50)					
<i>P</i> -value dupilumab vs placebo			< .0001		< .0001					
n		243	469	127	136					
Median % change from baseline (95% CI) at end of treatment		-0.16 (-4.85 to 8.97)	-38.24 (-42.42 to -33.70)	8.30 (-1.75 to 19.64)	-51.47 (-57.21 to -45.24)					
<i>P</i> -value dupilumab vs placebo			< .0001		< .0001					
Serum total IgE (IU/mL)										
n	312	110	318	626	150	148	456	465	24	23
Baseline median (95% CI)	3477.00 (2471.00	4925.50 (3038.00	178.50 (138.00	174.00 (152.00	139.00 (104.00	122.50 (98.00 to	2849.00 (2174.00	2396.00 (2001.00	126.50 (58.00 to	67.00 (42.00 to

	to 4757.00)	to 10 000.00)	to 213.00)	to 200.00)	to 172.00)	157.00)	to 3817.00)	to 3025.00)	254.00)	220.00)
n	117	83	251	482	128	140	196	366	19	23
Median (95% CI) at end of treatment	2257.00 (1327.00 to 3711.00)	951.00 (520.00 to 1648.00)	176.00 (136.00 to 217.00)	48.50 (40.00 to 55.00)	144.00 (115.00 to 191.00)	29.50 (23.00 to 40.00)	1592.00 (927.00 to 3022.00)	1107.00 (739.00 to 1518.00)	159.00 (64.00 to 388.00)	53.00 (33.00 to 147.00)
n	116	83	248	478	128	139	196	366	19	23
Median change from baseline (95% CI) at end of treatment	0 (-7.00 to 0)	-2549.00 (-5481.0 to -735.00)	-2.50 (-7.00 to 1.00)	-116.50 (-135.00 to -102.00)	4.00 (0 to 8.00)	-84.00 (-106.00 to -62.00)	17.10 (3.00 to 75.00)	-976.00 (-1331.0 to -696.00)	9.00 (-4.00 to 28.00)	-17.00 (-40.00 to -9.00)
<i>P</i> -value dupilumab vs placebo		< .0001		< .0001		< .0001		< .0001		< .0001
n	116	83	248	478	128	139	196	366	19	23
Median % change from baseline (95% CI) at end of treatment	0 (-6.5 to 0)	-76.7 (-80.9 to -72.7)	-4.42 (-10.00 to 0.51)	-69.97 (-72.22 to -67.60)	7.54 (0 to 14.57)	-71.43 (-75.61 to -67.39)	7.8 (2.1 to 15.1)	-46.5 (-48.4 to -44.4)	8.3 (-6.9 to 18.0)	-24.8 (-33.2 to -14.5)
<i>P</i> -value dupilumab vs placebo		< .0001		< .0001		< .0001		< .0001		< .0001
Serum periostin (ng/mL)										
n			319	629	150	147			24	22

Baseline median (95% CI)			71.00 (66.30 to 75.10)	69.70 (67.30 to 72.80)	104.00 (95.70 to 113.00)	99.60 (86.60 to 114.00)		56.95 (49.70 to 68.20)	56.20 (48.70 to 76.90)	
n			253	490	120	128		18	23	
Median (95% CI) at end of treatment			66.50 (61.20 to 72.20)	55.50 (53.70 to 57.60)	94.15 (80.80 to 104.00)	61.55 (57.60 to 65.60)		61.60 (53.90 to 86.80)	47.00 (44.20 to 55.90)	
n			251	488	120	127		18	22	
Median change from baseline (95% CI) at end of treatment			-4.10 (-6.40 to -1.80)	-12.45 (-14.30 to -10.40)	-3.15 (-8.50 to 1.30)	-39.00 (-52.70 to -30.40)		7.50 (-3.70 to 14.10)	-8.95 (-13.30 to 2.10)	
<i>P</i> -value dupilumab vs placebo				< .0001		< .0001			.0016	
n			251	488	120	127		18	22	
Median % change from baseline (95% CI) at end of treatment			-6.94 (-9.93 to -3.16)	-18.93 (-21.48 to -16.23)	-3.52 (-8.29 to 1.62)	-41.08 (-46.22 to -31.14)		10.1 (-6.5 to 25.8)	-13.6 (-22.5 to 3.0)	
<i>P</i> -value dupilumab vs placebo				< .0001		< .0001			.0005	
Blood eosinophils (Giga/L)										
n	314	110	320	632	150	148	454	465	24	23

Baseline median (95% CI)	0.42 (0.36 to 0.49)	0.45 (0.37 to 0.52)	0.27 (0.24 to 0.30)	0.25 (0.22 to 0.28)	0.36 (0.32 to 0.40)	0.34 (0.30 to 0.40)	0.40 (0.40 to 0.50)	0.40 (0.40 to 0.50)	0.40 (0.20 to 0.50)	0.30 (0.20 to 0.40)
n	120	83	245	476	129	140	195	360	21	22
Median (95% CI) at end of treatment	0.28 (0.25 to 0.32)	0.30 (0.23 to 0.35)	0.25 (0.22 to 0.29)	0.19 (0.17 to 0.22)	0.34 (0.29 to 0.39)	0.26 (0.21 to 0.31)	0.30 (0.30 to 0.40)	0.30 (0.30 to 0.40)	0.30 (0.20 to 0.60)	0.15 (0.10 to 0.20)
n	119	83	245	476	129	139	194	360	21	22
Median change from baseline (95% CI) at end of treatment	-0.08 (-0.16 to -0.04)	-0.15 (-0.20 to -0.09)	-0.03 (-0.04 to -0.01)	-0.03 (-0.04 to -0.01)	-0.03 (-0.07 to 0.02)	-0.07 (-0.11 to -0.04)	-0.10 (-0.10 to 0)	0.00 (-0.10 to 0)	0.00 (-0.20 to 0.10)	-0.10 (-0.20 to -0.10)
<i>P</i> -value dupilumab vs placebo		.5718		.2916		.0814		.0755		.0455
n	119	83	242	469	128	139	190	346	20	21
Median % change from baseline (95% CI) at end of treatment	-29.8 (-36.8 to -18.2)	-40.7 (-50.0 to -31.5)	-11.44 (-17.78 to -3.57)	-14.58 (-20.00 to -7.69)	-11.88 (-17.86 to 5.88)	-29.41 (-40.00 to -16.33)	-20.0 (-30.8 to 0)	0.0 (-15.8 to 0)	-12.5 (-50.0 to 33.3)	-50.0 (-50.0 to -33.3)
<i>P</i> -value dupilumab vs placebo		.1817		.0552		.0174		.0552		.0282

Note: Gray cells indicate that data were not collected or available for these patients.

Abbreviations: AD, atopic dermatitis; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; IU, international

units; ICS, inhaled corticosteroids; INCS, intranasal corticosteroids; LABA, long-acting β_2 -agonist; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; TARC, thymus and activation-regulated chemokine; TCS, topical corticosteroids.

FIGURE 1 The roles of biomarkers in type 2 inflammation. DC, dendritic cell; IL, interleukin; ECP, eosinophil cationic protein; iNOS, inducible nitric oxide synthase; PARC, pulmonary and activation-regulated chemokine; TARC, thymus and activation-regulated chemokine.

FIGURE 2 The effect of dupilumab on concentrations of serum TARC (pg/mL). $*P < .05$; $**P < .01$, $***P < .001$ vs matched placebo. *P*-values represent differences between dupilumab and placebo in change from baseline (A and C) or percentage change from baseline (B and D) and were analyzed using a rank ANCOVA model. CHRONOS, SOLO-1, and SOLO-2 included the corresponding baseline values as covariates and the treatment, geographic region, baseline Investigator Global Assessment (IGA) strata, and study identifier as fixed factors. EoE PoC included the corresponding baseline values as covariates and the treatment, baseline Straumann Dysphagia Instrument (SDI) strata study identifier as fixed factors. QUEST included the corresponding baseline value, age, sex, geographic region (pooled country), baseline eosinophil strata, and baseline inhaled corticosteroid dose level as covariates. SINUS-52 included the corresponding baseline value, age, geographic region, asthma/nonsteroidal anti-inflammatory drugs–exacerbated respiratory disease (NSAID-ERD) status and prior surgery history as covariates. AD, atopic dermatitis; ANCOVA, analysis of covariance; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; TARC, thymus and activation-regulated chemokine.

