



## Airway inflammation in COPD- progress to precision medicine

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Abstract:	<p>Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-inflammatory therapy is not first line treatment. This inflammation has many guises and phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD with activation of the inflammasome, T1 and T17 immunity is the most common phenotype with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and revealed that corticosteroids are most effective in those with evidence of eosinophilic inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD has been disappointing with smaller benefits for the same intensity of eosinophilic inflammation questioning its role in COPD. Biological therapies beyond T2-mediated inflammation have not demonstrated benefit and in some cases increased risk of infection suggesting that neutrophilic inflammation and inflammasome activation might be largely driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future biomarker approaches to assess inflammation in COPD and how this might reveal tractable approaches to precision medicine and unmask important host-environment interactions leading to airway inflammation.</p>

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3 **1 Airway inflammation in COPD- progress to precision medicine**  
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55 24 the submitted work.  
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## 26 **Summary**

27 Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and  
28 mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent  
29 feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-  
30 inflammatory therapy is not first line treatment. This inflammation has many guises and  
31 phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD  
32 with activation of the inflammasome, T1 and T17 immunity is the most common phenotype  
33 with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed  
34 in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and  
35 revealed that corticosteroids are most effective in those with evidence of eosinophilic  
36 inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD  
37 has been disappointing with smaller benefits for the same intensity of eosinophilic  
38 inflammation questioning its role in COPD. Biological therapies beyond T2-mediated  
39 inflammation have not demonstrated benefit and in some cases increased risk of infection  
40 suggesting that neutrophilic inflammation and inflammasome activation might be largely  
41 driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future  
42 biomarker approaches to assess inflammation in COPD and how this might reveal tractable  
43 approaches to precision medicine and unmask important host-environment interactions leading  
44 to airway inflammation.

45  
46 **Key words:** COPD, ACOS, eosinophil, neutrophil, macrophage, inflammasome, biologics,  
47 eosinophil, interleukin (IL)5, benralizumab, mepolizumab, tumour necrosis factor (TNF) $\alpha$ ,  
48 IL6, IL8, IL1 $\beta$ , IL33, IL13, IL17, anti-IgE antibody, thymic stromal lymphopoietin (TSLP),  
49 prostaglandin D<sub>2</sub> receptor type 2 (DP2)

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51 **Take home message**

52 Airway inflammation drives COPD, but corticosteroids only work in those with eosinophilic  
53 inflammation. There is a need to better understand the patterns of inflammation, the reason for  
54 its persistence and the opportunities for new treatments.

55

## 56 **Introduction**

57 Chronic obstructive pulmonary disease (COPD) is a common disease of chronic lung  
58 inflammation that results in persistent symptoms and fixed airflow obstruction [1]. This is  
59 caused by an inflammatory response following inhalation of cigarette smoke or other noxious  
60 external particles such as air pollution and biomass fuel [1]. Airway and systemic inflammation  
61 in COPD is related to disease progression and mortality [1-2]. Current diagnostic criteria do  
62 not capture the heterogeneity of COPD in terms of the complex pathological changes occurring  
63 within lung, the different airway inflammatory patterns or the airway microbial ecology.  
64 Airway inflammation is a consistent feature of COPD and is present in both the large and small  
65 airways [1, 3-6]. The airway inflammation can persist after smoking cessation and is likely a  
66 consequence of altered immunity [6] and changes in the airway microenvironment [8-10].

67  
68 Despite the long-standing recognition that airways inflammation is a key driver of COPD  
69 progression and exacerbations, first-line treatment strategies are aimed at symptomatic  
70 treatment of bronchoconstriction in the form of bronchodilators, rather than anti-inflammatory  
71 therapy [1]. In this review we shall describe the heterogeneity of airway inflammation in  
72 COPD, current and future biomarker approaches to dissect this heterogeneity and redefine  
73 COPD using multi-dimensional phenotyping and how this might reveal tractable approaches  
74 to precision medicine and provide important insights into the host-environment interactions.

## 76 **Multi-dimensional COPD phenotyping providing insights into pathophysiology**

77 COPD is a consequence of complex host-environment interactions that occur over time  
78 summarised in **Figure 1**. Smoking, and other pollutants, pathogens and allergens insult the  
79 lung promoting airway inflammation and damage in a susceptible host as a consequence of

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2  
3 80 genetic predisposition and altered immunity [6, 10-12]. This in turn leads to irreversible  
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5 81 damage resulting in fixed airflow obstruction and the consequent typical symptoms of COPD.  
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10 83 ***Approaches to phenotyping airway inflammation and damage in COPD***

11  
12 84 Insights into airway inflammation and damage to the airways have been derived from lung  
13  
14 85 specimens obtained from surgical resection and at post mortem. Importantly *in vivo* measures  
15  
16 86 of airway and systemic inflammation have been characterised longitudinally, at exacerbations  
17  
18 87 and in response to therapies through invasive sampling of the airway by bronchoscopy (large  
19  
20 88 airway brush and biopsy and smaller airways by bronchoalveolar lavage), non-invasive sputum  
21  
22 89 sampling (mostly large airways) which is safe even in severe COPD [13], breath analysis (large  
23  
24 90 and small airways), lung imaging (large airways directly and small airways indirectly) and  
25  
26 91 beyond the lung by assessing upper airway samples and systemically using blood and urine [5,  
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28 92 14] (**Figure 2**).  
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35 94 ***Neutrophil-associated airway inflammation***

