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An airway epithelial IL-17A response signature identifies a steroid-unresponsive COPD patient subgroup

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BACKGROUND. Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous smoking-related disease characterized by airway obstruction and inflammation. This inflammation may persist even after smoking cessation and responds variably to corticosteroids. Personalizing treatment to biologically similar “molecular phenotypes” may improve therapeutic efficacy in COPD. IL-17A is involved in neutrophilic inflammation and corticosteroid resistance, and thus may be particularly important in a COPD molecular phenotype.

METHODS. We generated a gene expression signature of IL-17A response in bronchial airway epithelial brushings (“BAE”) from smokers with and without COPD (n = 238), and validated it using data from two randomized trials of IL-17 blockade in psoriasis. This IL-17 signature was related to clinical and pathologic characteristics in two additional human studies of COPD: (1) SPIROMICS (n = 47), which included former and current smokers with COPD, and (2) GLUCOLD (n = 79), in which COPD participants were randomized to placebo or corticosteroids.

RESULTS. The IL-17 signature was associated with an inflammatory profile characteristic of an IL-17 response, including increased airway neutrophils and macrophages. In SPIROMICS the signature was associated with increased airway obstruction and functional small airway disease on quantitative chest CT. In GLUCOLD the signature was associated with decreased response to corticosteroids, irrespective of airway eosinophilic or Type 2 inflammation.

CONCLUSION. These data suggest that a gene signature of IL-17 airway epithelial response […]

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53 **Abstract**

54

55 **Background**: Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous smoking-
56 related disease characterized by airway obstruction and inflammation. This inflammation may
57 persist even after smoking cessation and responds variably to corticosteroids. Personalizing
58 treatment to biologically similar "molecular phenotypes" may improve therapeutic efficacy in
59 COPD. IL-17A is involved in neutrophilic inflammation and corticosteroid resistance, and thus
60 may be particularly important in a COPD molecular phenotype.

61 **Methods**: We generated a gene expression signature of IL-17A response in bronchial airway
62 epithelial brushings ("BAE") from smokers with and without COPD (n=238), and validated it
63 using data from two randomized trials of IL-17 blockade in psoriasis. This IL-17 signature was
64 related to clinical and pathologic characteristics in two additional human studies of COPD: (1)
65 SPIROMICS (n=47), which included former and current smokers with COPD, and (2) GLUCOLD
66 (n=79), in which COPD participants were randomized to placebo or corticosteroids.

67 **Results**: The IL-17 signature was associated with an inflammatory profile characteristic of an IL-
68 17 response, including increased airway neutrophils and macrophages. In SPIROMICS the
69 signature was associated with increased airway obstruction and functional small airway disease
70 on quantitative chest CT. In GLUCOLD the signature was associated with decreased response
71 to corticosteroids, irrespective of airway eosinophilic or Type 2 inflammation.

72 **Conclusion**: These data suggest that a gene signature of IL-17 airway epithelial response
73 distinguishes a biologically, radiographically, and clinically distinct COPD subgroup that may
74 benefit from personalized therapy.

75 **Trial Registration:** ClinicalTrials.gov NCT01969344

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77

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Brief Summary (25 words): A COPD subgroup displays an enhanced IL-17A airway epithelial response associated with increased airway obstruction, neutrophilic inflammation, and a poor response to corticosteroids.

101 **Introduction**

102 Personalizing treatment to "molecular phenotypes", *i.e.* to subsets of patients with shared
103 underlying biology, is an emerging strategy to guide therapeutic choices in chronic disease (1,
104 2). In respiratory disease, this strategy has particularly gained traction in severe asthma where
105 subgroups of patients with Type 2 and eosinophilic inflammation can be targeted using new
106 biologics (1-4). Much of the inflammation in chronic respiratory disorders, however, does not
107 respond to therapies directed against Type 2 inflammation. Identifying subgroups that display
108 enhanced non-Type 2 inflammatory pathways may lead to the repurposing of available biologics
109 indicated for other inflammatory disorders to target these subgroups.
110
111 Chronic obstructive pulmonary disease (COPD) is a highly prevalent respiratory disease, most
112 commonly associated with smoking. COPD is a major cause of morbidity and mortality
113 worldwide for which few interventions have been found that prevent disease progression (5).
114 Yet, molecular phenotyping has been less studied in COPD than in asthma and has focused on
115 eosinophilic and type 2 inflammation based on the previous work in asthma (2). Type 2
116 inflammation is likely relevant in only a minority of COPD patients (6). Nonetheless, this work
117 suggests that biologically distinct COPD subgroups exist and are clinically relevant. COPD
118 patients with high eosinophil counts or an airway epithelial genomic signature of Type 2
119 inflammation are more likely to respond to corticosteroids, and potentially to biologics targeting
120 eosinophils (6-9). These studies suggest the promise of molecular phenotyping in COPD, but
121 responses beyond Type 2 inflammation require further investigation.
122
123 The IL-17 family of cytokines includes six members that play various roles in mucosal host
124 defense and chronic inflammation (10). IL-17A stimulates the airway epithelium to produce
125 chemokines and other mediators which recruit and activate neutrophils and macrophages, cells

126 crucial to COPD pathogenesis (11). IL-17A is implicated in COPD-associated pathogenic
127 responses, including emphysema, lymphoid neogenesis, corticosteroid resistance, dysbiosis,
128 mucus hypersecretion, and ongoing inflammation despite smoking cessation (12-19). However,
129 many of these responses have not been investigated in human studies. By identifying the
130 COPD subgroup that manifests IL-17A associated inflammation (hereafter referred to as IL-17),
131 we hypothesize that we can distinguish a corticosteroid-unresponsive subgroup that may benefit
132 from anti-IL17 biologics. Anti-IL-17 biologics are now approved for the treatment of autoimmune
133 diseases, specifically psoriasis and psoriatic arthritis, and are being studied in COPD (20, 21).
134 Non-targeted trials of biologic therapies in COPD have failed to meet clinical endpoints,
135 suggesting the importance of directing therapy to appropriate subgroups (22).
136
137 Here we studied the transcriptional response of the airway epithelium to IL-17. We have found
138 that direct measurement of interleukin proteins, including IL-17A, can be difficult in human blood
139 and bronchoalveolar lavage fluid (23, 24). These challenges may contribute to the inconsistent
140 evidence for IL-17A protein levels being increased in COPD (25-30). Conversely, airway
141 epithelial cells have reproducible transcriptional responses to many interleukins. Thus our
142 general strategy has been to assay this epithelial response, which we have validated for IL-13
143 (24, 31, 32) and interferons (33).
144
145 We examined a genomic signature of the airway epithelial IL-17 response in three separate
146 human COPD studies in which bronchial airway samples were collected during bronchoscopy.
147 We first fit the IL-17 genomic signature, generated using bronchial epithelial cells exposed to IL-
148 17, to a cross-sectional study of ever-smokers with and without COPD (Bronchial Airway
149 Epithelial (BAE) dataset, n=237). We next established that the signature specifically identified
150 IL-17 associated inflammation by determining its response to other airway epithelial adaptive
151 immune responses (type 1 and 2) and to IL-17-directed biologic therapies in psoriatic skin

152 lesions. We then tested the associations between this IL-17 signature and clinical features in
153 two independent COPD studies which collected rich phenotypic data (GLUCOLD: n= 79 and
154 SPIROMICS: n=47, Study design in **Figure 1**). We hypothesized that our airway epithelial IL-17
155 genomic signature would be increased in a COPD subset, and associated with distinct clinical,
156 pathologic and radiographic characteristics.
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178 **Results**

179 *Generation of an airway epithelial IL-17 associated gene expression signature in COPD*

180 We first characterized the airway epithelial response to IL-17 using whole transcriptome profiling
181 of IL-17 versus vehicle control-stimulated human bronchial epithelial cell (HBEC) cultures grown
182 at air liquid interface (ALI). The 100 genes most upregulated by $\log_2$ fold change in response to
183 IL-17 were studied as candidate IL-17 signature genes.