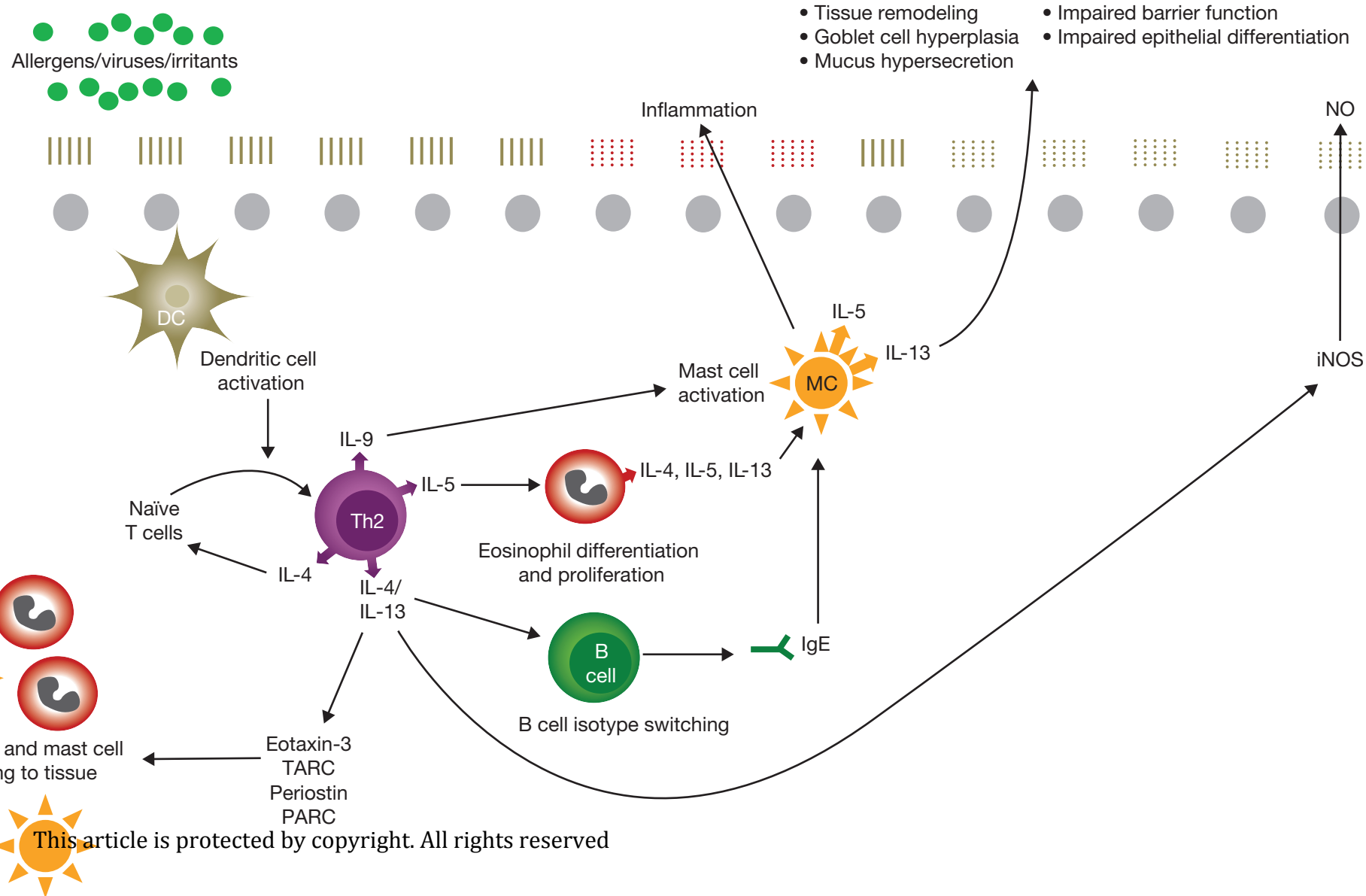
FIGURE 3 The effect of dupilumab on concentrations of plasma eotaxin-3 (pg/mL). $***P < .001$ vs matched placebo. *P*-values represent differences between dupilumab and placebo in change from baseline (A) or percentage change from baseline (B) and were analyzed using a rank ANCOVA model. QUEST included the corresponding baseline value, age, sex, geographic region (pooled country), baseline eosinophil strata, and baseline inhaled corticosteroid dose level as covariates. SINUS-52 included the corresponding baseline value, age, geographic region (pooled country), asthma/NSAID-ERD status, and prior surgery history as covariates. ANCOVA, analysis of covariance; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; NSAID-ERD, nonsteroidal anti-inflammatory drugs–exacerbated respiratory disease; q2w, every 2 weeks.

FIGURE 4 The effect of dupilumab on concentrations of serum total IgE (IU/mL). $*P < .05$; $**P < .01$, $***P < .001$ vs matched placebo. *P*-values represent differences between dupilumab and placebo in change from baseline (A and C) or percentage change from baseline (B and D) and were analyzed using a rank ANCOVA model. CHRONOS, SOLO-1, and SOLO-2 included the corresponding baseline values as covariates and the treatment, geographical region, baseline IGA strata, and study identifier as fixed factors. EoE PoC included the corresponding baseline values as covariates and the treatment, baseline SDI strata study identifier as fixed factors. QUEST included the corresponding baseline value, age, sex, geographic region (pooled country), baseline eosinophil strata, and baseline inhaled corticosteroid dose level as covariates. SINUS-52 included the corresponding baseline value, age, geographic region (pooled country), asthma/NSAID-ERD status, and prior surgery history as covariates. AD, atopic dermatitis; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; NSAID-ERD, nonsteroidal anti-inflammatory drugs–exacerbated respiratory disease; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SDI, Straumann Dysphagia Instrument.

FIGURE 5 The effect of dupilumab on concentrations of serum periostin (ng/mL). $*P < .05$; $**P < .01$; $***P < .001$ vs matched placebo. *P*-values represent differences between dupilumab and placebo in change from baseline (A and C) or percentage change from baseline (B and D) and were analyzed using a rank ANCOVA model. EoE PoC included the corresponding baseline values as covariates and the treatment, baseline SDI strata study identifier as fixed factors. QUEST included the corresponding baseline value, age, sex, geographic region (pooled country), baseline eosinophil strata, and baseline inhaled corticosteroid dose level as covariates. SINUS-52 included the corresponding baseline value, age, geographic region (pooled country), asthma/NSAID-ERD status, and prior surgery history as covariates. ANCOVA, analysis of covariance; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; NSAID-ERD, nonsteroidal anti-inflammatory drugs–exacerbated respiratory disease; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SDI, Straumann Dysphagia Instrument.

FIGURE 6 The effect of dupilumab on concentrations of blood eosinophils (Giga/L). $*P < .05$; $**P < .01$; $***P < .001$ vs matched placebo. *P*-values represent differences between dupilumab and placebo in change from baseline (A and C) or percentage change from baseline (B and D) and were analyzed using a rank ANCOVA model. CHRONOS, SOLO-1, and SOLO-2 included the corresponding baseline values as covariates and the treatment, geographic region, baseline IGA strata, and study identifier as fixed factors. EoE PoC included the corresponding baseline values as covariates and the treatment, baseline SDI strata study identifier as fixed factors. QUEST included the corresponding baseline value, age, sex, geographic region (pooled country), baseline eosinophil strata, and

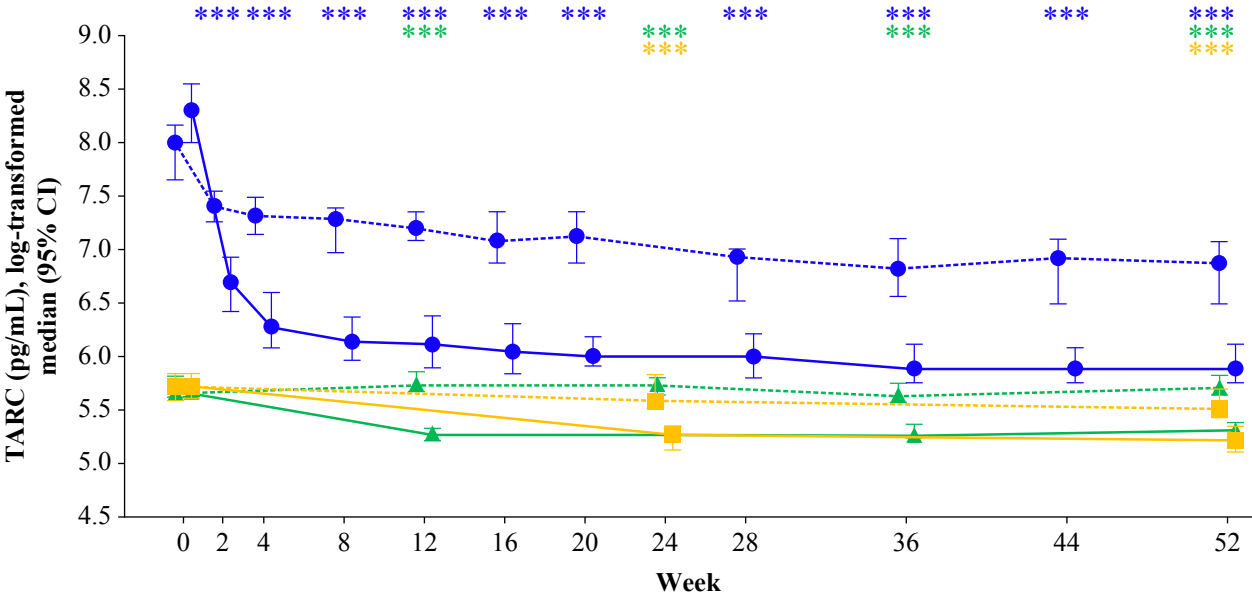
baseline inhaled corticosteroid dose level as covariates. SINUS-52 included the corresponding baseline value, age, geographic region (pooled country), asthma/NSAID-ERD status, and prior surgery history as covariates. AD, atopic dermatitis; ANCOVA, analysis of covariance; CI, confidence interval; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; NSAID-ERD, nonsteroidal anti-inflammatory drugs–exacerbated respiratory disease; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SDI, Straumann Dysphagia Instrument.



AD patients from CHRONOS Asthma patients from QUEST CRSwNP patients from SINUS-52 AD patients from SOLO-1 and SOLO-2 EoE patients from EoE PoC

Placebo qw Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo qw Dupilumab 300 mg q2w Placebo qw Dupilumab 300 qw

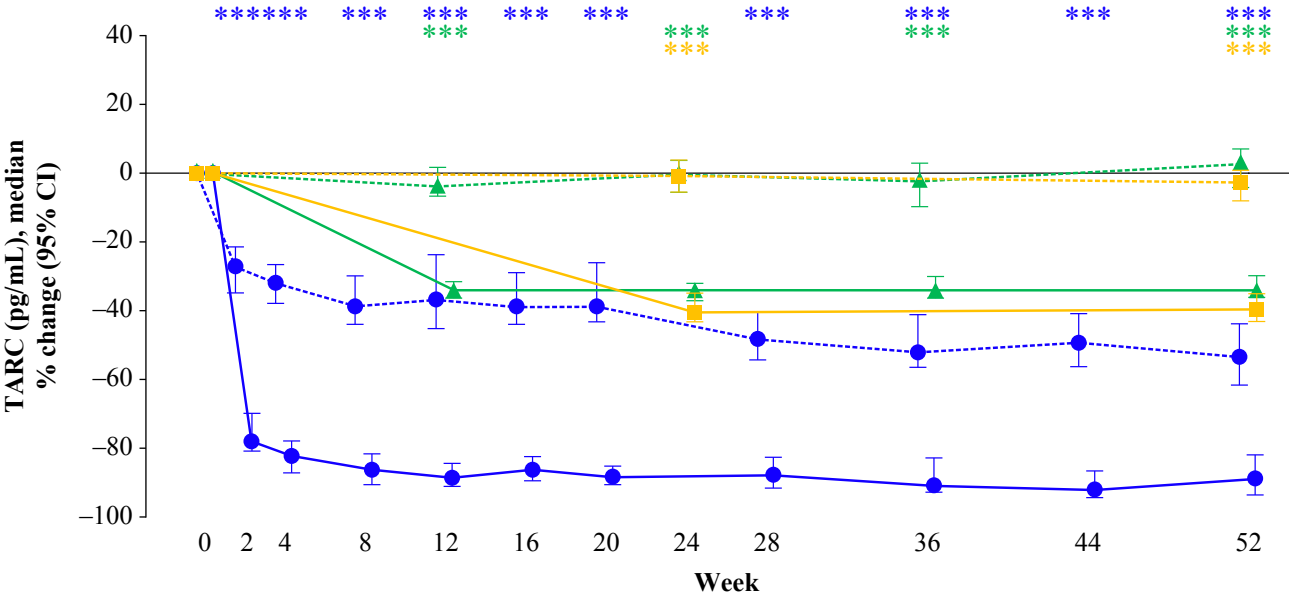
(A) 52-week studies in AD, asthma, and CRSwNP