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37 95 The inflammatory response in COPD involves both innate and adaptive immunity with  
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39 96 neutrophilic inflammation the commonest inflammatory phenotype in COPD. Following  
40  
41 97 exposure to cigarette smoke, other pollutants, and oxidants there is airway damage [15] leading  
42  
43 98 to release of pro-inflammatory mediators and damage-associated molecular patterns (DAMPs)  
44  
45 99 such as interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP) [15]. The distribution of  
46  
47 100 the IL-33 receptor ST2 is altered in response to cigarette smoke with down-regulation in innate  
48  
49 101 type-2 innate lymphoid cells with up-regulation by macrophages leading to a triggering of an  
50  
51 102 IL-33-dependent exaggerated pro-inflammatory cascade [16]. As a consequence of airway  
52  
53 103 damage the altered barrier function predisposes the airway to infection and bacterial dysbiosis  
54  
55 104 which together with pollutants drive switching of ILC2 cells towards ILC1 cells further  
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3 105 amplifying the type-1 inflammatory cascade [17]. In COPD there is an increase in the phyla  
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5 106 proteobacteria and the emergence of *H. Influenzae* predominance such that the ratio of  
6  
7 107 gammaproteobacteria to firmicutes ( $\gamma$ P:F) increase [7-9, 18]. These pathogens themselves  
8  
9 108 promote an inflammatory response via activation of pathogen-associated molecular patterns  
10  
11 109 (PAMPs) and further amplification of the airway inflammation with the intensity of airway  
12  
13 110 inflammation related to the abundance of *H. Influenzae* [19, 20]. In this scenario, epithelial  
14  
15 111 cells are activated and are involved in the release of inflammatory mediators, such as tumor  
16  
17 112 necrosis factor (TNF), IL-1 $\beta$ , IL-6 and IL-8. Macrophages are recruited with further release or  
18  
19 113 pro-inflammatory cytokines and activation of the NLRP3 inflammasome with caspase-1-  
20  
21 114 dependent release of pro-inflammatory IL-1-like cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-33 and IL-18 [6,  
22  
23 115 15]. Activation of the inflammasome can lead to persistence of an inflammatory response by  
24  
25 116 triggering an auto-inflammatory response with intrinsic production of pro-inflammatory  
26  
27 117 mediators independent of exogenous stimuli [6]. Interestingly activation of type 1 responses  
28  
29 118 are more closely related to COPD severity than inflammasome activation and thus  
30  
31 119 autoimmunity can occur across disease severity [21]. Neutrophils are recruited as the  
32  
33 120 predominant cells with consequent release of proteases and airway damage as well as activation  
34  
35 121 of innate lymphoid type 3 cells (ILC3). The adaptive immune response is also involved with  
36  
37 122 polarization and subsequent recruitment of CD4<sup>+</sup> Th1 and Th17 cells, which produce IFN- $\gamma$   
38  
39 123 and IL-17A and IL-17F [6, 15, 22] respectively with a later predominance of CD8<sup>+</sup> T-cells. In  
40  
41 124 concert or independent of the auto-inflammatory response there is an auto-immune response  
42  
43 125 which can also promote persistence of inflammation [6]. In more severe disease there is an  
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45 126 accumulation of B cells particularly in the smaller airways which together with T cells and  
46  
47 127 follicular dendritic cells comprise aggregates organised into tertiary lymphoid follicles [23].  
48  
49 128 These lymphoid follicles support the priming and clonal expansion of T and B-cells with an  
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51 129 increase proportion of IgA<sup>+</sup> B-cells perhaps in response to increased persistent airway infection  
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3 130 or auto-antigens [24, 25]. The cytokine network in neutrophil-associated COPD is summarised  
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5 131 in **Figure 3A**.

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10 133 ***Eosinophil-associated airway inflammation***

11  
12 134 Even though neutrophil-associated COPD is the most common inflammatory phenotype,  
13  
14 135 consistent with the heterogeneity of the disease 10-40% of COPD patients demonstrate  
15  
16 136 increased eosinophilic inflammation in the sputum and or blood [5, 26, 27] with increased T2-  
17  
18 137 transcriptome signatures [28]. The broad range in prevalence is in part due to differences in  
19  
20 138 patient populations but also due to different cut-offs applied in sputum (>2 or >3% eosinophils)  
21  
22 139 or blood (2% or >250, 300, 400 eosinophils/ $\mu$ L). Increased eosinophilic inflammation in  
23  
24 140 peripheral blood and sputum samples in COPD, like asthma, is associated with a greater future  
25  
26 141 risk of severe exacerbations [29, 30]. The aetiology of eosinophilic inflammation in COPD is  
27  
28 142 uncertain. As with neutrophil-associated COPD eosinophilic COPD is also likely to be a  
29  
30 143 combination of innate and adaptive immunity summarised in **Figure 3B**. These pathways are  
31  
32 144 well-described for asthma [5, 30]. Following allergic sensitisation and T-cell polarisation TH2  
33  
34 145 cells produce interleukin (IL)-4, IL-5, and IL-13. IL-5 is an obligate cytokine for the survival  
35  
36 146 and maturation of eosinophils, and IL-4 and IL-13 promote IgE production from B cells and  
37  
38 147 have direct effects upon structural cells. Recruitment of eosinophils to the lung mucosa is  
39  
40 148 mediated via production of predominantly epithelium-derived CCR3 chemokines and other  
41  
42 149 eosinophil chemoattractants, such as mast cell-derived prostaglandin (PG)D2. PGD2 amplifies  
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44 150 T2 immunity via activation of PGD2 type 2 receptors (DP2 or CRTH2). Total IgE is elevated  
45  
46 151 in eosinophilic COPD even though atopy is not increased. Whether this reflects a hitherto  
47  
48 152 undescribed allergen is unclear. Eosinophilic inflammation can also occur via activation of  
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50 153 ILC2 cells, which produce IL-5 and IL-13 in response to PGD2 and the epithelial-derived  
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52 154 'alarmins' IL-33, IL-25, and TSLP released after epithelial damage by pollutants and microbes.  
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3 155 Additional contributions might be from macrophage-derived IL33 released following  
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5 156 inflammasome activation. Whether these innate and acquired T2-mediated immune  
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7 157 mechanisms occur in COPD, the predominance of one over another is more important in COPD  
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9 158 versus asthma or whether there are alternative mechanisms driving eosinophilic inflammation  
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11 159 in COPD remains unclear.  
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### 161 *Biological clustering to dissect heterogeneity of airways inflammation*