184

185 We examined these 100 genes in a previously generated bronchial airway epithelial
186 transcriptome profiling dataset derived from bronchoscopic brushing samples from ever-
187 smokers with (n=85) and without COPD (n=152, Bronchial Airway Epithelial (BAE) dataset,
188 demographics in **Table 1**). Candidate IL-17 signature genes were enriched amongst smokers
189 with COPD compared to those without (mean of the zero-centered $\log_2$ gene expression in
190 those with COPD=0.11 (±0.27) versus without= -0.60 (±0.19), $p=6.10*10^{-6}$, **Figure S1**).

191

192 Next, we generated a genomic signature of the IL-17 response specific to in vivo brushing
193 samples from smokers by restricting the 100 gene signature identified in the culture model to
194 those tightly correlated in the BAE dataset using an elastic net (34, 35). We took this additional
195 step because cell culture models cannot optimally reproduce the complexity of the in vivo
196 environment in which multiple mechanistic pathways impact gene expression, often with
197 disparate affect. This signature refinement process is based on the premise that highly inter-
198 correlated genes are co-regulated by the same molecular processes, a premise also used by
199 pathway analysis tools such as weighted gene co-expression analysis (36, 37). Starting with the
200 100 candidate genes as predictors, elastic net regression with leave-one-out cross validation
201 selected 10 genes highly correlated with a representative IL-17 related gene, *CCL20,* in the
202 BAE dataset (**Figure 2**). We chose *CCL20 a priori* to guide the elastic net gene selection to

203 specifically identify an IL-17/*CCL20*-associated response. *CCL20* was chosen for this role
204 based on: 1) biological relevance, as an epithelial gene known to be the only ligand for CCR6, a
205 chemokine receptor preferentially expressed by Th17 cells, and thus thought to be more specific
206 for an IL-17 response as compared to other adaptive immune responses (38), and 2) statistical
207 relevance, because it was highly upregulated (log_2 fold change=2.92, FDR=0.0006) following IL-
208 17 stimulation in vitro. Importantly, this IL-17 associated gene was chosen to guide gene
209 selection as our goal was to retain co-associated genes due to their potential biologic relevance,
210 independent from outcomes of interest. We confirmed that the 10 genes selected by elastic net
211 and *CCL20* were all inter-correlated (**Figure S2**), verifying that the elastic net procedure
212 removed loosely correlated genes. Nearly all of the 10 genes have previously been shown to be
213 associated with IL-17 related inflammation (38-42). We thus used these 10 genes, along with
214 *CCL20,* to construct a gene signature of airway epithelial response to IL-17 using the mean of
215 their zero-centered log_2 expression values.
216
217 We confirmed that the genes selected for the signature are measuring an IL-17 response not
218 just specific to *CCL20* in two ways. First, we evaluated the correlation between our IL-17
219 signature and a 5 gene airway epithelial IL-17 gene expression metric previously examined in
220 asthma (39). In the BAE dataset the two signatures were well-correlated ($\rho=0.51$, $p<2.2*10^{-16}$) in
221 COPD participants, **Figure S3, Table S1**). The signatures were also correlated in an additional
222 COPD dataset, GLUCOLD (demographics in **Table 1**), in which transcriptomic profiles from
223 endobronchial biopsies were obtained from 79 participants with COPD ($\rho=0.49$, $p=5.0*10^{-6}$,
224 **Figure S3, Table S1**). Second, we repeated the elastic net procedure using *SLC26A4*, the gene
225 most upregulated with IL-17 stimulation in cell culture also measured in the COPD array data
226 (log_2 fold change=8.51, FDR=0), to guide the elastic net. The *SLC26A4*-based signature
227 incorporated 16 genes, 6 of which were also in the 11 gene *CCL20*-based signature, and was
228 highly correlated with the *CCL20*-based signature in the BAE and GLUCOLD datasets ($\rho=0.97$

229 and $p<2.2*10^{-16}$, ρ =0.87 and $p<2.2*10^{-16}$, respectively, **Figure S4, Table S1**). Thus, removal of
230 loosely associated genes from the 100 gene signature using *CCL20* to guide the elastic net
231 measured a response that does not appear to be exclusive to *CCL20*. However, we used the
232 *CCL20*-based signature for our subsequent analyses as it had clear advantages over the
233 others. The asthma signature was generated in a cell culture model and never fit to the in vivo
234 environment. *SLC26A4 is of* unclear significance in IL-17 biology, and thus we considered the
235 *CCL20*-based signature more biologically relevant.
236
237 *Validation of the IL-17 signature*
238 *IL-17 related gene expression confirmed in an additional Airway Epithelial Culture Dataset*
239 We validated the association between the 10 genes selected by elastic net and IL-17 stimulation
240 in another publicly available microarray dataset of HBECs grown at ALI and stimulated with IL-
241 17 for 24 hours (as opposed to the 7 day stimulation in our culture model) (GSE10240) (43).
242 Although two of the 10 genes (*SAA1* and *SAA2*) were poorly annotated on this array and could
243 not be measured, the rest were significantly upregulated after IL-17 stimulation in this validation
244 dataset (7 of 8 were within the top 50 genes by $\log_2$ fold change) despite differences in cytokine
245 stimulation time.
246
247 *IL-17 related gene expression measures a response distinct from Type 1 and 2 immune*
248 *responses*
249 Only three of the 11 IL-17 signature genes were significantly altered after HBECs at ALI were
250 stimulated with interferon gamma, the main cytokine released from Th1 and Tc1 cells, and thus
251 indicative of a Type 1 (T1) response. Two of the genes were repressed and one induced with an
252 overall mean $\log_2$ fold change of -0.19 (**Table S2**). None of the genes were significantly
253 upregulated in steroid-naïve mild-moderate asthmatics previously shown to have high type 2

254 (T2) gene expression (n=40) compared to asthmatics with low T2 expression (n=22) and
255 healthy controls (n=43) (**Table S3)**.
256
257 *Decreased IL-17 signature expression following IL-17 blockade in psoriatic lesions*
258 To further validate that our IL-17 signature reflects an IL-17 response, we examined it in two
259 publicly available transcriptomic datasets of psoriatic skin lesions before and after controlled
260 treatment with anti-IL-17 biologics.
261
262 In the first dataset (GSE31652) (44), psoriatic skin lesion biopsies were taken at baseline and
263 after two weeks of Ixekizumab, an anti-IL-17 monoclonal antibody (n=6), or placebo (n=4). All
264 Ixekizumab-treated participants, but none of the placebo-treated, showed clinical improvement
265 of at least 75% at 6 weeks. The skin IL-17 gene signature decreased over 2 weeks in lesions
266 from Ixekizumab but not placebo-treated participants (p=0.003 for the interaction between
267 treatment and time, **Figure 3A and B**).
268
269 In the second dataset (GSE53552) (45), biopsies were taken from psoriatic skin lesions and
270 matched non-lesional skin at baseline (n=25). Psoriatic lesions were then sampled over 6
271 weeks after treatment with placebo (n=5) or a dose range of Brodalumab (n=20), an IL-17
272 receptor α-blocking monoclonal antibody. Psoriatic lesions showed higher IL-17 signature
273 expression compared to matched non-lesional skin (p=0.001, **Figure 3C-F**). The signature
274 decreased over time in psoriatic lesions in those who received 350 or 700mg compared to
275 placebo, but not in those who received 140mg (350mg: p=0.005 at 1 week, p=0.02 at 2 weeks,
276 and p=0.12 at 6 weeks, 700mg: p=0.002 at 2 weeks and 0.0006 at 6 weeks for the interaction
277 between treatment and time, **Figure 3C-F**). This was consistent with clinical treatment response
278 (all placebo-treated and three of four 140mg-treated participants showed no clinical treatment
279 response, all 700mg-treated and all but one 350mg-treated showed at least 70% clinical

280 improvement). The observation that our putative IL-17 signature tracked with clinical response
281 to an IL-17 inhibitor in two psoriasis clinical trials provides independent confirmation of its value
282 as a metric of IL-17 driven inflammation
283
284 *Characterization of the IL-17 signature in COPD transcriptional profiling datasets*