Number of patients

AD, placebo qw	313	295	272	241	213	188	173	151	137	126	120
AD, dupilumab 300 mg q2w	110	104	101	98	94	93	91	88	85	83	84
Asthma, placebo q2w	319				317			289	295		248
Asthma, dupilumab 300 mg q2w	622				623			581	564		479
CRSwNP, placebo q2w	149							143			127
CRSwNP, dupilumab 300 mg q2w	147							142			139

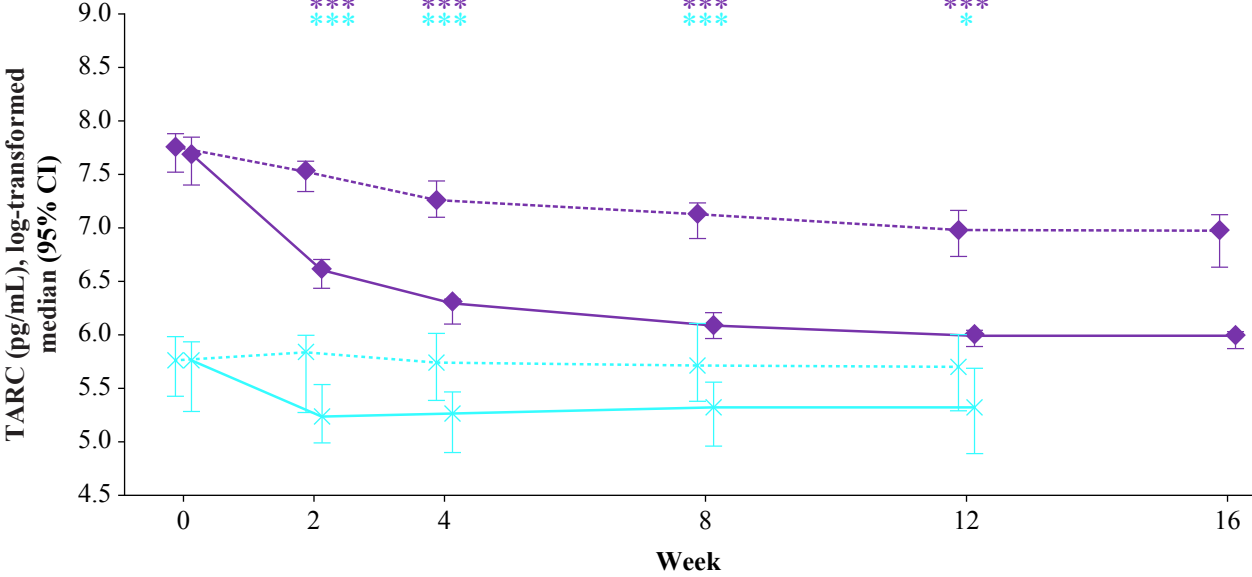
(B) 52-week studies in AD, asthma, and CRSwNP



Number of patients

AD, placebo qw	313	293	271	240	212	187	172	150	136	125	119
AD, dupilumab 300 mg q2w	110	104	101	98	94	93	91	88	85	83	84
Asthma, placebo q2w	319				315			288	293		246
Asthma, dupilumab 300 mg q2w	622				613			572	557		473
CRSwNP, placebo q2w	149							142			126
CRSwNP, dupilumab 300 mg q2w	147							140			137

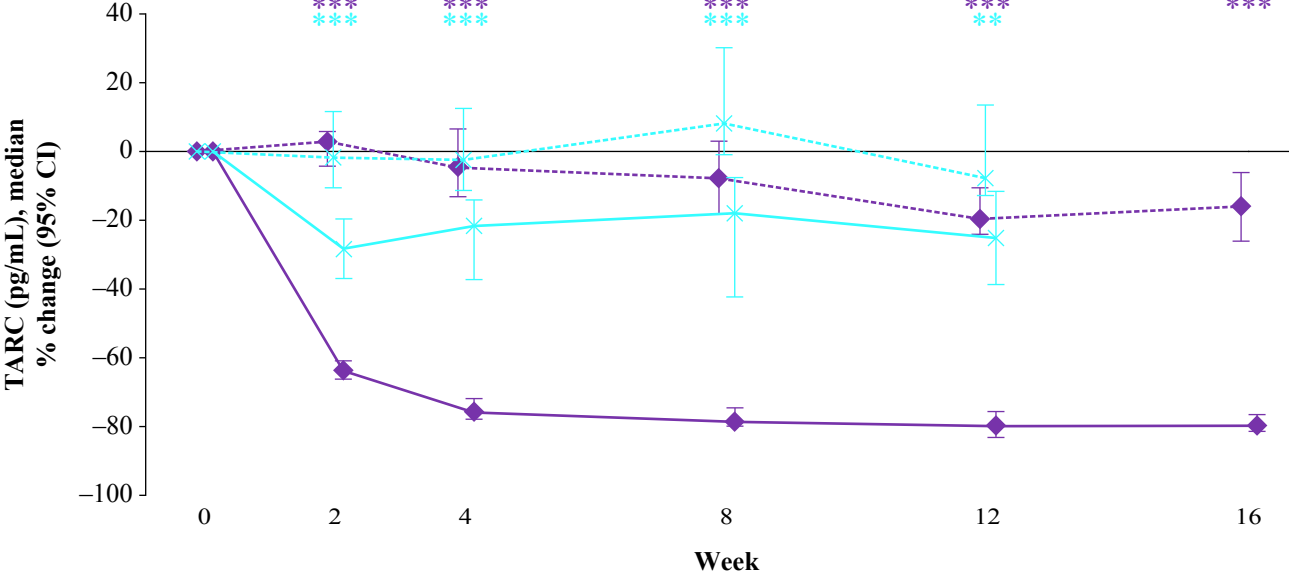
(C) 12- to 16-week studies in EoE and AD



Number of patients

AD, placebo qw	454	379	311	239	208	194
AD, dupilumab 300 mg q2w	464	424	401	390	370	361
EoE, placebo qw	24	23	19	19	19	
EoE, dupilumab 300 mg qw	22	22	20	19	22	

(D) 12- to 16-week studies in EoE and AD



Number of patients

AD, placebo qw	454	379	311	237	208	193
AD, dupilumab 300 mg q2w	464	423	401	389	369	360
EoE, placebo qw	24	23	19	19	19	
EoE, dupilumab 300 mg qw	22	21	19	18	21	