162 These eosinophilic versus neutrophilic associated inflammatory profiles represent extreme  
163 phenotypes. However they are consistently reproducible and demonstrate phenotype stability  
164 [20, 26]. The neutrophil and eosinophil-associated phenotypes also exhibit distinct microbial  
165 ecology with  $\gamma$ P:F predominance in the neutrophilic phenotype [8, 9, 31]. However, to describe  
166 extremes can be an over-simplification of a complex underlying biology. To validate these  
167 phenotypes and to further inform the understanding of the heterogeneity of COPD in stable  
168 state unbiased statistical approaches such as cluster analysis have been applied to large clinical  
169 and biological datasets [18, 32, 33]. Interestingly these have underscored the importance of  
170 eosinophilic airway inflammation in asthma, COPD and the asthma-COPD overlap syndrome  
171 (ACOS) [32, 34]. Combined data from asthma and COPD revealed three biological clusters  
172 [32]. Cluster 1 consisted of asthma subjects with increase IL-5, IL-13 and CCL26 mediators  
173 and eosinophil predominance. Cluster 2 consisted of an overlap between asthma and COPD  
174 with neutrophil predominance. Cluster 3 consisted mainly of COPD patients with a mixed  
175 granulocytic airway inflammation. The differences seen between neutrophilic COPD in cluster  
176 2 and eosinophilic COPD in cluster 3 included the presence of increased bacterial colonisation  
177 with an increased  $\gamma$ P:F ratio in the former and increased CCL13 in the latter possibly explaining  
178 the observed airway inflammation differences seen between these clusters (**Figure 4A**).

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3 180 Using a similar unbiased cluster analysis approach for COPD exacerbations four biological  
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5 181 clusters were identified and these validated the *a priori* aetiological groups: 'Pro-inflammatory'  
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7 182 bacterial-associated, 'Th1' viral-associated, 'Th2' eosinophilic-associated and a fourth group  
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9 183 that were termed 'pauci-inflammatory' as this was associated with limited changes in the  
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11 184 inflammatory profile (**Figure 4B**) [33]. Disease severity was not different between these  
12  
13 185 biological clusters and the biomarkers were associated with their respective potential  
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15 186 aetiologies. In the pro-inflammatory bacterial-associated group the strongest discriminating  
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17 187 inflammatory mediator was sputum IL-1 $\beta$  with increased  $\gamma$ P:F consistent with bacterial  
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19 188 dysbiosis. The blood eosinophil count was the best predictor of sputum eosinophilic  
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21 189 inflammation (>3% eosinophils) at the time of the exacerbation in this study although the  
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23 190 correlations are typically weaker in stable disease [35] Interestingly Bafadhel *et al* found that  
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25 191 patients experienced more bacterial exacerbations if their stable sputum samples contained  
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27 192 more bacteria and high  $\gamma$ P:F and more eosinophilic exacerbations if eosinophilic inflammation  
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29 193 was present in the stable state suggesting that the exacerbation event was an amplification of  
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31 194 the underlying phenotype [33]. Thus these biomarkers in addition to directing therapy during  
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33 195 the exacerbation event might identify subgroups to target therapy in stable state with the aim  
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35 196 of reducing future risk. The exception to this was a viral infection representing a new event  
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37 197 and a new inflammatory profile with increased blood and sputum concentrations of the  
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39 198 interferon-inducible chemokines CXCL10 and CXCL11.

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#### 200 ***Airway damage and remodelling- emphysema and small airway obliteration***

201 Airway inflammation in COPD contributes to airway damage, remodelling, loss of small  
202 airways and emphysema (tissue damage with permanent dilatation distal to the terminal  
203 bronchiole). Chronic airflow obstruction is due to a combination of emphysema and small  
204 airway obliteration. Small airways are the major site of airway obstruction in COPD [48]. This

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3 205 small airways obliteration is due to a combination of remodelling and accumulation of  
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5 206 inflammatory exudates within the airway lumen, both of which increase with disease severity  
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8 207 [36, 37]. Remodelling changes observed in COPD include disruption and loss of epithelial cilia,  
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10 208 squamous metaplasia of the respiratory epithelium, goblet cell hyperplasia and mucous gland  
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12 209 enlargement, bronchiolar smooth muscle hypertrophy, airway wall fibrosis and inflammatory  
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15 210 cell infiltration [36, 37].  
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19 212 Computed tomography (CT) and micro CT has demonstrated a reduction in the luminal area  
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21 213 of terminal bronchioles in COPD, but also substantial loss of terminal airways [38-40]. This is  
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23 214 consistent with the view that the inflammation and remodelling of the small airways largely as  
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26 215 a consequence of inflammation leads to destruction of the terminal followed by respiratory  
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28 216 bronchioles to form centrilobular lesions. This in turn can result in destruction of entire lung  
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30 217 lobules which coalesce to form bullous emphysema. Thus narrowing and consequent  
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33 218 disappearance of small conducting airways can explain the increased peripheral airway  
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35 219 resistance reported in COPD prior to the development of emphysema [38-40]. The distribution  
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37 220 of emphysema can be centrilobular or panacinar. It is uncertain if these represent a spectrum  
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39 221 with panacinar a consequence of centrilobular emphysema or if they represent distinct  
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42 222 conditions. Panacinar is observed in individuals with alpha-1-anti-trypsin deficiency perhaps  
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44 223 suggesting this form of emphysema might be largely a consequence of the imbalance between  
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47 224 protease and anti-protease activity whereas centrilobular is largely due to loss of and  
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49 225 remodelling of small airways caused by persistent airway inflammation. Quantitative CT has  
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51 226 demonstrated that gas trapping due to small airway disease moreover than emphysema is  
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53 227 related to lung function impairment [41, 42]. These mechanisms of small airway obliteration  
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56 228 and emphysema are important when considering anti-inflammatory therapy as only the  
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3 229 remaining inflamed airways can be targeted in contrast to the airways and alveoli that are  
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5 230 already destroyed in patients with COPD.  
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10 232 **Airway inflammation in COPD- progress to precision medicine**

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12 233 Increasing knowledge of disease pathology and inflammatory phenotypes will inform our  
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14 234 understanding of COPD and enable phenotype-specific clinical management beyond the first  
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17 235 line bronchodilator therapy for COPD.  
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21 237 ***Eosinophilic COPD- corticosteroids***