285 *Cross-sectional characterization of an IL-17 gene signature in the BAE dataset*
286 In the BAE dataset, our 11 gene IL-17 signature was higher in former smokers (mean of the
287 zero-centered log_2 gene expression=0.29±0.46) compared to current smokers (-0.42±0.48,
288 $p<2.2*10^{-16}$, **Figure 4A Table S4**), and associated with older age (ρ=0.19, p=0.004). The
289 signature was increased in COPD compared to ever-smokers without COPD (i.e. those with
290 preserved lung function, 0.21±0.66 and -0.12±0.51 respectively, $p=1.34*10^{-5}$), even after
291 adjustment for smoking status and age ($p=6.2*10^{-6}$). The signature was also higher with
292 decreasing lung function (defined as the volume of air exhaled in the first second of a forced
293 expiratory maneuver, or FEV_1). Specifically, a higher gene signature was associated with lower
294 FEV_1 expressed as a percentage of the predicted value (FEV_1% predicted) across all
295 participants (1 unit increase in the IL-17 signature is associated with a 12 ml decrease in FEV_1,
296 $p=1.40*10^{-5}$) and amongst only COPD participants (associated with a 5.5 ml decrease in FEV_1,
297 p= 0.04), suggesting an association with increasing COPD severity (**Figure 4B**).
298
299 *Cross-sectional characterization in GLUCOLD and SPIROMICS*
300 We next studied baseline clinical characteristics associated with the IL-17 signature in
301 GLUCOLD and another COPD dataset, SPIROMICS (demographics in **Table 1**). GLUCOLD
302 included endobronchial biopsy transcriptomic profiles from steroid-naïve participants with
303 moderate to severe COPD (n=79). SPIROMICS included bronchial epithelial brushing profiles
304 from ever smokers with mild to moderate COPD (n=47). Similar to the BAE dataset, in both

305 GLUCOLD and SPIROMICS the IL-17 signature was associated with increasing age (ρ=0.24,
306 p=0.039 and ρ=0.20, p=0.046, respectively) and was higher in former compared to current
307 smokers ($p=2.42*10^{-6}$ and $1.35*10^{-5}$ respectively, **Table S4**). We performed subsequent
308 analyses before and after adjustment for age and smoking status.
309

310 *Association with increased airway neutrophils and macrophages*

311 In GLUCOLD, the IL-17 signature was associated with increased airway biopsy neutrophil
312 ($p=6.41*10^{-5}$, **Figure 5A**) and macrophage counts (p=0.009, **Figure 5B**), but not eosinophils,
313 mast cell counts, or our previously described T2 genomic score (**Table 2**). Tissue cell counts
314 and the T2S score were not measured in SPIROMICS, but the T2S score was also not
315 associated with the IL-17 signature in the BAE dataset (**Table 2**). The IL-17 signature was
316 moderately associated with sputum neutrophil counts in both GLUCOLD (p=0.041, **Figure 5C**)
317 and SPIROMICS (p=0.033, **Figure 5D**) although this did not stand up to multiple comparisons
318 adjustment. There was no association with sputum eosinophil counts or any blood cell counts
319 (**Table 2**).
320

321 *Association with airway obstruction*

322 Similar to the BAE dataset, in SPIROMICS we found that a higher IL-17 signature was
323 associated with slightly greater airway obstruction in COPD (p=0.038 after adjustment for
324 smoking and age, **Figure S5, Table 3**), although this was not significant after adjustment for
325 multiple comparisons. In GLUCOLD we found a trend towards an association (p=0.06 before
326 and p=0.12 after adjustment for smoking and age, **Figure S5, Table 3).**
327

328 *Association with CT measurements of functional small airway disease*

329 In SPIROMICS, we obtained inspiratory and expiratory quantitative Chest CT scans at study
330 entry. We found that the IL-17 signature was associated with an increase in air-trapping in areas

331 devoid of emphysema (known as functional small airways disease (PRM^{fsad}) by parametric
332 response mapping (PRM) analysis (p=0.01, **Figure 6A**, **Table 3**) (46).The IL-17 signature was
333 not associated with PRM-measured emphysema (PRM^{emph}). However, almost all participants
334 who underwent bronchoscopy had mild disease with very few displaying significant emphysema
335 (**Figure 6B**).

336

337 *Association with decreased response to inhaled corticosteroids in GLUCOLD*

338 Following baseline bronchoscopy in GLUCOLD, 49 participants with available baseline biopsies
339 were randomized to treatment with 30 months of ICS-containing medication (n=33) or placebo
340 (n=16). A higher baseline IL-17 signature was associated with lack of improvement in post-
341 bronchodilator FEV_1 on ICS, whereas a lower IL-17 signature was associated with improvement
342 in FEV_1, as compared to placebo (p=0.028 for the interaction between treatment and time,
343 **Figure 7, Table 3**). We identified 28% of GLUCOLD participants as having high IL-17 gene
344 expression ("IL-17 high") by cluster partitioning (31% of COPD participants over all three
345 studies, including 33% of BAE and 34% of SPIROMICS participants, were "IL-17 high", **Figure**
346 **S6**). After categorization of participants based on this cluster partitioning, those with an "IL-17
347 low" designation were more likely to respond to ICS with an improvement in lung function while
348 "IL-17 high" was associated with lack of response to ICS at 30 months (p=0.047 for the
349 interaction between IL-17 status and percent change in FEV_1 after ICS compared to placebo).
350 We found that a high IL-17 signature is specific but not sensitive for steroid unresponsiveness.
351 Using the dichotimization into IL-17 high and low by cluster partitioning the specificity for steroid
352 unresponsiveness was 75% (**Table S5**). When the IL-17 high group is restricted to a slightly
353 higher cut-off at the top quartile of IL-17 signature values, the specificity increases to 94%
354 (**Table S6**).

355

356 The association between the IL-17 signature and change in FEV_1 amongst ICS-treated
357 participants was not due to those participants with low IL-17 signature expression reciprocally
358 exhibiting high Type 2 inflammation. The significance of the relationship between the IL-17
359 signature and ICS response persisted even after we adjusted for markers of steroid-responsive
360 Type 2 inflammation using either airway tissue eosinophils (p=0.027) or our previously identified
361 airway epithelial genomic signature of Type 2 inflammation (p=0.018, **Figure S7, Table 3**) (6).
362 The association also does not appear to be explained by IL-17 inflammation simply reflecting
363 tissue neutrophils or macrophages as adjustment for neutrophil or macrophage counts in the
364 model also did not change the relationship between the IL-17 signature and ICS response
365 (p=0.016 and 0.030, respectively, **Table 3**).
366
367 The IL-17 signature alone explained 23% of the variation in change in FEV_1 with corticosteroids
368 (r^2=0.23, **Table 3**). As expected given the low sensitivity of the IL-17 signature for steroid
369 unresponsiveness, the Area Under the Receiver Operator Characteristic Curve (AUC) was
370 modest (63%, **Figure S8**). However, there were no significant associations between other
371 biomarkers of inflammation (including sputum and blood cell counts) and change in FEV_1 in ICS
372 versus placebo-treated participants after adjustment for age and smoking status. Furthermore,
373 the AUCs for these other potential biomarkers (sputum eosinophils: 51%, blood eosinophils:
374 55%, sputum neutrophils: 52%, blood neutrophils: 45%) suggest that they lack any predictive
375 power for corticosteroid responsiveness in this dataset (supplemental **Figure S8**). Although
376 limited by small sample size, these proof-of-concept analyses suggest that our airway epithelial
377 signature of IL-17 response in COPD may mark FEV_1 response to ICS better than easily
378 measured cell differentials or other genomic markers of the adaptive immune response.
379
380
381