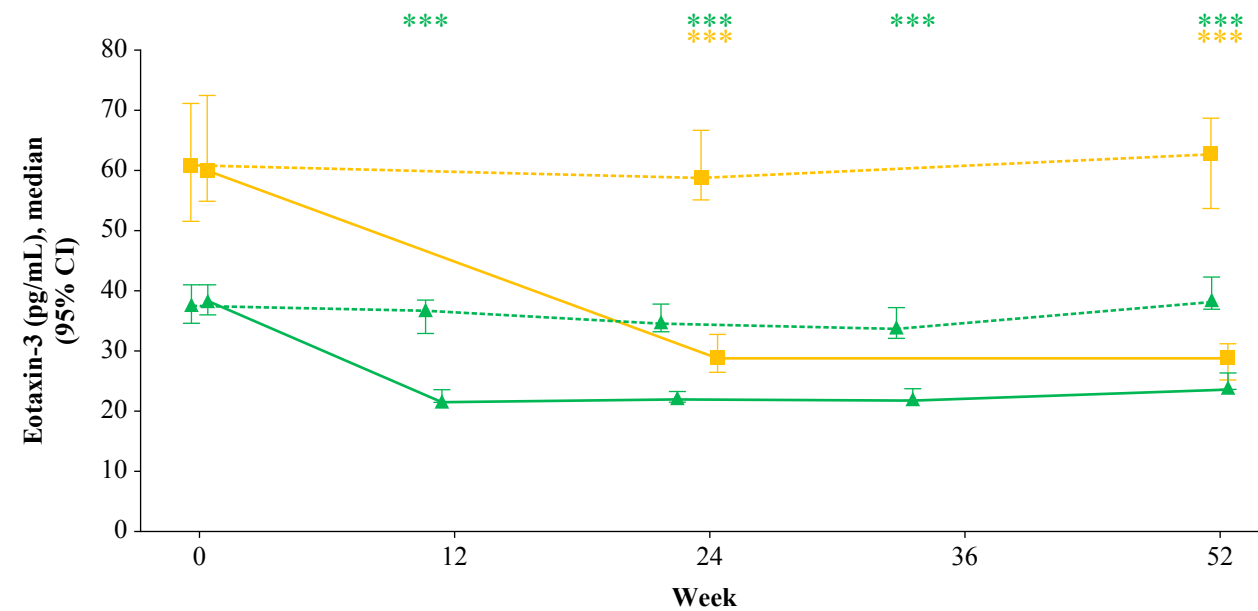
Asthma patients from QUEST

CRSwNP patients from SINUS-52

Placebo q2w Dupilumab 300 mg q2w

Placebo q2w Dupilumab 300 mg q2w

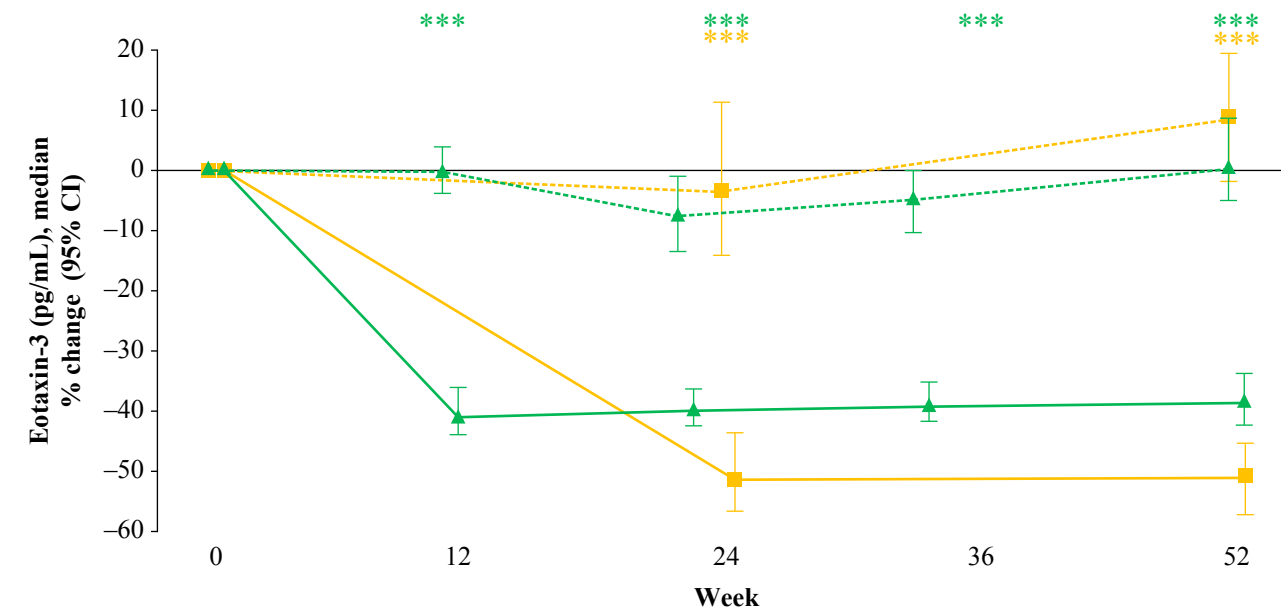
(A) 52-week studies in asthma and CRSwNP



Number of patients

Asthma, placebo q2w	318	316	297	298	246
Asthma, dupilumab 300 mg q2w	625	623	592	575	473
CRSwNP, placebo q2w	150	142	142		127
CRSwNP, dupilumab 300 mg q2w	146		142		139

(B) 52-week studies in asthma and CRSwNP



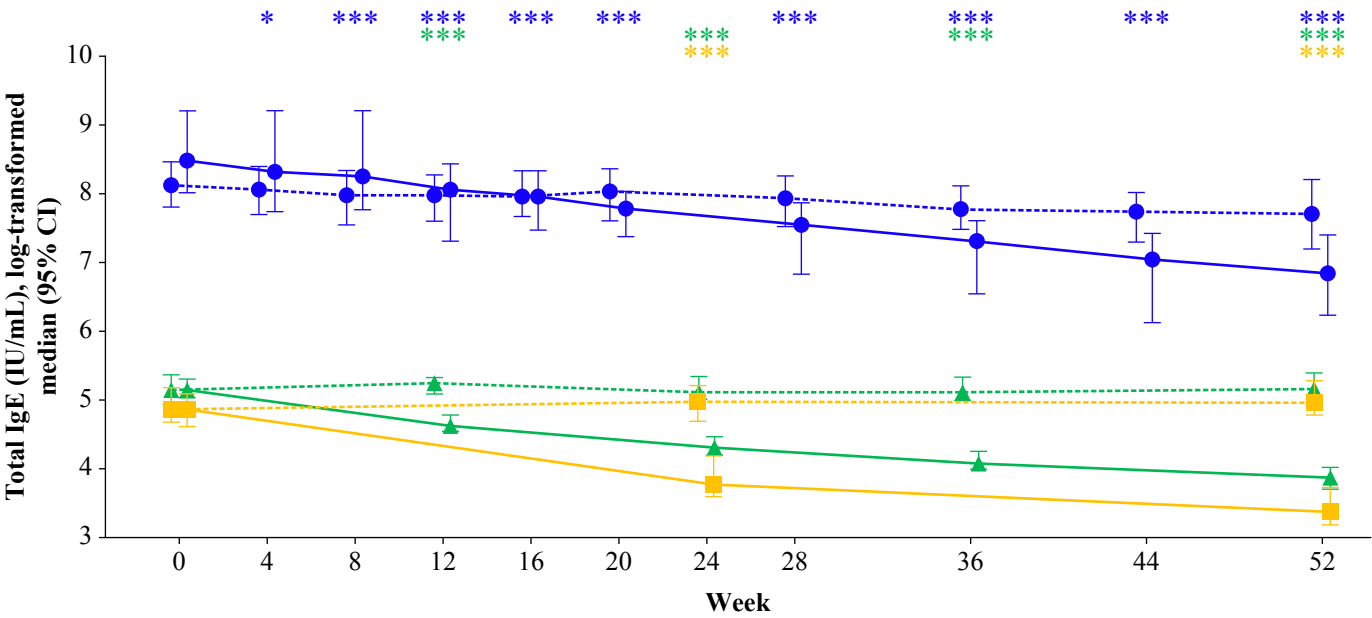
Number of patients

Asthma, placebo q2w	318	313	295	295	243
Asthma, dupilumab 300 mg q2w	625	616	587	570	469
CRSwNP, placebo q2w	150		142		127
CRSwNP, dupilumab 300 mg q2w	146		139		136

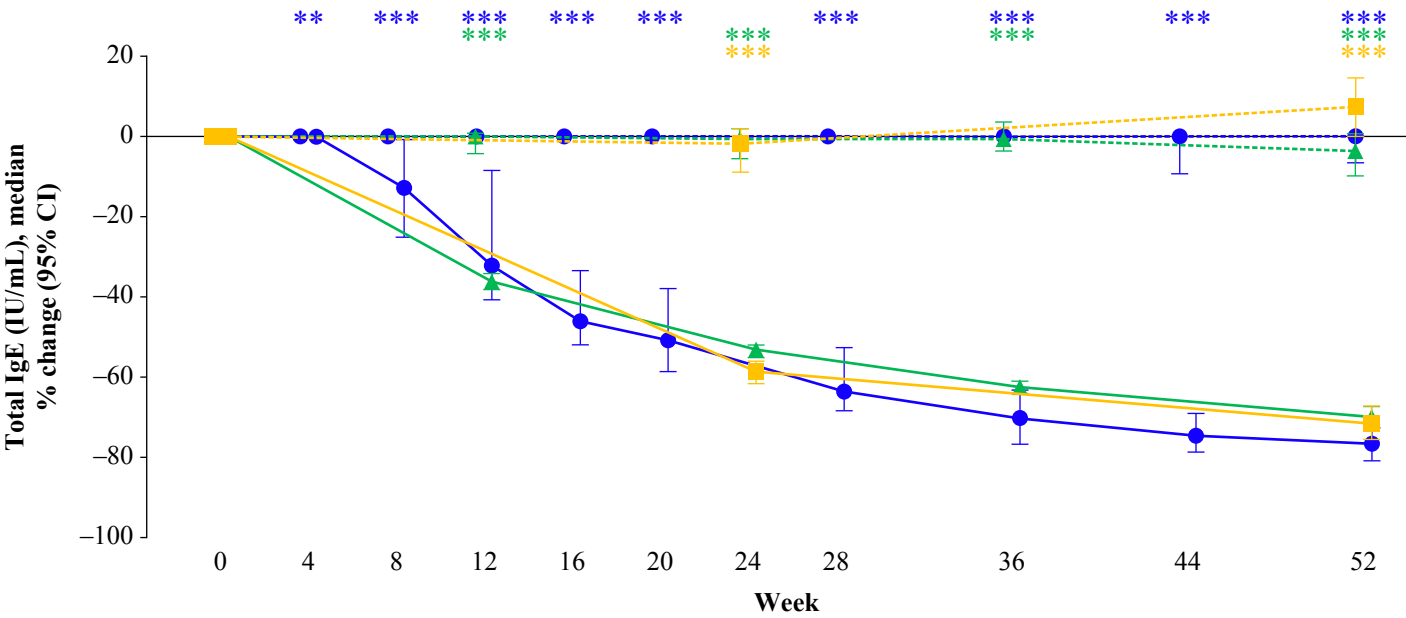
AD patients from CHRONOS Asthma patients from QUEST CRSwNP patients from SINUS-52 AD patients from SOLO-1 and SOLO-2 EoE patients from EoE PoC

Placebo qw Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo qw Dupilumab 300 mg q2w Placebo qw Dupilumab 300 mg qw

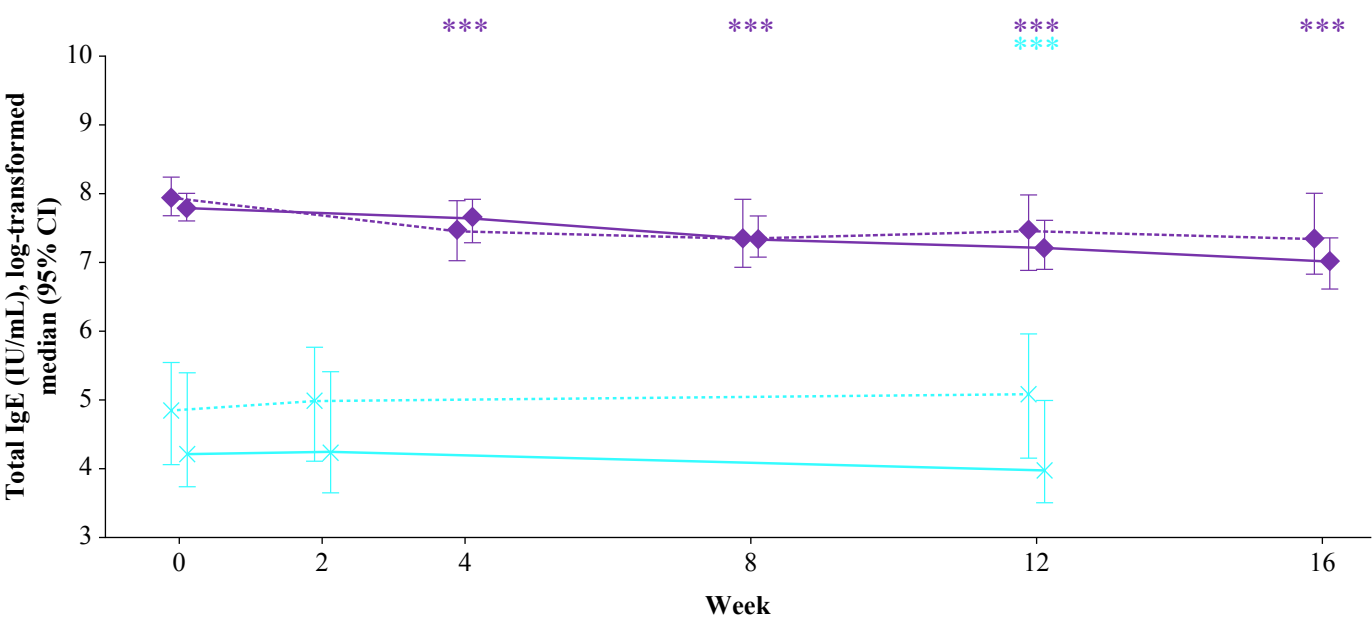
(A) 52-week studies in AD, asthma, and CRSwNP