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24 238 Corticosteroids have been used in the treatment of COPD for more than 40 years with moderate  
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26 239 overall benefit in terms of improvement in lung function, health status, 6 minute walk distance  
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28 240 and exacerbation frequency [1]. More recently a differential response in patients has been seen  
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30 241 based on eosinophil count. An elevated sputum eosinophil count is associated with a greater  
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33 242 response to both inhaled and oral corticosteroids in stable disease [43, 44], whilst blood  
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35 243 eosinophil count can be used to predict response to corticosteroid response in stable [45, 46]  
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37 244 and acute COPD [47] and titration of corticosteroids directed by sputum eosinophil counts  
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39 245 reduces hospital admissions [48]. Importantly most of these studies have recruited COPD  
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42 246 subjects with frequent exacerbations and thus whether findings can be generalised to all COPD  
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44 247 subjects is uncertain. Additionally it is unclear if the clinical benefits, such as lung function  
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46 248 and health status, with corticosteroids are independent of the reduction of exacerbations. In  
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48 249 contrast non-T2 pathways such as IL-17 activation as determined by the epithelial IL-17A  
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50 250 response transcriptome signature are associated with a decreased response to corticosteroids  
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52 251 [49]. Whether the benefit from corticosteroids in COPD associated with eosinophilic  
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54 252 inflammation is restricted to its effects upon the eosinophil or due to other broader anti-  
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56 253 inflammatory effects is uncertain. GOLD now includes the blood eosinophil count as a  
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3 254 biomarker to direct the use of ICS in COPD patients with frequent exacerbations [1]. Benefits  
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5 255 in response to roflumilast are also possibly due to attenuation of eosinophilic inflammation  
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8 256 [50].  
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### 12 258 *Eosinophilic COPD- T2 targeted therapies*

14 259 Evidence for targeting T2-mediated inflammation using biologics has revolutionised clinical  
16 260 practice in severe asthma [30, 51]. As described above significant eosinophilic inflammation  
18 261 does exist in COPD, albeit in a smaller proportion of patients than in asthma. However, the  
20 262 findings from the phase 2 and 3 trials of T2-directed therapies for COPD summarised in  
22 263 **Table 1** have been disappointing compared to asthma [52].  
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28 265 In the first anti-IL5R biologic (benralizumab) trial in COPD while a reduction in eosinophilic  
29 266 inflammation was observed, the primary outcome annual rate of acute exacerbations was not  
30 267 met, which included all patients with COPD, irrespective of baseline eosinophil count [53].  
31 268

32 269 Importantly the sample size was small to study exacerbations and was underpowered to observe  
33 270 small effects. Secondary outcomes showed an improvement in FEV<sub>1</sub> in those receiving  
34 271 benralizumab but no difference was observed in health status. In a pre-specified post hoc  
35 272 analysis improvements in exacerbation frequency, lung function and health status were related  
36 273 to the intensity of baseline blood and sputum eosinophil count. In the yet to be fully reported  
37 274 phase 3 trials of benralizumab in COPD the primary outcome of exacerbations in those with  
38 275 increased blood eosinophil count ( $\geq 220$  cells/ $\mu$ L) was also not met [54]. In a small single centre  
39 276 study mepolizumab reduced sputum eosinophil count, but did not improve lung function or  
40 277 health status [55]. In two phase 3 trials of mepolizumab in COPD (METREX and METREO)  
41 278 there were small reductions in moderate or severe exacerbations in the eosinophilic sub-group  
42 ( $\geq 150$  cells/ $\mu$ L), which was statistically significant in the METREX (18% reduction) but not  
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3 279 in METREO [56]. In a *post hoc* analysis there was no reduction in exacerbation events treated  
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5 280 with antibiotics alone in those receiving mepolizumab versus placebo but the reduction in  
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8 281 exacerbations treated with oral corticosteroids with or without antibiotics was ~35% in those  
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10 282 with blood eosinophil counts >300 eosinophils/ $\mu$ L. No improvements in lung function and  
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12 283 health status in those receiving mepolizumab versus placebo were observed.  
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17 285 Importantly, both the mepolizumab and benralizumab studies suggest that the effect size is  
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19 286 smaller than that seen in severe asthma (**Figure 5**) although, like asthma, the magnitude of  
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21 287 benefit is directly related to the intensity of eosinophilic inflammation [57]. The sub-population  
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23 288 of COPD patients most likely to respond to anti-IL-5(R) therapy remains unclear, although it  
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25 289 is most likely those with a greater disease burden and higher degree of eosinophilic  
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27 290 inflammation. Importantly in those with a low blood eosinophil count there was a suggestion  
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29 291 of a poorer outcome following treatment with ant-IL5(R) which was not observed in asthma.  
30  
31 292 Whether this reflects a role for the eosinophil in host defence in COPD or the importance of  
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33 293 IL-5 in IgA B cell differentiation [58] as a possible reason for this adverse effect in the low  
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35 294 eosinophil group and an attenuated response in those with the same degree of eosinophilic  
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37 295 inflammation as asthma or because the eosinophil is less important in COPD needs to be further  
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39 296 explored. However, a small *post hoc* study of the effects of benralizumab upon the airway  
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41 297 microbiome from samples obtained in the phase 2a study suggest that benralizumab does not  
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43 298 have an adverse effect on the bacterial load or composition [59].  
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51 300 Other T2-directed therapies have been tested in COPD or are ongoing. GATA 3 inhibition  
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53 301 reduces the sputum eosinophil count in COPD but like ant-IL5 did not affect clinical endpoints  
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55 302 [60]. A single trial of an anti-IL-13 (Lebrikizumab) has been tested in COPD. The full result  
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57 303 of the study is yet to be published but the press release reported that COPD exacerbations were  
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3 304 not reduced in those receiving lebrikizumab versus placebo ([NCT02546700](#)). In phase 3 studies  
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5 305 for asthma, anti-IL-13 [51] failed to meet their primary outcome for reduction in exacerbations,  
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7 306 whereas in contrast anti-IL4R $\alpha$  substantially reduced exacerbations. Whether anti-IL4R $\alpha$  has  
8  
9 307 efficacy in COPD is currently being tested. The role of the DAMPs thymic stromal  
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11 308 lymphopoietin (TSLP) and IL33 are also being tested in COPD. DP2 antagonism in COPD  
12  
13 309 reduced the intensity of eosinophilic inflammation [61]. Whether DP2 antagonists are  
14  
15 310 beneficial in a subgroup of COPD patients with underlying eosinophilic inflammation requires  
16  
17 311 future studies.  
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### 313 ***Specific pro-inflammatory and pro-neutrophilic cytokines and chemokines in COPD***