382 **Discussion**

383 In this study, we used three complementary human COPD studies to characterize the clinical
384 significance of the airway epithelial response to IL-17 in COPD. We showed that a signature of
385 IL-17 associated airway inflammation is upregulated in a subset of participants with COPD (31%
386 across studies), and is associated with distinct inflammatory, physiologic, and clinical features.
387 Increases in this signature are associated with an inflammatory profile characteristic of an IL-17
388 response, including increased airway neutrophils and macrophages but not eosinophils, Type 2
389 markers, or Type 1 gene expression. Decreases in the signature occur in response to
390 therapeutic blockade of IL-17 in psoriatic skin lesions, and this response corresponds to clinical
391 improvement in that disease. In COPD, the signature is further associated with more severe
392 airway obstruction and a novel CT biomarker of functional small airway disease that is predictive
393 of worsening airway disease over time (46, 47). Finally, higher IL-17 signature expression is
394 associated with a lack of response to ICS in COPD, whereas low expression may identify those
395 patients who benefit from ICS. This association does not simply appear to be due to reciprocal
396 alterations in Type 2 inflammation as the interaction between our IL-17 signature and treatment
397 was unaffected by adjustments for airway eosinophils or our Type 2 airway genomic signature.
398 Thus, our findings suggest that enhanced IL-17 inflammation characterizes a distinct subset of
399 COPD, and that identifying this subgroup may be important for therapeutic decisions.
400
401 In COPD, chronic exposure to smoking, microbial insults, and recurrent mucosal injury may all
402 contribute to immune activation with IL-17A producing T cells, supported by innate IL-17A
403 producing cells (17). This likely contributes to ongoing neutrophilic inflammation and
404 macrophage recruitment with subsequent airway remodeling and tissue destruction (48). We
405 found that our IL-17 gene expression signature is associated with increases in airway
406 neutrophils and macrophages, indicating an IL-17 response. It is related to worse clinical

407 outcomes across former and current smokers. These findings provide evidence for the
408 contribution of IL-17 inflammation to COPD pathology despite smoking cessation.
409
410 We found that the IL-17 response in COPD is heterogeneous, enhanced in a subgroup. Prior
411 studies found variability in IL-17-related inflammation within COPD (13, 25-30), and our data
412 suggest that this variability is clinically significant. Other studies have identified some
413 characteristics of IL-17 associated inflammation in COPD including more severe obstruction,
414 emphysema, and lymphoid neogenesis (13, 15). Here we comprehensively investigated the
415 associations between IL-17 driven inflammation and COPD patient characteristics. In addition to
416 an association with increased airway obstruction, we found associations with a novel CT
417 biomarker of functional small airways disease and corticosteroid unresponsiveness. COPD
418 phenotypes are heterogeneous and complex. Thus we hypothesize that multiple overlapping
419 molecular phenotypes underlie the complex clinical phenotypes we observe in chronic airway
420 diseases and that there will be an upper bound to the predictive power of any one biological
421 pathway (33, 49). However, a strength here is that we observe correlations that are reproducible
422 across our transcriptional datasets (for associations with neutrophils and FEV_1).
423
424 We had hypothesized that the IL-17 signature would be associated with increased emphysema,
425 as found in a previous study (13). We evaluated this using the recently developed PRM CT
426 analysis method (46). By matching inspiratory and expiratory scans, PRM improves the ability to
427 distinguish emphysema from functional small airway disease, both of which are associated with
428 low radio-density lung regions on expiration (i.e. air trapping). Our IL-17 signature is associated
429 with PRM^{fsad} but not PRM^{emph} in SPIROMICS. As the participants generally had mild to
430 moderate disease with minimal emphysema, the lack of association with PRM^{emph} is not
431 surprising. The association with PRM^{fsad} is of interest as the small airways are likely the main
432 site of airway inflammation in COPD, and small airway disease is thought to precede

433 emphysema (50). Studies using PRM have supported these findings. PRM^{fsad} is associated with
434 more rapid FEV_1 decline, particularly in mild to moderate disease (47). PRM^{fsad} is also the
435 greater contributor to radiographic abnormalities in mild to moderate COPD with both PRM^{fsad}
436 and PRM^{emph} contributing in severe disease (46). Thus, an association between our IL-17
437 signature and PRM^{fsad} in mild to moderate COPD does not preclude an association with
438 emphysema in more severe disease. In fact, it signifies an association with a more severe
439 phenotype amongst participants with milder airway obstruction and suggests that IL-17 related
440 inflammation may be a pathway on which to intervene to prevent the progression to emphysema
441 and severe airway obstruction.
442
443 Our IL-17 signature, when measured at baseline, is associated with a poor lung function
444 response to corticosteroids at 30 months. This corticosteroid responsiveness is not simply due
445 to participants with low IL-17 signature expression exhibiting low neutrophil counts or
446 reciprocally exhibiting high Type 2 inflammation. In murine models, Th-17 cell-mediated airway
447 inflammation has been shown to be corticosteroid resistant, in contrast to Th2 cell-mediated
448 inflammation (51). Here we show the association between an IL-17 inflammatory signature and
449 corticosteroid unresponsiveness for the first time in a longitudinal randomized controlled trial in
450 humans. Many patients with COPD do not respond to corticosteroids, and ICS are only
451 indicated in exacerbation-prone symptomatic COPD. However, corticosteroids are still used
452 broadly despite possible increases in adverse outcomes such as pneumonia (52). The
453 corticosteroid unresponsiveness finding suggests that a more easily measurable surrogate for
454 our IL-17 signature could serve as a biomarker for therapeutics in COPD. While it may be useful
455 to predict who will not respond to corticosteroids, it may be even more useful to predict who will
456 respond to therapies targeting IL-17 or associated inflammatory pathways as we found in
457 psoriatic lesions.
458

459 Our study relied on the airway epithelial gene expression response to IL-17, where the cytokine
460 induces a major effect, and the first line of defense against injury to the lung. Other studies have
461 relied on cell counts or immunoreactivity which are poorly correlated in the human lung (13).
462 Additionally, Th17 cells display a high level of plasticity, and are thus more unstable than Th1 or
463 Th2 cells (53), suggesting cell numbers may not represent cytokine response. Data is also
464 conflicting on whether IL-17+ cell counts are elevated in COPD and related to key pathologic
465 characteristics such as airway neutrophilia (28, 29). We, however, show that IL-17 signature
466 genes are not only upregulated in two separate experiments in which HBE cells were stimulated
467 with IL-17, but that our signature is associated with increases in airway neutrophils as well. We
468 also show that our signature is decreased in response to IL-17 blocking agents in psoriatic skin
469 lesions but distinct from airway epithelial Type 1 and 2 responses, further indicating that we are
470 marking an IL-17 specific epithelial response.
471
472 We acknowledge that fitting our IL-17 signature to *CCL20* could have limited its generalizability.
473 However, the signature generalized well in that: 1) it was highly correlated with two other IL-17
474 gene signatures (a signature previously studied in asthma (39) and a signature fit to *SLC26A4*,
475 the most significantly upregulated gene in our IL-17 stimulated HBE culture experiments) and 2)
476 our IL-17 signature was responsive to anti-IL17 therapy and reflective of clinical response in 2
477 randomized controlled trials in psoriasis. The advantage of fitting this gene signature to *CCL20*
478 is that it improved its “fit” to a more complex in vivo tissue environment rather than a simple cell
479 culture model. In a COPD patient this complex environment may be further compounded by
480 multiple airway insults (e.g. smoking, microbial colonization, exacerbations, medications) that
481 are not modelled well in culture. By retaining only tightly inter-correlated genes, a well-
482 established method for identifying genes in the same molecular pathway (36, 37), we removed
483 those genes that may be non-specific to an IL-17 response in vivo.
484