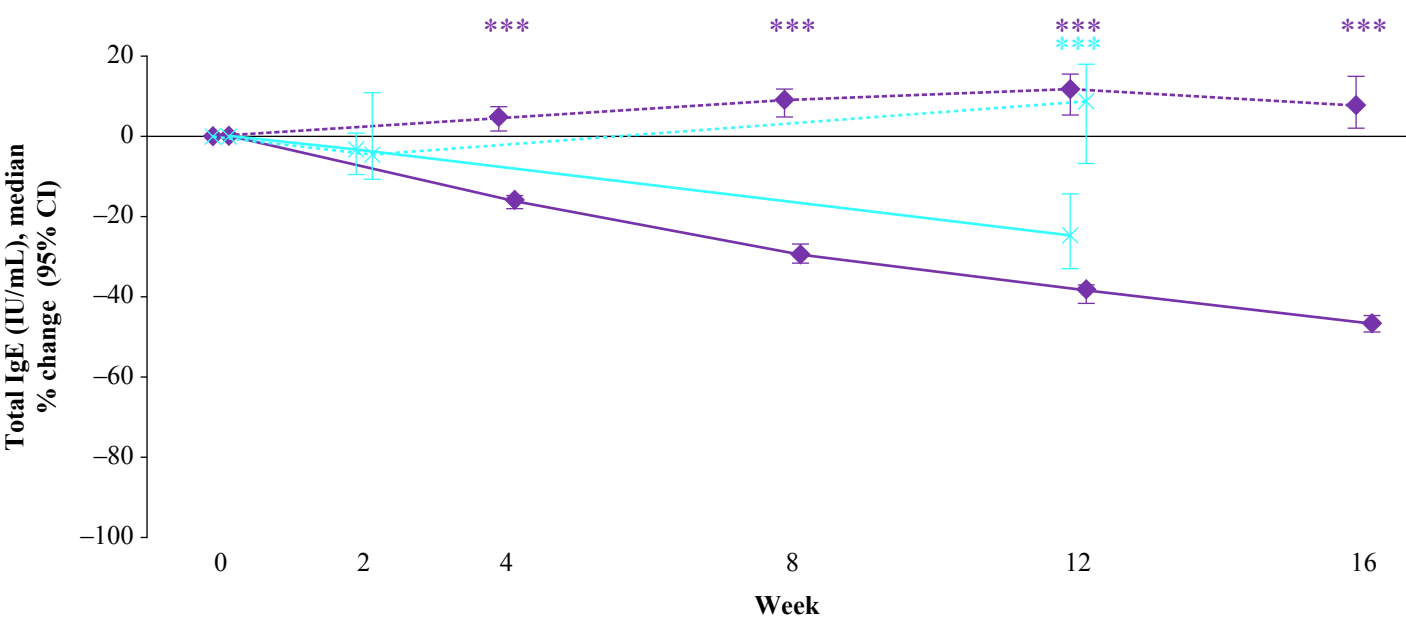
(B) 52-week studies in AD, asthma, and CRSwNP



(C) 12- to 16-week studies in EoE and AD



(D) 12- to 16-week studies in EoE and AD



Asthma patients from QUEST

CRSwNP patients from SINUS-52

EoE patients from EoE PoC

Placebo q2w

Dupilumab 300 mg q2w

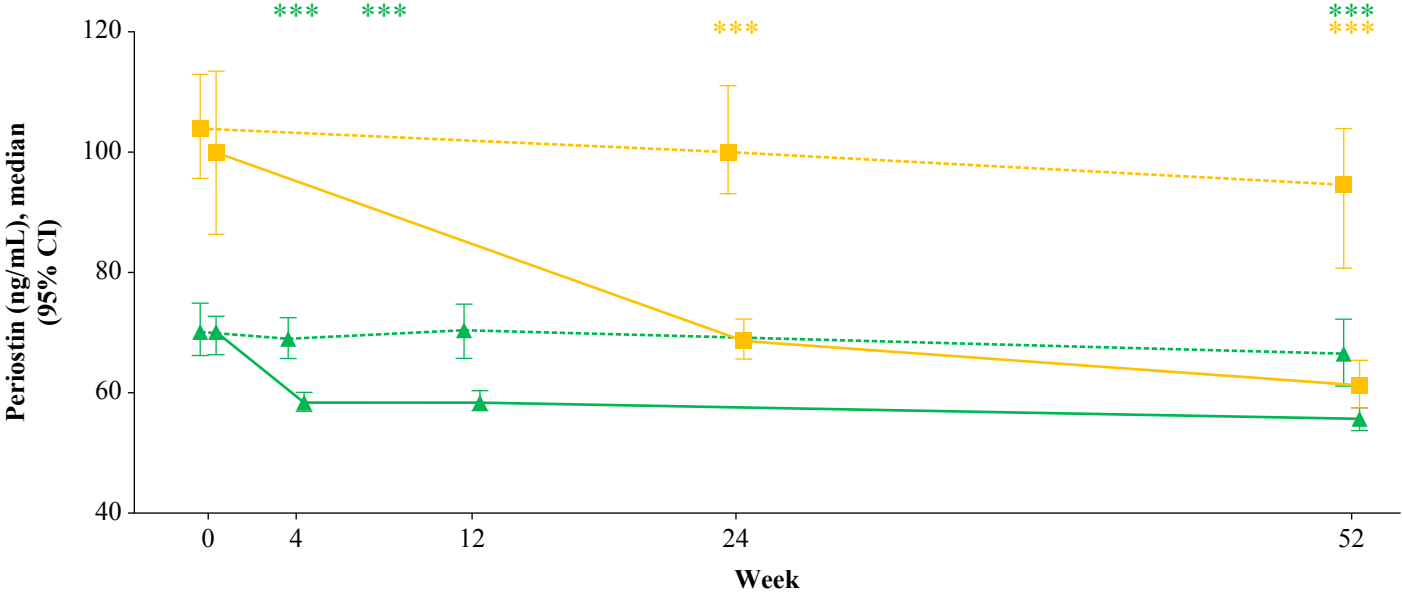
Placebo q2w

Dupilumab 300 mg q2w

Placebo qw

Dupilumab 300 mg qw

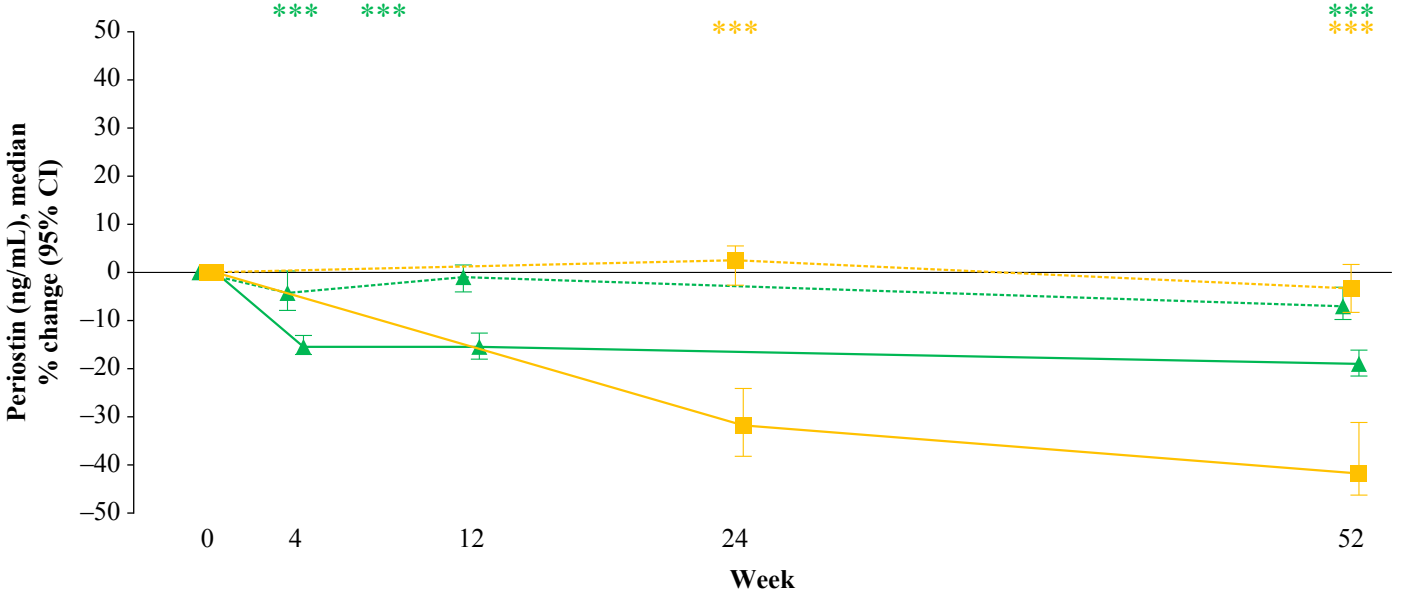
(A) 52-week studies in asthma and CRSwNP



Number of patients

Asthma, placebo q2w	319	314	314	253
Asthma, dupilumab 300 mg q2w	629	615	614	490
CRSwNP, placebo q2w	150			120
CRSwNP, dupilumab 300 mg q2w	147			128