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26 314 While the main inflammatory pathway in COPD is neutrophilic in nature, studies targeting  
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28 315 neutrophilic inflammation have been disappointing to date (**Table 2**). The chemokine CXCL8  
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30 316 (IL-8) is known to attract and activate neutrophils during an inflammatory response via the  
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32 317 CXC chemokine receptor 1 (CXCR1) and CXCR2. In a small study a monoclonal antibody  
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34 318 targeting IL-8 in COPD showed improved dyspnoea measured using the transitional dyspnoea  
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36 319 index [62]. Anti-CXCR2 demonstrated small improvements in lung function particularly in  
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38 320 those who were current smokers but did reduce exacerbations and led to increased infection  
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40 321 rates in longer-term follow-up [63, 64]. Anti-TNF (infliximab) in COPD showed no  
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42 322 improvements in health status, lung function, symptoms nor exacerbation frequency [65-67].  
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44 323 Importantly, increased adverse events were noted in those receiving infliximab, including  
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46 324 cancer and pneumonia [67]. Targeting IL-17 with biological therapy has also been ineffective  
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48 325 in COPD [68]. The inflammasome has been targeted with two independent anti-IL-1R1  
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50 326 biologics [69, 70]. In both trials there were neither benefits nor increased adverse events in  
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52 327 those COPD subjects that received the biologic versus placebo.  
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3 329 Thus targeting neutrophilic inflammation, the inflammasome, TNF and IL17 have been  
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5 330 ineffective in COPD and in some cases have increased risk of infection. This suggests that  
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7 331 intrinsic activation of these pathways driving an auto-inflammatory process is probably less  
8  
9 332 important than their activation secondary to persistent airway colonisation and infection. It  
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11 333 remains a possibility that targeting auto-immunity with B-cell targeted biologics could be  
12  
13 334 beneficial in COPD. However, it is more likely that targeting bacterial dysbiosis in stable state  
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15 335 and infection at exacerbation events will be more efficacious and will consequently impact  
16  
17 336 upon airway inflammation. Indeed benefits with long-term anti-microbials such as  
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19 337 azithromycin might exert their effects largely upon the airway ecology and then ameliorate  
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21 338 airway inflammation rather than having substantial direct anti-inflammatory effects [71, 72].  
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#### 27 28 340 **Future Directions**

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30 341 Our current understanding of the role of different inflammatory phenotypes in COPD  
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32 342 demonstrate that the identification of eosinophilic COPD has value in directing the use of  
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34 343 corticosteroids in COPD. This fits with the concept of a ‘treatable trait’ [73]. This suggests that  
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36 344 in some COPD sufferers targeting T2-immunity beyond corticosteroids might have value.  
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38 345 However as described above it is not straightforward to extrapolate findings in asthma to COPD  
39  
40 346 and the response to T2-targeted therapies is likely to be different and will need to be carefully  
41  
42 347 tested for each mechanism. Notwithstanding this limitation it would seem likely that this  
43  
44 348 approach will uncover further effective therapies for eosinophilic COPD patients. The impact  
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46 349 on the airway ecology and potential risk of promoting airway infection as observed with non-  
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48 350 T2 targeted anti-inflammatory therapies needs to be carefully studied. However eosinophilic  
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50 351 associated inflammation remains a minority of patients with COPD, meaning therapies to target  
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52 352 other pathways are a priority. Targeting neutrophilic and inflammasome mediated  
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54 353 inflammation in COPD does not seem to be an attractive strategy and more attention should be  
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3 354 focussed upon trying to normalise the airway ecology either through novel anti-microbials or  
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5 355 alternative strategies such as vaccines and phage therapy [74, 75].  
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10 357 The multi-dimensional phenotyping strategy also suggests that the impact of the airway  
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12 358 inflammation might have led to airway and alveoli loss which is then not amenable to anti-  
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14 359 inflammatory therapy. This suggests that again in contrast to asthma the degree to which the  
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16 360 COPD is reversible in response to anti-inflammatory therapy in established disease is limited.  
17  
18 361 This will require a paradigm shift in identifying disease early and having biomarkers that are  
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20 362 predictive of high risk of progression in order to intervene early and change the natural history  
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22 363 of the disease. This would be similar to approaches for inflammatory joint diseases and other  
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24 364 chronic inflammatory conditions. Genome-wide association studies have revealed multiple  
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26 365 genes that are associated with lung function and implicated some genes involved in tissue repair  
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28 366 and immunity. Together these genes have formed a genetic risk score for COPD. This risk  
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30 367 score needs to be extended to identify genetic risk of disease progression or under-development  
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32 368 of full lung function with altered lung function trajectories [76] and increased likelihood of  
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34 369 response to treatment. To date the clinical impact of COPD genetic studies has been limited.  
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36 370 However, the genetic risk score together with early disease biomarkers of changes in small  
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38 371 airway disease such as oscillometry and imaging which have been extensively validated in the  
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40 372 asthma study ATLANTIS [77] could identify at risk groups. The longitudinal study of airway  
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42 373 inflammation and airway ecology in these at risk groups with 'early' COPD [78] would help  
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44 374 to define mechanism for disease onset and progression such as whether changes in bacterial  
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46 375 dysbiosis trigger inflammation and airway damage or a consequence of these features.  
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48 376 Improved adoption of current biomarkers into clinical practice and the development of new  
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50 377 simple, safe, repeatable and preferably near-patient biomarkers will provide insights of the  
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52 378 inflammatory profile in the patient and their airway microenvironment. This will mean that the  
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3 379 tests could be done serially to help with clinical decision making in stable state but also predict  
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5 380 exacerbation events [79] prior to their onset. Breathomics is a particularly attractive approach  
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8 381 with early findings suggesting this could be applied to measure airway and systemic  
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10 382 inflammation as well as microbial dysbiosis with pathogen- and inflammatory profile-specific  
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12 383 breath signatures beginning to be described [80]. Urine biomarkers of systemic inflammation  
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14 384 are more distant from the lung but with the development of home monitoring strategies for  
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16 385 multiple inflammatory mediators coupled to artificial intelligence algorithms to provide risk  
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18 386 stratification of future events could become part of clinical care [81].  
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## 23 24 388 **Conclusion**