485 Our study has some potential limitations. For instance, some analyses were cross-sectional,
486 and those analyses can only show associations, not causality. Our longitudinal analyses were
487 limited by sample size. Thus, while we did find a strong association between our IL-17 signature
488 and lack of response to inhaled steroids over 30 months, an assessment of the predictive power
489 of the signature for corticosteroid responsiveness was quite limited. Furthermore, our definitions
490 of “high” and “low” for the IL-17 signature are highly dependent on the population in which they
491 were developed. Therefore, further studies will be needed to determine if the signature could be
492 used as a biomarker for steroid unresponsiveness, and to determine the best cut-off for IL-17
493 “high” and “low”. We were also not powered to study the association between the signature and
494 exacerbation rates, which will be important to study in relation to therapeutic response. It was
495 not within the scope of this paper to identify the cause of the increased IL-17 response. We do,
496 however, see associations in current and former smokers, suggesting that more than just smoke
497 exposure is playing a role. The contributions of stimuli, such as alterations in the microbiome or
498 autoimmunity, to enhanced IL-17 related gene expression will require further study.
499 Furthermore, COPD phenotypes are heterogeneous and complex, and thus we hypothesize that
500 multiple overlapping molecular phenotypes underlie the complex clinical phenotypes we
501 observe in chronic airway diseases. Finally, future work will be needed to identify surrogate
502 biomarkers in more easily obtained specimens than airway brushings. This is similar to the
503 approach we took in our asthma studies in which we initially identified a Type 2 high asthma
504 molecular phenotype based on airway gene expression, and then expanded this work to identify
505 the best associated biomarkers (periostin, eosinophils, FeNO).
506
507 In summary, we show here that a signature of IL-17 associated airway inflammation is
508 upregulated in approximately a third of COPD participants and is associated with distinct
509 inflammatory, physiologic, and clinical features. Our findings suggest that the IL-17 signature

510 defines a molecular COPD phenotype that responds poorly to corticosteroid therapy, and which
511 could instead be the target of emerging therapies that interfere with IL-17 (44, 45, 48).
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536 **Materials and Methods**

537 *Transcriptomic Datasets:*

538 Eight transcriptomic datasets were used for these analyses.

539 ***1. UCSF Human bronchial epithelial cell (HBEC) culture dataset:***

540 Human bronchial epithelial cells obtained from the proximal airways of 6 lung donors
541 rejected for transplant (5 without airway disease, 1 with asthma) were grown to confluence
542 in an air-liquid interface culture (ALI) for 28 days as described previously (54). Some
543 cultures were stimulated with IL-17A (10 ng/mL) for the final 7 days of culture or interferon
544 gamma (IFNγ, 10 ng/mL) for the final 24 hours of culture. Matched cultures maintained in
545 media without cytokine over the same time period were used as controls. Cultured cells
546 were then harvested and underwent RNA isolation using the Qiagen miRNeasy kit (Qiagen
547 Inc., Valencia, CA) as per manufacturer's protocol. RNA quality and quantity were assessed
548 using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and the
549 NanoDrop Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Library
550 preparation and multiplexing were done using the Illumina TruSeq Stranded Total RNA with
551 Ribo-zero Human/Mouse/Rat kit (Illumina Inc, San Diego, CA) as per manufacturer's
552 protocol at the UCSF Sandler Genomics Core Facility. 100 base pair paired-end sequencing
553 was done on multiplexed samples via the Illumina HiSeq 2500 at the UCSF Genomics Core.

554

555 2. ***Bronchial Airway Epithelial (BAE) dataset****:* Bronchial epithelial brushings obtained from
556 6th-8th generation bronchi of former and current smokers with a range of lung function
557 (COPD=85, no COPD=152) were previously profiled by Affymetrix (Santa Clara, CA) HG 1.0
558 ST Arrays (55). Spirometry was done in all participants. Raw microarray files may be
559 downloaded from the Gene Expression Omnibus (GEO, accession: GSE37147) (54).
560 Inclusion/exclusion criteria were previously published.

3. **Validation HBEC culture dataset:** Data were downloaded from GEO (GSE10240). Primary HBE cells provided by the Tissue Core Laboratory at the University of Pittsburgh or purchased from Cambrex (Lonza) were grown to confluence in ALI then stimulated apically and basolaterally with media control or IL-17A for 24 hours (3 replicates each) as previously described (43). Isolated RNA was profiled by Affymetrix HG U133A 2.0 Arrays.

4. ***Asthma Dataset**:* Bronchial airway epithelial brushings obtained by bronchoscopy from steroid-naive subjects with mild to moderate asthma (n = 62) and control subjects without asthma (n = 43) were previously profiled by Affymetrix HG U133 plus 2.0 Arrays (GSE67472) (24). Inclusion/exclusion criteria for this study were previously published (24). Subjects with asthma were divided into Type 2-high and -low subgroups (n = 40 and 22, respectively) using a validated standardized mean expression level of *POSTN*, *SERPINB2*, and *CLCA1* (24, 32). IL-17 associated genes were evaluated amongst those differentially expressed between Type 2-high asthma compared to Type 2-low asthma and healthy controls.

5. ***Ixekizumab Psoriasis Dataset:*** Data were downloaded from GEO (GSE31652). Biopsies of psoriatic skin lesions were taken at baseline and after treatment with two weeks of Ixekizumab (n=6) or placebo (n=4) and previously profiled by Affymetrix HG U133A 2.0 Arrays (44).

6. ***Brodalumab Psoriasis Dataset:*** Data were downloaded from GEO (GSE53552). Biopsies were taken from psoriatic skin lesions and matched non-lesional skin from 25 participants at baseline. The psoriatic lesions were then sampled over 6 weeks after treatment with

586 placebo (n=5) or a dose range of Brodalumab (140mg n=4, 350mg n=8, 700mg n=8). All
587 samples were previously profiled by Affymetrix HG U133 plus 2.0 Arrays (45).
588
589 ***7. Gronigen and Leiden Universities study of Corticosteroids in Obstructive Lung***
590 ***Disease (GLUCOLD) dataset****:* Endobronchial biopsies from steroid-naïve participants with
591 moderate to severe COPD (n=79) were previously obtained by bronchoscopy and profiled
592 by Affymetrix HG 1.0 ST Arrays (GSE36221) (56). Blood collection, sputum induction, and
593 spirometry were done at the first study visit via previously described methods (57). A subset
594 of these participants was randomized to receive 30 months of placebo (n=16) or ICS with or
595 without long acting beta agonist (salmeterol, LABA) (n=33). Inclusion/exclusion criteria were
596 previously published (57).
597
598 8. ***Subpopulations and InteRmediate Outcome Measures In COPD Study (SPIROMICS)***
599 ***dataset****:* A subgroup of participants in the SPIROMICS multi-center observational cohort
600 study underwent research bronchoscopy. RNA was obtained from bronchial epithelial
601 brushings from 3rd-4th generation bronchi of the right or left lower lobe of current and former
602 smokers with mild to moderate COPD (n= 47). RNA was used for profiling IL-17-associated
603 gene expression by two-step, nested-primer RT-qPCR as described previously (32). Primer
604 and probe sequences are listed in **Table S4**.
605
606 At least a 20 pack-year smoking history was required for inclusion, and participants were
607 classified as former smokers after one year of smoking cessation. Participants were
608 classified as having COPD based on spirometry, performed before and after four inhalations
609 each of albuterol (90µg dose per inhalation) and ipratropium (18µg dose per inhalation),
610 using the GOLD staging system (58). Full inclusion/exclusion criteria are included in **Table**
611 **S5**.

Blood collection, sputum induction, and CT scans were done at the first study visit. Sputum induction was performed as previously described (59). Parametric response mapping (PRM) of CT imaging was used to distinguish areas of normal lung (PRM^{norm}) from areas of functional small airways disease (PRM^{fsad}) and emphysema (PRM^{emph}) as previously described (46, 47). Briefly, PRM is a CT voxel-based imaging biomarker that utilizes dynamic image registration to spacially align paired inspiratory and expiratory scans. PRM^{fsad} is defined as areas of lung that are >-950 Hounsfield Units (HU) on inspiration and <-856 HU on expiration. PRM^{emph} is defined as areas of lung that are <-950 HU on inspiration and <-856 HU on expiration. PRM^{norm} is defined as areas of lung exceeding both thresholds on inspiration and expiration.