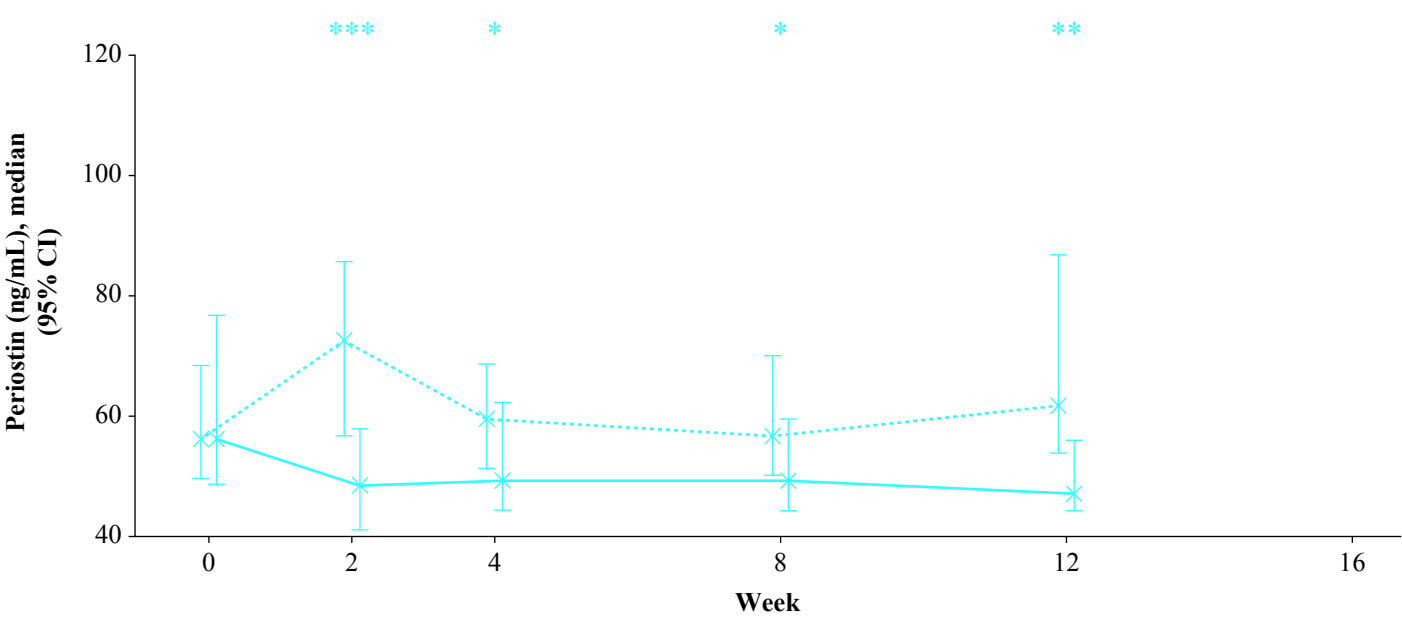
(B) 52-week studies in asthma and CRSwNP



Number of patients

Asthma, placebo q2w	319	312	312	251
Asthma, dupilumab 300 mg q2w	629	612	611	488
CRSwNP, placebo q2w	150			120
CRSwNP, dupilumab 300 mg q2w	147			127

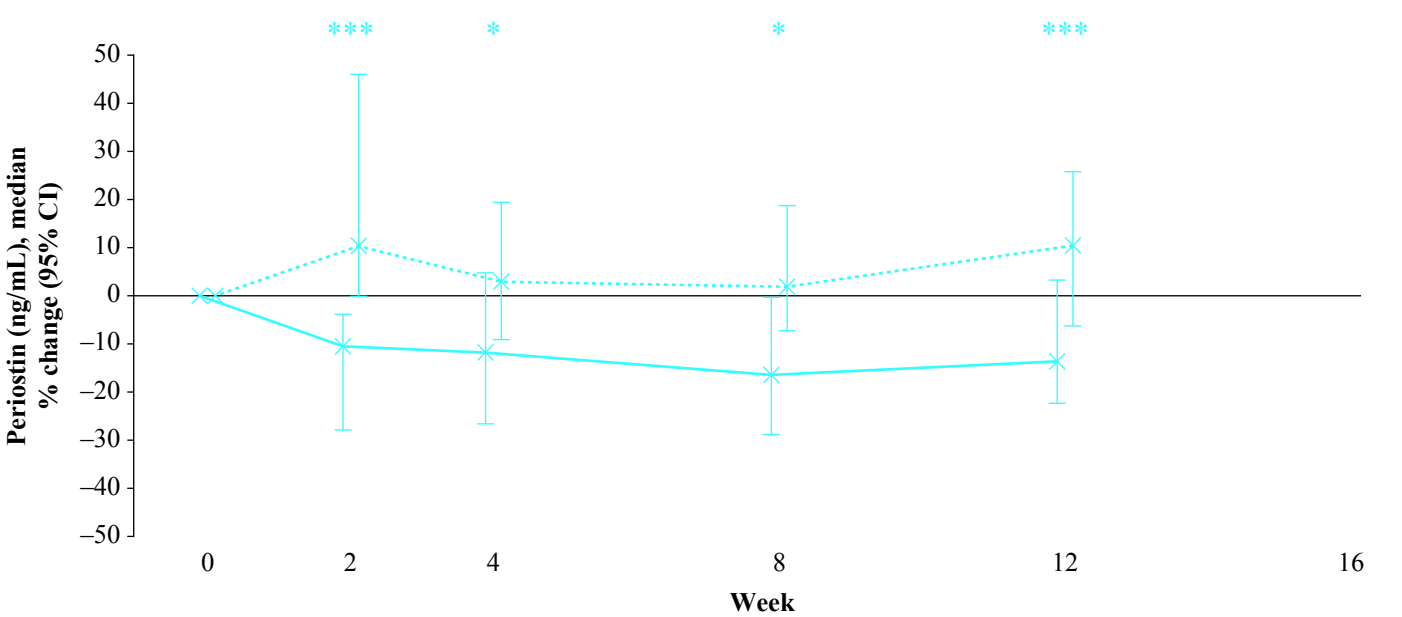
(C) 12-week study in EoE



Number of patients

EoE, placebo qw	24	23	20	18
EoE, dupilumab 300 mg qw	22	23	22	23

(D) 12-week study in EoE



Number of patients

EoE, placebo qw	24	23	20	19	18
EoE, dupilumab 300 mg qw	22	22	21	19	22

AD patients from CHRONOS

Asthma patients from QUEST

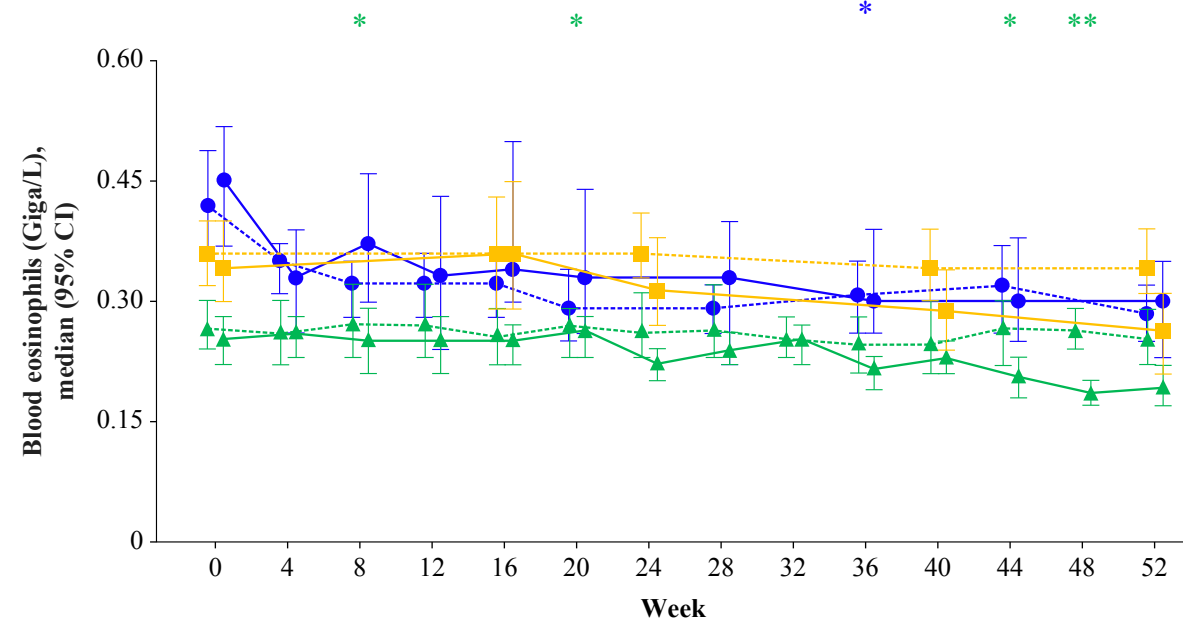
CRSwNP patients from SINUS-52

AD patients from SOLO-1 and SOLO-2

EoE patients from EoE PoC

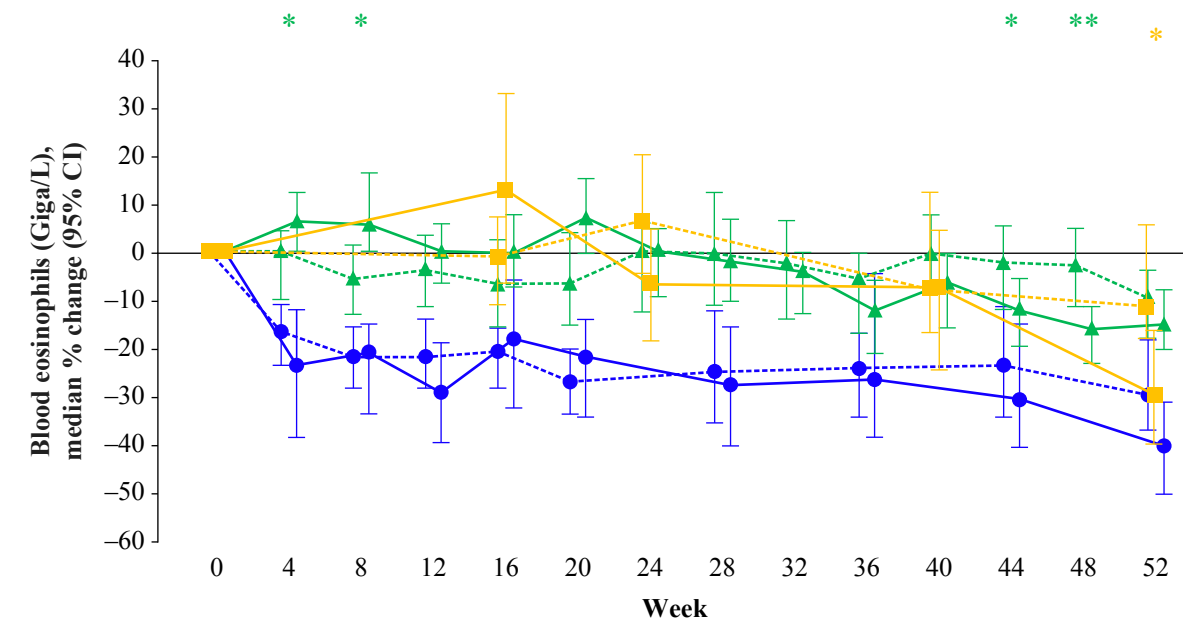
Placebo qw Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo q2w Dupilumab 300 mg q2w Placebo qw Dupilumab 300 mg q2w Placebo qw Dupilumab 300 mg q2w