25  
26 389 In conclusion, airway inflammation is a consistent feature of COPD and is implicated in the  
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28 390 pathogenesis and progression of COPD. Inflammation in COPD is heterogeneous underscoring  
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30 391 the need for a precision medicine approach [82]. Corticosteroids are most effective in those  
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32 392 with eosinophilic inflammation. Anti-IL5 biologics have been disappointing in COPD versus  
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34 393 asthma suggesting that the role of the eosinophil is different in COPD. However, the response  
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36 394 to corticosteroids and partial response to anti-IL5 in this group does suggest that it is a tractable  
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38 395 phenotype and further studies of mechanism and alternative interventions are warranted.  
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40 396 Therapies targeting neutrophilic inflammation and the inflammasome have been ineffective  
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42 397 and in some cases increased risk of infection suggesting that their activation might be a  
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44 398 consequence of bacterial colonisation and dysbiosis. Underscoring the need to focus on  
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46 399 bacterial dysbiosis as a target to then secondarily attenuate airway inflammation. Therefore to  
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48 400 realise anti-inflammatory precision medicine in COPD we need to stop chasing rainbows and  
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50 401 improve the characterisation of the disease to reflect the complexity of the multi-dimensional  
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52 402 mechanisms driving COPD in individual patients.  
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**Box 1: Key Points**

- COPD results from an abnormal inflammatory response which is highly heterogeneous in nature
- Eosinophilic COPD is responsive to corticosteroids and identifies those most likely to respond to T2 targeted biological therapy
- Treatments to target neutrophilic inflammation have failed to show efficacy
- Neutrophilic inflammation is likely to be a response to changes in microbial ecology

405

406 **Table 1. Randomised placebo-controlled trials of anti-T2 therapies in COPD**

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Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Benralizumab; anti-IL-5R 100mg every 4 weeks (3 doses) then every 8 weeks (5 doses), 56 week N= 82 [53]	↔ Moderate-to-severe exacerbations	↑ FEV1 in intervention group ↔ health status ↓ Blood and sputum eosinophils
Benralizumab (TERRANOVA); anti-IL5R (NCT02155660) 10, 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=2255 [54]	↔ Exacerbations	Not yet reported
Benralizumab (GALATHEA); anti-IL5R (NCT02138916) 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=1656 [54]	↔ Exacerbations	Not yet reported
Mepolizumab; anti-IL-5 (NCT01463644) 750mg/month, for 6 months N= 18 [55]	↓ Sputum eosinophils	↓ Blood eosinophil ↔ FEV1, CAT, CRQ, exacerbations
Mepolizumab; anti-IL-5 (METREX) (NCT02105961) 100mg or 300mg every 4 weeks, 52 weeks N= 1070 [56]	↓ Exacerbations in pre-specified (n= 462) eosinophilic group	↑ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Mepolizumab; anti-IL-5 (METREO) (NCT02105948) 100mg or 300mg every 4 weeks, 52 weeks N= 674 [56]	↔ Exacerbations	↔ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Anti-GATA3 Inhaled 10 mg SB010 BID 28 days, N=23 [60]	Feasibility study	↓ Sputum eosinophil ↔ FEV1, FENO, symptoms

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409

410 **Table 2. Randomised placebo-controlled trials of anti-neutrophil, TNF and**  
 411 **inflammasome targeted therapies in COPD**

412

Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Anti-IL8; IL-8 (NCT00035828) 800mg loading dose, 400mg/month for 3 months, 5 month follow-up N= 109 [62]	↓ Severity of dyspnoea as measured by transition dyspnoea index	↔ Health status, lung function, 6-min walk test, rescue use of albuterol
Anti-CXCR2 50mg BD OR 80mg BD, 4 weeks [63]	Safety and tolerability	↓ Blood neutrophil counts
AntiCXCR2 Dose 10mg, 30mg or 50mg, 6 months [64]	↑ FEV1 at 6 months	↔ Time to first exacerbation ↓ absolute and percent sputum neutrophil counts ↔ SGRQ score ↑ Rate of respiratory infection
Infliximab; anti-TNF (NCT00244192) 5mg/kg, for 8 weeks N= 22 [65]	↔ Sputum inflammatory cells	↔ FEV1, SGRQ
Etanercept; anti-TNF (NCT 00789997) 50mg, for 90 days N= 81 [66]	↔ FEV1 over 14 days from exacerbation onset	↔ 90 day treatment failure, dyspnoea, health status
Infliximab; TNF (NCT00056264) 3mg/kg or 5mg/kg, 44 weeks. N= 157 [67]	↔ CRQ	↔ FEV1, 6 mins walk test, TDI ↑ Malignancy, pneumonia
CNTO 6785(61); anti-IL-17 (NCT01966549) 6mg/kg every two weeks for 4 weeks then every 4 weeks for remaining 8 weeks N= 186 [68]	↔ pre-BD % predicted FEV1	↔ Post-BD % predicted FEV1 ↔ SGRQ-C ↔ frequency of AECOPD ↔ weekly usage of rescue medication
MEDI 8968; anti-IL-1 (NCT01448850) 300 mg every 4 weeks, 52 weeks N= 160 [69]	↔ Moderate-to-severe exacerbations	↔ SGRQ-C
Canakinumab/ IL-1 (NCT00581945) 1x 1mg/kg, 2x 3mg/kg, , 42x 6mg/kg, 45 weeks [70]	Changes from baseline in FEV1, FVC No statistical analysis provided for changes in FEV1, FVC from baseline	Serious adverse events  No statistical analysis provided