Derivation of gene expression datasets:

RNA-Seq (HBEC culture dataset):

.fastq files were quality filtered and aligned to the human genome using STAR and the ENSEMBL GRCh38 genome build (60, 61). Read counts were normalized and differential expression analyses on matched samples were performed between 1) IL-17A stimulated samples and controls, and 2) IFNγ-stimulated samples and controls using the DESeq2 package in R (62). Differential expression in DESeq2 is carried out using generalized linear models following a negative binomial distribution. Results were trimmed to transcripts indexed in the HGNC database and with a Ensembl gene biotype label of “protein_coding”. Multiple comparisons corrections were done using False Discovery Rate by the Benjamini-Hochberg method (63).

Microarray (BAE, Asthma, GLUCOLD, Ixekizumab, and Brodalumab datasets):

637 Each microarray dataset independently underwent background adjustment (without the use
638 of mismatch probes), quantile normalization, and probe summarization using the RMA
639 algorithm (affy package, Bioconductor, R) (64, 65). Entrez gene custom chip definition files
640 available for the appropriate microarray for each dataset at
641 http://brainarray.mbni.med.umich.edu were used for annotation. Batch effect was minimized
642 using Combat when appropriate (66).
643

644 ***qPCR (SPIROMICS dataset):***

645 Data were normalized to the mean of *PPIA*, *RPL13A*, *ACTB*, and *DNAJA1*, determined using
646 the SLqPCR package in R, as described previously (32, 67).
647

648 ***Derivation of the IL-17 genomic signature***

649 An IL-17 genomic signature specific to bronchial epithelial brushings from smokers was
650 generated using elastic net regression for feature selection in the BAE dataset. The 100 genes
651 most up-regulated in ALI models after IL-17A stimulation were used as candidate predictor
652 variables ("features"). Genes highly correlated with a representative IL-17 gene, *CCL20*, were
653 selected as features for inclusion into the IL-17 signature using elastic net regression via the
654 glmnet package in R with alpha=0.75 and leave-one-out cross-validation (68). Alpha was
655 selected at just below one to maximize sparsity (and thus limit feature selection) while allowing
656 for selection of closely correlated genes. *CCL20* and the 10 genes selected by elastic net
657 regression were used for generation of the IL-17 signature. The mean of the zero-centered $\log_2$-
658 scale gene expression values of these 11 genes was used as the IL-17 airway epithelial
659 signature metric, a previously validated method (33, 39). To confirm that our IL-17 signature
660 was not just specific to *CCL20*, two alternative IL-17 signatures were generated. One was
661 generated using the above procedure with *SLC26A4*, the most upregulated gene following IL-17
662 stimulation in cell culture also measured in the COPD array data, guiding the elastic net. The

other was an IL-17 signature previously studied in asthma and was generated in the same way as previously reported, using the mean value of the zero-centered gene expression of five IL-17 associated genes (39).

For the Ixekizumab and Brodalumab studies, four genes were excluded prior to deriving the IL-17 signature metric: two genes that were poorly annotated in the microarray platform used (*SAA1*, *SAA2*), and two genes that were not expressed above background (*CSF3*, *MTNR1A1*) in these skin biopsies. As there was 100% concordance between the two psoriasis studies on genes not expressed above background, we concluded that these genes were poorly expressed in the resident skin cells. We did not, however, change the signature in any way based on knowledge of the genes or relevance in psoriasis. The IL-17 skin signature was thus derived using the mean value of the zero-centered log_2-scale gene expression values of the remaining seven genes (*CCL20*, *SLC26A4*, *TNIP3*, *CXCL3*, *CXCL5*, *CXCL6*, and *VNN1*).

Statistical analyses of the IL-17 genomic signature

All regression analyses were performed using the limma package in R (69). For cross-sectional analyses of the associations between the IL-17 signature and clinical variables (in the BAE, GLUCOLD, and SPIROMICS datasets) linear or logistic regression were used, as appropriate. Analyses were done before and after adjustment for age and smoking status. Race, gender, pack-years, and inhaled corticosteroid use were evaluated as potential confounders as well. These variables were, however, left out of the final models as they were not significantly associated with IL-17 signature expression, and did not significantly alter the relationships between the IL-17 signature and outcomes beyond adjustments for age and smoking status. Data was transformed when necessary for normal distribution. A P value less than 0.05 was considered significant. However, multiple hypothesis testing was done using a false discovery rate when appropriate (63). For the Ixekizumab and Brodalumab studies mixed effects models were used to relate the

689 IL-17 signature (as the outcome variable) to the interaction between treatment and time (fixed
690 effects) across participants (random effect). For longitudinal analyses in GLUCOLD the
691 interaction between treatment (ICS or placebo) and the baseline IL-17 signature was related to
692 change in FEV_1 over 30 months. The ICS and ICS + long acting beta agonist groups were
693 combined to improve power as the long-term effects in these groups were comparable. In
694 secondary analyses, the interactions between the IL-17 score and A) tissue eosinophils, B) our
695 previously generated metric of Type 2 inflammation (the T2S score), or C) tissue neutrophils were
696 related to change in FEV_1 over 30 months amongst those GLUCOLD participants that received
697 ICS (6).
698
699 ***Clustering***
700 All clustering analyses were performed using euclidean distance with average linkage as the
701 distance metric. The NbClust package (R, bioconductor) was used to determine the best
702 participant clustering of the IL-17 signature genes, based on a majority vote of 30 indices that
703 evaluate partitioning (70). NbClust deals with the inherent variability in the many indices
704 available to determine the optimal number of clusters by requiring a consensus vote amongst
705 these indices on best partitioning. Participants with relatively high expression who clustered
706 separately from the majority of participants were considered "IL-17 High". Prior to determining
707 the best number of partitions the datasets were first stratified by smoking status given the large
708 effect of smoking on gene expression. Differences amongst indices in deciding best clustering
709 were generally due to separation of those with "IL-17 High" expression into one or more
710 categories, while those with low expression clustered together. The exception were two
711 participants in the SPIROMICS dataset with low expression that were partitioned into their own
712 groups. Six of 35 participants with relatively high IL-17 gene expression in the BAE dataset, 3 of
713 23 participants in the GLUCOLD dataset, and 5 of 18 participants in the SPIROMICS dataset

were partitioned out as the highest for IL-17 expression. For simplicity, all IL-17 high, including these highest participants, were grouped together.

The IL-17 signature was then discretized into two categories: “IL-17 high” and “IL-17 low” using two different methods to use as a categorical predictor for longitudinal analyses in GLUCOLD. First, discretization was based on the best partitioning decided by NbClust, and then, alternatively based on the top quartile of signature expression. Ten percent of samples with IL-17 signatures closest to the partition were removed prior to discretization to diminish overlap.

Study Approval

The included human studies were all approved by the institutional review boards at the institutions involved in sample and data collection. All participants provided written informed consent prior to inclusion in the study.

740

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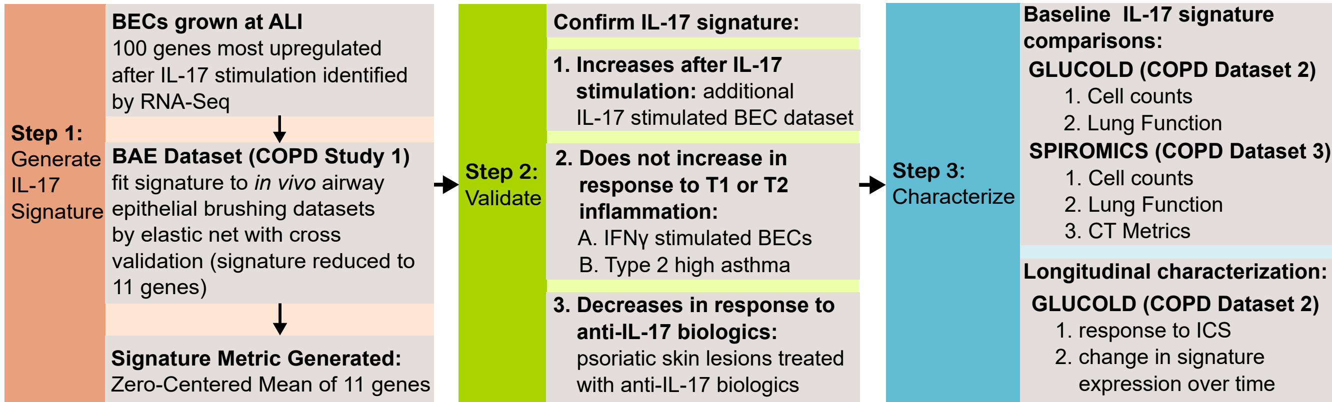


Figure 1. Study Design. Abbreviations: BEC: Bronchial Epithelial Cell, ALI: air-liquid interface, RNA-Seq: RNA Sequencing, BAE: Bronchial Airway Epithelial, T1: Type 1 Inflammation, T2: Type 2 Inflammation, IFNγ: Interferon Gamma, ICS: Inhaled Corticosteroid.