(A) 52-week studies in AD, asthma, and CRSwNP



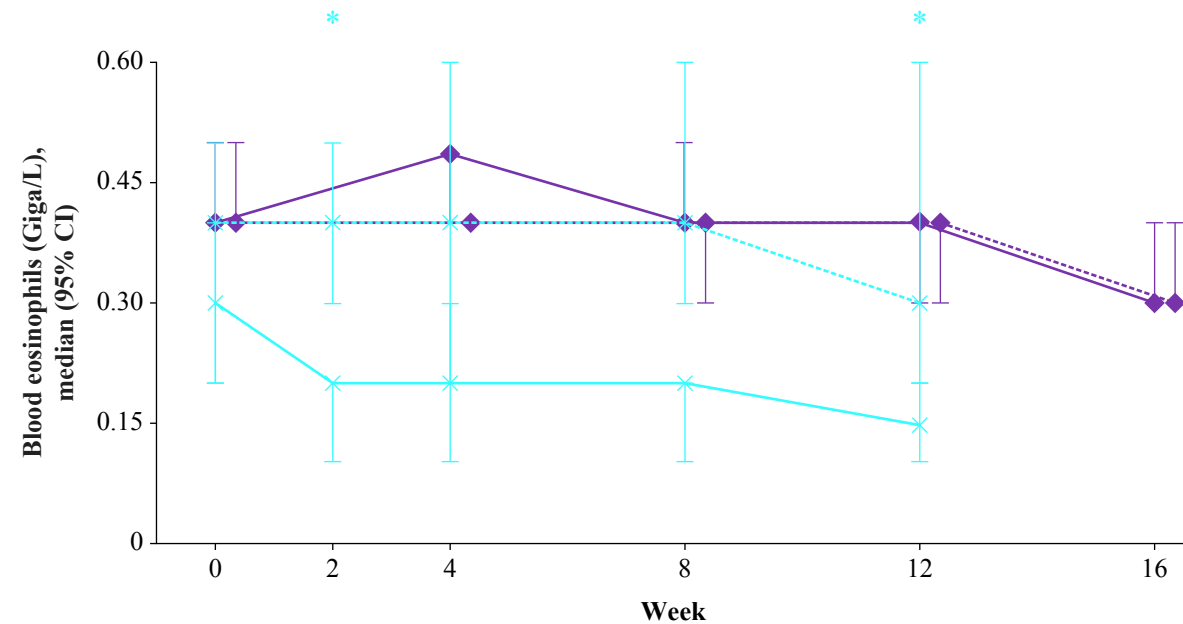
Number of patients												
AD, placebo qw	314	269	240	207	187	172		151	135	125	120	
AD, dupilumab 300 mg q2w	110	98	97	90	91	90		86	84	83	83	
Asthma, placebo q2w	320	307	305	302	300	291	289	294	294	290	281	277
Asthma, dupilumab 300 mg q2w	632	616	587	595	596	581	577	576	560	561	552	537
CRSwNP, placebo q2w	150				143		137			131		129
CRSwNP, dupilumab 300 mg q2w	148				141		142			140		140

(B) 52-week studies in AD, asthma, and CRSwNP



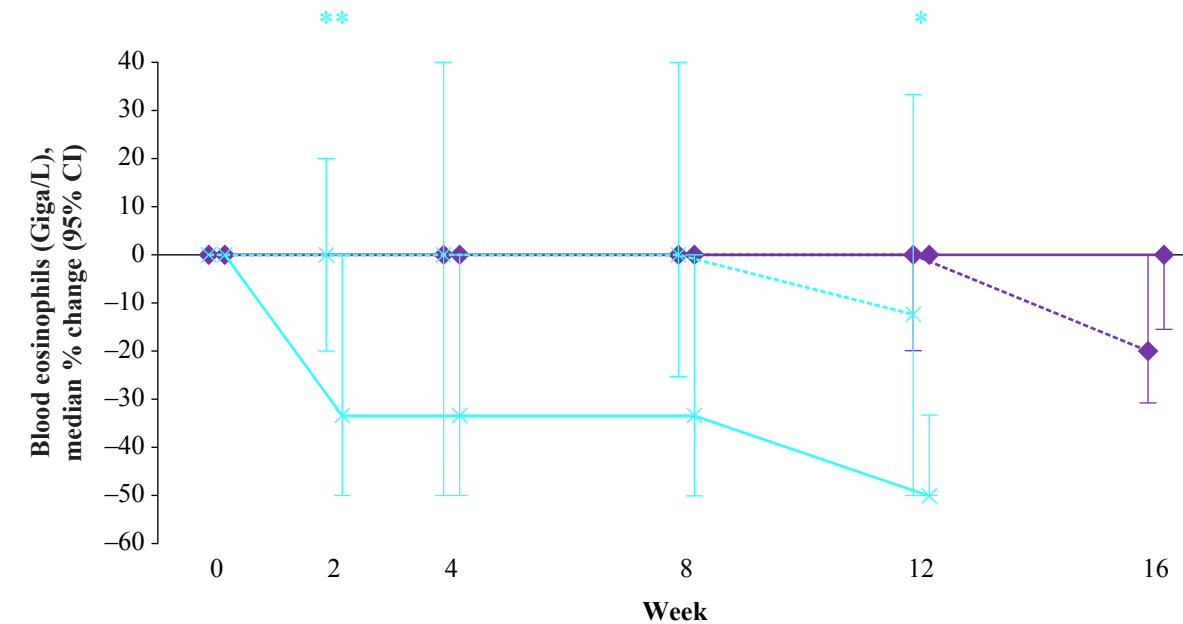
Number of patients												
AD, placebo qw	314	268	239	206	186	171		150		134	124	119
AD, dupilumab 300 mg q2w	110	98	97	90	91	90		86		84	83	83
Asthma, placebo q2w	320	304	301	298	297	287	285	290	290	286	277	242
Asthma, dupilumab 300 mg q2w	632	609	580	588	589	574	570	569	554	561	545	469
CRSwNP, placebo q2w	150				142		136			130		128
CRSwNP, dupilumab 300 mg q2w	148				140		141			139		139

(C) 12- and 16-week studies in EoE and AD



Number of patients					
AD, placebo qw	454		324	235	208
AD, dupilumab 300 mg q2w	465		412	392	382
EoE, placebo qw	24	23	21	20	21
EoE, dupilumab 300 mg q2w	23	23	23	21	22

(D) 12- and 16-week studies in EoE and AD



Number of patients					
AD, placebo qw	454		317	230	204
AD, dupilumab 300 mg q2w	465		399	378	368
EoE, placebo qw	24	22	20	19	20
EoE, dupilumab 300 mg q2w	23	22	22	20	21

Supplementary materials for the article:
Dupilumab suppresses type 2 inflammatory biomarkers across multiple atopic, allergic diseases

Jennifer D. Hamilton, PhD; Sivan Harel, PhD; Brian N. Swanson, PhD; William Brian, PhD; Zhen Chen, PhD, MS, MA; Megan S. Rice, ScD; Nikhil Amin, MD; Marius Ardeleanu, MD; Allen Radin, MD; Brad Shumel, MD; Marcella Ruddy, MD; Naimish Patel, MD; Gianluca Pirozzi, MD; Leda Mannent, MD; and Neil M.H. Graham, MD

This appendix has been provided by the authors to give readers additional information about their work.

APPENDICES

Appendix S1. Baseline demographics and history of atopic disease in patients with atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyps, and eosinophilic esophagitis	3
Appendix S2. Mean biomarkers at baseline and end of treatment for patients with atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyps, and eosinophilic esophagitis	6
Appendix S3. The effect of dupilumab on mean ($\pm$ SE) concentrations of blood eosinophils (Giga/L)	11

APPENDIX S1 Baseline demographics and history of atopic disease in patients with atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyps, and eosinophilic esophagitis

Type 2 disease	Atopic dermatitis		Asthma		Chronic rhinosinusitis with nasal polyps		Atopic dermatitis		Eosinophilic esophagitis	
Study name	CHRONOS		QUEST		SINUS-52		SOLO-1 and SOLO-2		EoE PoC	
Study duration	52 weeks		52 weeks		52 weeks		16 weeks		12 weeks	
Treatment group	Placebo	Dupilumab	Placebo	Dupilumab	Placebo	Dupilumab	Placebo	Dupilumab	Placebo	Dupilumab
	qw +	300 mg	2mL q2w +	300 mg q2w +	q2w	300 mg	qw, n =	300 mg	qw, n =	300 mg
	TCS, n =	q2w +	ICS/LABA	ICS/LABA	+ INCS, n	q2w +	460	q2w, n =	24	qw, n =
	315	TCS, n =	n = 321	n = 633	= 153	INCS, n =		457		23
		106				150				
Baseline demographics										
Age, mean (SD), years	36.6 (13.0)	39.6 (14.0)	48.2 (14.7)	47.7 (15.6)	51.7 (12.7)	51.9 (11.9)	38.4 (14.0)	38.3 (14.4)	36.1 (12.8)	33.1 (8.7)
Male, n (%)	193 (61.3)	62 (58.5)	103 (32.1)	239 (37.8)	95 (62.1)	97 (64.7)	250 (54.3)	267 (58.4)	10 (41.7)	13 (56.5)