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3 414 **Figure Legends**  
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8 416 **Figure 1.** COPD is a heterogeneous complex disease as a consequence of complex host-  
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10 417 environment interactions due to multiple environmental exposures over time the host's  
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12 418 underlying susceptibility and various host responses at the protein-to-cell and tissue-to-organ  
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14 419 scales leading onto the clinical presentation of daily symptoms and exacerbations.  
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19 421 **Figure 2.** Sampling approaches to study inflammation in COPD illustrating how these  
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21 422 approaches in concert provide insights into the host airway and systemic inflammatory  
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23 423 response and the local airway ecology  
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28 425 **Figure 3.** Cytokine networks in a) Neutrophil-associated inflammasome mediated COPD and  
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30 426 b) eosinophil-associated T2-mediated COPD illustrating the immunological responses to the  
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32 427 multiple environmental stimuli.  
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37 429 **Figure 4.** a) Biological cluster analysis of COPD exacerbations derived from multiplex of  
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39 430 sputum mediators revealing 4 clusters: T2-mediated eosinophilic inflammation, T1-mediated  
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41 431 viral associated, Inflammasome mediated bacteria associated neutrophil associated and pauci-  
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43 432 inflammatory without evidence of increased airway inflammation. Ellipsoid size is reflective  
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45 433 of the number of patients in each cluster. b) Principal component analysis of biological clusters  
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47 434 derived from subjects with asthma and COPD illustrating that the viral, bacterial and  
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49 435 eosinophilic clusters are present in asthma and COPD exacerbations with different proportions  
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51 436 represented in each cluster for each disease.  
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3 438 **Figure 5.** Forest-plot of the effect of mepolizumab versus placebo in severe asthma derived  
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5 439 from the MENSA trial and in COPD from the METREX and METREO trials illustrating the  
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7 440 greater reduction in exacerbations in asthma versus COPD for the same blood eosinophil counts  
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3 **1 Airway inflammation in COPD- progress to precision medicine**  
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## 26 **Summary**

27 Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and  
28 mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent  
29 feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-  
30 inflammatory therapy is not first line treatment. This inflammation has many guises and  
31 phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD  
32 with activation of the inflammasome, T1 and T17 immunity is the most common phenotype  
33 with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed  
34 in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and  
35 revealed that corticosteroids are most effective in those with evidence of eosinophilic  
36 inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD  
37 has been disappointing with smaller benefits for the same intensity of eosinophilic  
38 inflammation questioning its role in COPD. Biological therapies beyond T2-mediated  
39 inflammation have not demonstrated benefit and in some cases increased risk of infection  
40 suggesting that neutrophilic inflammation and inflammasome activation might be largely  
41 driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future  
42 biomarker approaches to assess inflammation in COPD and how this might reveal tractable  
43 approaches to precision medicine and unmask important host-environment interactions leading  
44 to airway inflammation.

45  
46 **Key words:** COPD, ACOS, eosinophil, neutrophil, macrophage, inflammasome, biologics,  
47 eosinophil, interleukin (IL)5, benralizumab, mepolizumab, tumour necrosis factor (TNF) $\alpha$ ,  
48 IL6, IL8, IL1 $\beta$ , IL33, IL13, IL17, anti-IgE antibody, thymic stromal lymphopoietin (TSLP),  
49 prostaglandin D<sub>2</sub> receptor type 2 (DP2)

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51 **Take home message**

52 Airway inflammation drives COPD, but corticosteroids only work in those with eosinophilic  
53 inflammation. There is a need to better understand the patterns of inflammation, the reason for  
54 its persistence and the opportunities for new treatments.

55

## 56 **Introduction**

57 Chronic obstructive pulmonary disease (COPD) is a common disease of chronic lung  
58 inflammation that results in persistent symptoms and fixed airflow obstruction [1]. This is  
59 caused by an inflammatory response following inhalation of cigarette smoke or other noxious  
60 external particles such as air pollution and biomass fuel [1]. Airway and systemic inflammation  
61 in COPD is related to disease progression and mortality [1-24]. Current diagnostic criteria do  
62 not capture the heterogeneity of COPD in terms of the complex pathological changes occurring  
63 within lung, the different airway inflammatory patterns or the airway microbial ecology.  
64 Airway inflammation is a consistent feature of COPD and is present in both the large and small  
65 airways [1, 3-65-8]. The airway inflammation can persist after smoking cessation and is likely  
66 a consequence of altered immunity [68] and changes in the airway microenvironment [8-109-  
67 14].

68  
69 Despite the long-standing recognition that airways inflammation is a key driver of COPD  
70 progression and exacerbations, first-line treatment strategies are aimed at symptomatic  
71 treatment of bronchoconstriction in the form of bronchodilators, rather than anti-inflammatory  
72 therapy [1]. In this review we shall describe the heterogeneity of airway inflammation in  
73 COPD, current and future biomarker approaches to dissect this heterogeneity and redefine  
74 COPD using multi-dimensional phenotyping and how this might reveal tractable approaches  
75 to precision medicine and provide important insights into the host-environment interactions.

## 77 **Multi-dimensional COPD phenotyping providing insights into pathophysiology**

78 COPD is a consequence of complex host-environment interactions that occur over time  
79 summarised in **Figure 1**. Smoking, and other pollutants, pathogens and allergens insult the  
80 lung promoting airway inflammation and damage in a susceptible host as a consequence of

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2  
3 81 genetic predisposition and altered immunity [[6](#), [10-128](#), [15-17](#)]. This in turn leads to  
4  
5 82 irreversible damage resulting in fixed airflow obstruction and the consequent typical symptoms  
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8 83 of COPD.  
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### 11 12 85 *Approaches to phenotyping airway inflammation and damage in COPD*

13  
14 86 Insights into airway inflammation and damage to the airways have been derived from lung  
15  
16 87 specimens obtained from surgical resection and at post mortem. Importantly *in vivo* measures  
17  
18 88 of airway and systemic inflammation have been characterised longitudinally, at exacerbations  
19  
20 89 and in response to therapies through invasive sampling of the airway by bronchoscopy (large  
21  
22 90 airway brush and biopsy and smaller airways by bronchoalveolar lavage), non-invasive sputum  
23  
24 91 sampling (mostly large airways) which is safe even in severe COPD [[138](#)], breath analysis  
25  
26 92 (large and small airways), lung imaging (large airways directly and small airways indirectly)  
27  
28 93 and beyond the lung by assessing upper airway samples and systemically using blood and urine  
29  
30  
31  
32  
33 94 [[5](#), [147](#), [19](#)] (**Figure 2**).  
34