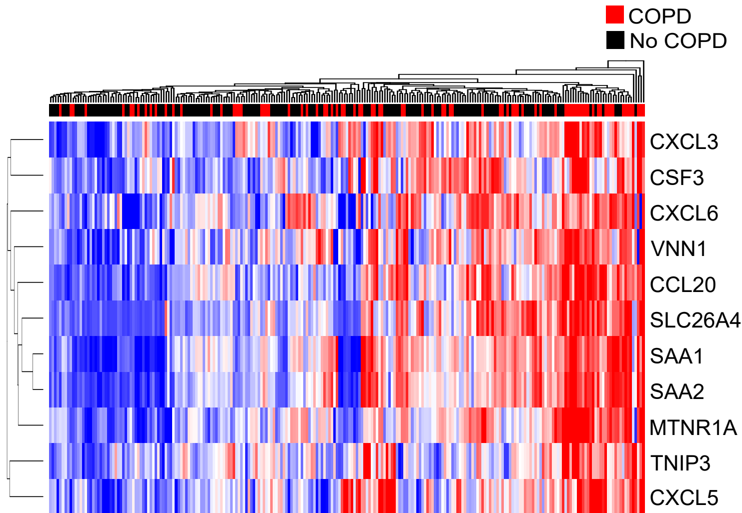
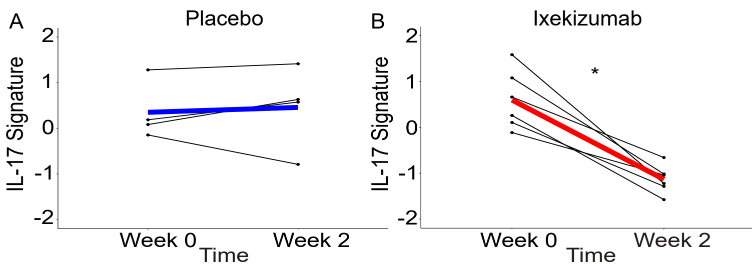


Figure 2. Hierarchical clustering of the 11 IL-17 signature genes in the BAE dataset (n=237). Signature genes shown in rows across participants in columns. Blue and red indicates low and high relative gene expression, respectively. Smokers with and without COPD are indicated by red and black in the above color bar, respectively. Clustering across participants and genes was done by Euclidean distance with average linkage.

Ixekizumab



Brodalumab

Figure 3. IL-17 blockade in psoriasis. Ixekizumab: The IL-17 signature was (A) decreased in psoriatic skin lesions (n=6) after 2 weeks of Ixekizumab compared to (B) placebo (n=4). Brodalumab: compared to (C) placebo (n=5), Brodalumab (n=20) at a dose of (D) 140mg did not, but (E) 350mg (at 1 and 2 weeks) and (F) 700mg (at 2 and 6 weeks) did result in a decrease in the IL-17 signature, consistent with clinical response. The IL-17 signature was higher in psoriatic lesions than matched non-lesional skin samples (C-F, dashed line). *$p<0.05$ using mixed effects models

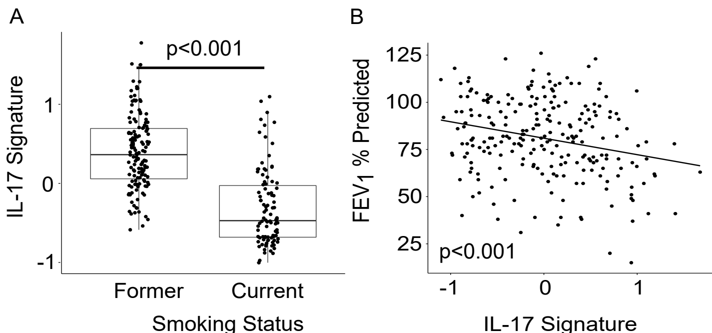


Figure 4. The IL-17 gene signature in the BAE dataset (n=237) is (A) increased in former (0.29±0.46) compared to current smokers (-0.42±0.48, $p<0.001$ by Wilcoxon Rank Sum test), and (B) associated with decreasing FEV_1% predicted ($\rho=-0.23$, $p<0.001$ by Spearman's correlation).

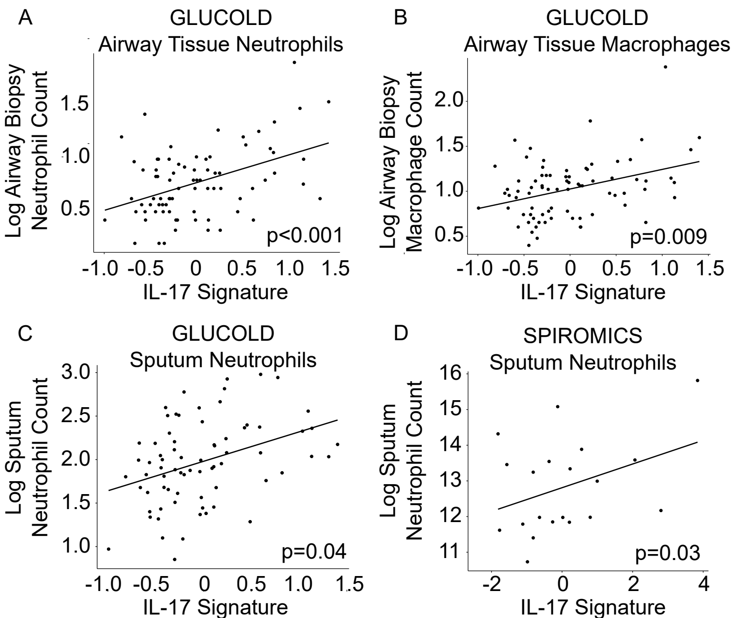
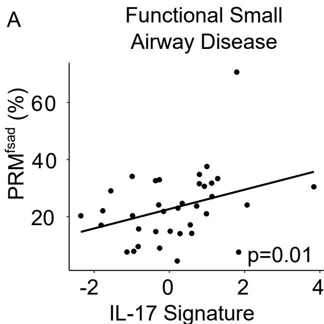


Figure 5. Airway neutrophils and macrophages. GLUCOLD (n=79): the IL-17 signature was associated with increasing log2 counts of (A) airway tissue neutrophils, (B) airway tissue macrophages, and (C) sputum neutrophils (n=72 with measured neutrophils). (D) SPIROMICS: the signature was associated with log2 sputum neutrophil counts (n=20). P-values shown for linear models adjusted for age and smoking status.



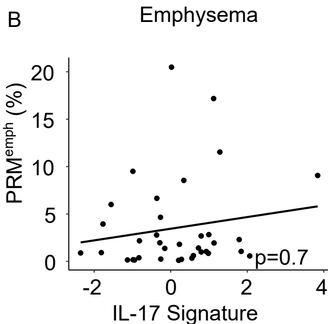
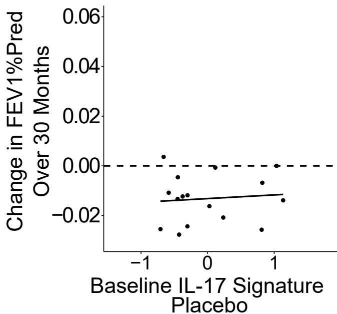


Figure 6. CT Biomarkers. The IL-17 signature was associated with increasing percent of lung area with (A) functional small airways disease (PRM^{fsad}) but not (B) emphysema (PRM^{emph}) by parametric response mapping of baseline CT scans (n=35). P-values shown for linear models adjusted for age and smoking status.