Female, n (%)	122 (38.7)	44 (41.5)	218 (67.9)	394 (62.2)	58 (37.9)	53 (35.3)	210 (45.7)	190 (41.6)	14 (58.3)	10 (43.5)	
Current history of comorbid type 2 immune diseases, ^a n (%)											
Allergies (other than food allergy)	201 (63.8)	68 (61.8)							9 (37.5)	14 (60.9)	
Allergic conjunctivitis (kerato-conjunctivitis)	68 (21.6)	31 (28.2)	49 (15.3)	89 (14.1)	10 (6.5)	6 (4.0)			3 (12.5)	3 (13.0)	
Allergic rhinitis	134 (42.5)	53 (48.2)	225 (70.1)	438 (69.2)	74 (48.4)	72 (48.0)	224 (48.7)	224 (49.0)	14 (58.3)	16 (69.6)	
Asthma	130 (41.3)	45 (40.9)				91 (59.5)	85 (56.7)	205 (44.6)	226 (49.5)	8 (33.3)	8 (34.8)
Atopic dermatitis	310 (98.4)	108 (98.2)	38 (11.8)	62 (9.8)	10 (6.5)	4 (2.7)	—	—	4 (16.7)	3 (13.0)	
Chronic rhinosinusitis	26 (8.3)	7 (6.4)	63 (19.6)	113 (17.9)	—	—			8 (33.3)	2 (8.7)	
Eosinophilic esophagitis	0	1 (0.9)	2 (0.6)	0	0	1 (0.7)			—	—	
Food allergy	96 (30.5)	39 (35.5)	36 (11.2)	47 (7.4)	16 (10.5)	8 (5.3)	174 (37.8)	177 (38.7)	16 (66.7)	14 (60.9)	

Hives	34 (10.8)	14 (12.7)	15 (4.7)	30 (4.7)	6 (3.9)	3 (2.0)			5 (20.8)	6 (26.1)
Nasal polyps	7 (2.2)	2 (1.8)	40 (12.5)	81 (12.8)	–	–	18 (3.9)	19 (4.2)	2 (8.3)	1 (4.3)

Note: SOLO: full analysis set; CHRONOS and EoE PoC: safety analysis set; QUEST and SINUS-52: randomized population. Gray cells indicate that data were not collected or available for these patients.

Abbreviations: EoE, eosinophilic esophagitis; ICS, inhaled corticosteroids; INCS, intranasal corticosteroids; LABA, long-acting β_2 -agonist; n, number; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SD, standard deviation; TCS, topical corticosteroids.

^aFor asthma, this refers to a medical history.

APPENDIX S2 Mean biomarkers at baseline and end of treatment for patients with atopic dermatitis, asthma, chronic rhinosinusitis with nasal polyps, and eosinophilic esophagitis

Type 2 disease	Atopic dermatitis		Asthma		Chronic rhinosinusitis with nasal polyps		Atopic dermatitis		Eosinophilic esophagitis	
Study name	CHRONOS		QUEST		SINUS-52		SOLO-1 and SOLO-2		EoE PoC	
Study duration	52 weeks		52 weeks		52 weeks		16 weeks		12 weeks	
Treatment group	Placebo qw + TCS	Dupilumab 300 mg q2w + TCS	Placebo 2mL q2w + ICS/LABA	Dupilumab 300 mg q2w + ICS/LABA	Placebo q2w + INCS	Dupilumab 300 mg q2w + INCS	Placebo qw	Dupilumab 300 mg q2w	Placebo qw	Dupilumab 300 mg qw
Serum TARC (pg/mL)										
n	313	110	319	622	149	147	454	464	24	22
Mean (SE) at baseline	7291.72 (738.03)	9678.78 (1828.09)	398.00 (19.27)	364.66 (11.84)	370.20 (21.03)	372.27 (20.94)	5933.28 (487.89)	6864.12 (703.81)	350.83 (43.43)	275.54 (27.82)
n	120	84	248	479	127	139	194	361	19	22
Mean (SE) at end of treatment	1960.14 (377.87)	479.11 (43.13)	520.36 (143.08)	234.72 (7.95)	332.49 (20.13)	219.43 (10.77)	3239.13 (738.27)	572.33 (38.71)	322.63 (43.09)	203.38 (22.29)

n	119	84	246	473	126	137	193	360	19	21
Mean change (SE) from baseline at end of treatment	-2450.77 (592.10)	-10341.70 (2315.52)	137.66 (137.49)	-122.78 (10.02)	-15.09 (11.67)	-158.53 (14.93)	-1043.61 (446.81)	-5370.36 (700.55)	-10.11 (24.98)	-71.23 (17.90)
n	119	84	246	473	126	137	193	360	19	21
Mean % change (SE) from baseline at end of treatment	-29.14 (11.50)	-78.37 (3.97)	25.90 (9.43)	-22.74 (2.31)	1.28 (2.99)	-36.16 (1.91)	10.22 (9.49)	-69.80 (1.71)	0.14 (6.70)	-21.06 (5.73)

Plasma eotaxin-3 (pg/mL)										
n			318	625	150	146				
Mean (SE) at baseline			50.90 (3.25)	69.53 (19.26)	90.84 (9.08)	70.61 (3.74)				
n			246	473	127	139				
Mean (SE) at end of treatment			50.46 (2.89)	29.81 (1.73)	82.62 (8.13)	30.40 (1.28)				
n			243	469	127	136				

Mean change (SE) from baseline at end of treatment			0.28 (2.76)	-48.00 (24.33)	1.40 (4.75)	-39.95 (3.60)				
n			243	469	127	136				
Mean % change (SE) from baseline at end of treatment			57.13 (20.38)	-20.95 (5.39)	14.88 (5.42)	-39.10 (7.01)				

Serum total IgE (IU/mL)										
n	312	110	318	626	150	148	456	465	24	23
Mean (SE) at baseline	5082.55 (249.43)	5653.27 (423.00)	448.47 (44.67)	415.05 (28.03)	228.59 (21.94)	211.79 (21.16)	6999.88 (481.27)	6715.38 (485.50)	478.46 (198.56)	204.17 (66.31)
n	117	83	251	482	128	140	196	366	19	23
Mean (SE) at end of treatment	4203.74 (390.15)	2132.49 (331.55)	432.44 (49.46)	135.61 (14.64)	292.03 (46.24)	54.64 (5.78)	5676.28 (649.71)	3444.88 (295.04)	423.05 (169.52)	137.09 (40.04)
n	116	83	248	478	128	139	196	366	19	23

Mean change (SE) from baseline at end of treatment	-118.83 (219.48)	-3797.40 (399.55)	-4.03 (20.45)	-294.80 (23.76)	64.09 (32.08)	-156.92 (17.93)	366.67 (243.19)	-3027.83 (277.28)	-74.84 (76.03)	-67.09 (27.11)
n	116	83	248	478	128	139	196	366	19	23
Mean % change (SE) from baseline at end of treatment	68.42 (52.90)	-66.25 (3.17)	9.89 (4.33)	-58.43 (7.24)	20.82 (5.72)	-67.28 (1.63)	22.74 (4.25)	-41.90 (2.71)	4.9 (5.1)	-25.3 (2.8)

Serum periostin (ng/mL)								
n		319	629	150	147		24	22
Mean (SE)		80.94	78.42	113.00	109.70		62.16	61.18
at baseline		(2.28)	(1.51)	(4.03)	(4.06)		(6.20)	(3.90)
n		253	490	120	128		18	23
Mean (SE)		75.52	59.82	108.11	66.06		73.18	52.67
at end of treatment		(2.36)	(1.13)	(5.06)	(2.31)		(7.19)	(3.04)
n		251	488	120	127		18	22

Mean change (SE) from baseline at end of treatment			-5.05 (1.76)	-18.90 (1.40)	-4.73 (3.52)	-45.42 (3.91)			9.91 (5.20)	-8.25 (3.23)
n			251	488	120	127			18	22
Mean % change (SE) from baseline at end of treatment			-2.37 (1.88)	-17.80 (1.11)	0.15 (3.66)	-33.93 (2.64)			32.85 (19.31)	-8.22 (6.95)

Blood eosinophils (Giga/L)										
n	314	110	320	632	150	148	454	465	24	23
Mean (SE) at baseline	0.55 (0.03)	0.58 (0.06)	0.39 (0.02)	0.35 (0.02)	0.45 (0.03)	0.45 (0.03)	0.62 (0.03)	0.60 (0.03)	0.43 (0.06)	0.31 (0.04)
n	120	83	245	476	129	140	195	360	21	22
Mean (SE) at end of treatment	0.34 (0.02)	0.40 (0.04)	0.35 (0.02)	0.33 (0.02)	0.44 (0.04)	0.37 (0.03)	0.45 (0.04)	0.57 (0.04)	0.43 (0.07)	0.17 (0.02)
n	119	83	245	476	129	139	194	360	21	22

Mean change (SE) from baseline at end of treatment	-0.17 (0.03)	-0.20 (0.05)	-0.05 (0.02)	-0.01 (0.02)	0 (0.03)	-0.08 (0.04)	-0.07 (0.03)	0 (0.03)	0 (0.08)	-0.14 (0.03)
n	119	83	242	469	128	139	190	346	20	21
Mean % change (SE) from baseline at end of treatment	-10.02 (6.69)	-19.92 (7.07)	33.60 (13.26)	43.76 (13.93)	12.88 (8.85)	24.12 (18.25)	-1.49 (7.14)	12.89 (5.91)	-8.06 (11.94)	-43.71 (6.11)

Note: Gray cells indicate that data were not collected or available for these patients.

Abbreviations: EoE, eosinophilic esophagitis; IU, international units; ICS, inhaled corticosteroids; INCS, intranasal corticosteroids; LABA, long-acting β_2 -agonist; n, number; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SE, standard error; TARC, thymus and activation-regulated chemokine; TCS, topical corticosteroids.

APPENDIX S3 The effect of dupilumab on mean ($\pm$ SE) concentrations of blood eosinophils (Giga/L). AD, atopic dermatitis; CRSwNP, chronic rhinosinusitis with nasal polyps; EoE, eosinophilic esophagitis; PoC, proof of concept; qw, weekly; q2w, every 2 weeks; SE, standard error.