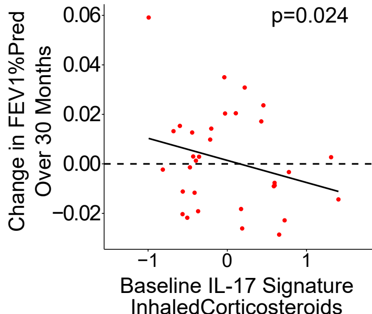


Figure 7. ICS response in GLUCOLD. An increased baseline IL-17 signature was associated with a greater decrease in percent change in FEV1 in the ICS ± long acting beta agonist group (n=33) compared to placebo (n=16) at 30 months (p=0.024 for the linear model interaction with adjustment for age and smoking status). Participants with low IL-17 signatures were more likely to show an improvement in FEV1 after ICS (greater than zero: above the dashed line).

Tables

Table 1. Demographic characteristics of the three datasets.

	BAE Current and Former Smokers ± COPD			GLUCOLD Current and Former Smokers with COPD Subset randomized to placebo or inhaled steroids				SPIROMICS Current and Former Smokers with COPD
	Smokers N=151	COPD N=87	p-val	All N=79	Placebo N=16	Steroid N=33	p-val	N=47
Age	65 (6)	64 (6)	0.22	61 (8)	58 (8)	61 (8)	0.22	63 (8)
Smoking Status								
Current	69	30	0.10	46	14	22	0.79	16
Former	82	57		33	7	15		29
Inhaled Steroid Use			0.0003					
Yes	7	18		0	0	0	NA	17
No	144	69		78	16	33		30
History of Asthma								
Yes	10	7	0.79	0	0	0	NA	11
No	141	80		78	16	33		33
FEV_1% Predicted	93 (13)	60 (14)	$<2.2*10^{-16}$	63 (9)	61 (9)	64 (9)	0.28	79 (18)

Means and (SD) or total counts are given for continuous and dichotomous variables respectively. For the BAE dataset p-values for differences between smokers with and without COPD by fischer's exact test or t-test as appropriate are given. GLUCOLD and SPIROMICS included only COPD participants, and point values are shown for all baseline participants. For GLUCOLD differences between those randomized to placebo or inhaled corticosteroid and the associated p-values are shown.

Table 2. Association between IL-17 metric and cell counts or T2S score.

	Unadjusted				Adjusted			
	R^2	Coefficient (SE)	p-val	FDR	R^2	Coefficient (SE)	p-val	FDR
Neutrophils								
GLUCOLD								
Endobronchial neutrophils	**0.19**	**0.27 (0.06)**	**$6.41*10^{-5}$**	**<0.001**	**0.20**	**0.26 (0.07)**	**0.0008**	**0.002**
Sputum neutrophils	**0.14**	**0.34 (0.10)**	**0.001**	**0.002**	**0.17**	**0.27 (0.13)**	**0.041**	**0.061**
Blood neutrophils (%)	0.002	-0.83 (2.00)	0.68	0.68	0.08	0.57 (2.26)	0.80	0.80
SPIROMICS								
Sputum neutrophils	**0.14**	**0.33 (0.19)**	**0.10**	**0.20**	**0.37**	**0.44 (0.19)**	**0.033**	**0.066**
Blood neutrophils	0.005	-0.02 (0.03)	0.63	0.63	0.09	0.03 (0.04)	0.43	0.43
Macrophages								
GLUCOLD								
Endobronchial macrophages	**0.13**	**0.22 (0.06)**	**0.0009**	**0.002**	**0.14**	**0.20 (0.07)**	**0.009**	**0.019**
Sputum macrophages	0.008	0.07 (0.09)	0.45	0.45	0.03	-0.02 (0.11)	0.84	0.84
SPIROMICS								
Sputum macrophages	0.008	0.05 (0.13)	0.71	N/A	0.02	0.06 (0.14)	0.68	N/A
Eosinophils								
GLUCOLD								
Endobronchial eosinophils	0.02	0.11 (0.10)	0.28	0.35	0.01	0.02 (0.12)	0.87	0.923
Sputum eosinophils	0.01	0.09 (0.08)	0.31	0.35	0.05	-0.01 (0.11)	0.92	0.923
Blood eosinophils	0.01	0.32 (0.34)	0.35	0.35	0.10	-0.21 (0.39)	0.59	0.923
SPIROMICS								
Sputum eosinophils	0.11	0.46 (0.40)	0.27	0.31	0.56	0.40 (0.41)	0.35	0.35
Blood eosinophils	0.02	0.07 (0.07)	0.31	0.31	0.07	0.12 (0.08)	0.15	0.30
Mast Cells								
GLUCOLD								
Endobronchial mast cells	0.007	-0.03 (0.04)	0.47	N/A	0.01	-0.02 (0.05)	0.67	N/A
T2S Score								
BAE	0.05	-1.49 (0.71)	**0.039**	N/A	0.10	-0.94 (0.85)	0.27	N/A
GLUCOLD	0.04	-1.01 (0.60)	0.096	N/A	0.14	-0.16 (0.67)	0.82	N/A

"Adjusted" models adjusted for smoking status and age. Count values for endobronchial tissue and sputum samples were logged prior to analysis. False discovery rates calculated on a cell type specific basis over each study.

FDR=False discovery rate, SE=standard error

Table 3. Association between IL-17 metric and clinical parameters in COPD participants.

	Unadjusted				Adjusted			
	R^2	Coefficient (SEM)	p-val	FDR	R^2	Coefficient (SEM)	p-val	FDR
Baseline								
BAE								
FEV1 % Predicted	0.01	-2.34 (2.28)	0.31	N/A	0.09	-5.52 (2.68)	**0.042**	**N/A**
GLUCOLD								
FEV1 % Predicted	0.04	-3.40 (1.81)	0.064	N/A	0.07	-3.23 (2.10)	0.129	N/A
SPIROMICS								
FEV1 % Predicted≠	0.03	-2.50 (2.1)	0.23	0.35	0.13	-5.24 (2.44)	**0.038***	**0.057**
CT PRM^{emph}≠	0.03	0.61 (0.65)	0.35	0.35	0.08	0.78 (0.91)	0.40	0.40
CT PRM^{fSAD}≠	0.12	3.40 (1.57)	**0.037**	**0.11**	0.26	5.70 (2.07)	**0.0097***	**0.029**
Longitudinal								
GLUCOLD: Change over 30 months after Inhaled steroid treatment compared to placebo (n=48)								
FEV_1 %	0.23	-0.014 (0.009)	0.11	N/A	0.36	-0.019 (0.008)	**0.024**	**N/A**
FEV_1 % also adjusted for airway tissue eosinophils	N/A	N/A	N/A	N/A	0.36	-0.020 (0.009)	**0.027**	**N/A**
FEV_1 % also adjusted for T2S score	N/A	N/A	N/A	N/A	0.38	-0.020 (0.008)	**0.018**	**N/A**
FEV_1 % also adjusted for airway tissue neutroophils	N/A	N/A	N/A	N/A	0.3	-0.022 (0.009)	**0.016**	**N/A**
FEV_1 % also adjusted for airway tissue macrophages	N/A	N/A	N/A	N/A	0.36	-0.019 (0.008)	**0.030**	**N/A**

"Adjusted" models adjusted for smoking status and age. Abbreviations: SE= Standard Error, FEV_1%Pred= Forced expiratory volume in one second percent predicited, PRM^{fSAD}= parameteric response mapping functional small airways disease biomarker, PRM^{emph}= parameteric response mapping emphysema biomarker, T2S= Type 2 Signature, N/A= Not Applicable, ≠ =parameters adjusted for multiple comparisons, *=p-value adjusted for FDR<0.1