35 95

### 36 37 96 *Neutrophil-associated airway inflammation*

38  
39 97 The inflammatory response in COPD involves both innate and adaptive immunity with  
40  
41 98 neutrophilic inflammation the commonest inflammatory phenotype in COPD. Following  
42  
43 99 exposure to cigarette smoke, other pollutants, and oxidants there is airway damage [[1520](#)]  
44  
45 100 leading to release of pro-inflammatory mediators and damage-associated molecular patterns  
46  
47 101 (DAMPs) such as interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP) [[1520-24](#)].  
48  
49 102 The distribution of the IL-33 receptor ST2 is altered in response to cigarette smoke with down-  
50  
51 103 regulation in innate type-2 innate lymphoid cells with up-regulation by macrophages leading  
52  
53 104 to a triggering of an IL-33-dependent exaggerated pro-inflammatory cascade [[1625](#)]. As a  
54  
55 105 consequence of airway damage the altered barrier function predisposes the airway to infection  
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3 106 and bacterial dysbiosis which together with pollutants drive switching of ILC2 cells towards  
4  
5 107 ILC1 cells further amplifying the type-1 inflammatory cascade [1726]. In COPD there is an  
6  
7 108 increase in the phyla proteobacteria and the emergence of *H. Influenzae* predominance such  
8  
9 109 that the ratio of gammaproteobacteria to firmicutes ( $\gamma$ P:F) increase [7-9-13, 1827, 28]. These  
10  
11 110 pathogens themselves promote an inflammatory response via activation of pathogen-associated  
12  
13 111 molecular patterns (PAMPs) and further amplification of the airway inflammation with the  
14  
15 112 intensity of airway inflammation related to the abundance of *H. Influenzae* [19, 2029, 30]. In  
16  
17 113 this scenario, epithelial cells are activated and are involved in the release of inflammatory  
18  
19 114 mediators, such as tumor necrosis factor (TNF), IL-1 $\beta$ , IL-6 and IL-8. Macrophages are  
20  
21 115 recruited with further release or pro-inflammatory cytokines and activation of the NLRP3  
22  
23 116 inflammasome with caspase-1-dependent release of pro-inflammatory IL-1-like cytokines IL-  
24  
25 117 1 $\alpha$ , IL-1 $\beta$ , IL-33 and IL-18 [6, 158, 20]. Activation of the inflammasome can lead to persistence  
26  
27 118 of an inflammatory response by triggering an auto-inflammatory response with intrinsic  
28  
29 119 production of pro-inflammatory mediators independent of exogenous stimuli [68].  
30  
31 120 Interestingly activation of type 1 responses are more closely related to COPD severity than  
32  
33 121 inflammasome activation and thus autoimmunity can occur across disease severity [231].  
34  
35 122 Neutrophils are recruited as the predominant cells with consequent release of proteases and  
36  
37 123 airway damage as well as activation of innate lymphoid type 3 cells (ILC3). The adaptive  
38  
39 124 immune response is also involved with polarization and subsequent recruitment of CD4+ Th1  
40  
41 125 and Th17 cells, which produce IFN- $\gamma$  and IL-17A and IL-17F [6, 15, 228, 20, 32, 33]  
42  
43 126 respectively with a later predominance of CD8+ T-cells. In concert or independent of the auto-  
44  
45 127 inflammatory response there is an auto-immune response which can also promote persistence  
46  
47 128 of inflammation [68, 34-36]. In more severe disease there is an accumulation of B cells  
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49 129 particularly in the smaller airways which together with T cells and follicular dendritic cells  
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51 130 comprise aggregates organised into tertiary lymphoid follicles [23]. These lymphoid follicles  
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3 131 support the priming and clonal expansion of T and B-cells with an increase proportion of IgA+  
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5 132 B-cells perhaps in response to increased persistent airway infection or auto-antigens [24, 2537].  
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7  
8 133 The cytokine network in neutrophil-associated COPD is summarised in **Figure 3A**.  
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### 12 135 *Eosinophil-associated airway inflammation*

14 136 Even though neutrophil-associated COPD is the most common inflammatory phenotype,  
15  
16  
17 137 consistent with the heterogeneity of the disease 10-40% of COPD patients demonstrate  
18  
19 138 increased eosinophilic inflammation in the sputum and or blood [5, 26, 277, 38, 39] with  
20  
21 139 increased T2-transcriptome signatures [2840]. The broad range in prevalence is in part due to  
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23  
24 140 differences in patient populations but also due to different cut-offs applied in sputum (>2 or  
25  
26 141 >3% eosinophils) or blood (2% or >250, 300, 400 eosinophils/ $\mu$ L). Increased eosinophilic  
27  
28 142 inflammation in peripheral blood and sputum samples in COPD, like asthma, is associated with  
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30  
31 143 a greater future risk of severe exacerbations [29, 3039, 41]. The aetiology of eosinophilic  
32  
33 144 inflammation in COPD is uncertain. As with neutrophil-associated COPD eosinophilic COPD  
34  
35 145 is also likely to be a combination of innate and adaptive immunity summarised in **Figure 3B**.  
36  
37  
38 146 These pathways are well-described for asthma [5, 307, 42]. Following allergic sensitisation and  
39  
40 147 T-cell polarisation TH2 cells produce interleukin (IL)-4, IL-5, and IL-13. IL-5 is an obligate  
41  
42 148 cytokine for the survival and maturation of eosinophils, and IL-4 and IL-13 promote IgE  
43  
44 149 production from B cells and have direct effects upon structural cells. Recruitment of  
45  
46  
47 150 eosinophils to the lung mucosa is mediated via production of predominantly epithelium-  
48  
49 151 derived CCR3 chemokines and other eosinophil chemoattractants, such as mast cell-derived  
50  
51 152 prostaglandin (PG)D2. PGD2 amplifies T2 immunity via activation of PGD2 type 2 receptors  
52  
53 153 (DP2 or CRTH2). Total IgE is elevated in eosinophilic COPD even though atopy is not  
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56 154 increased. Whether this reflects a hitherto undescribed allergen is unclear. Eosinophilic  
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58 155 inflammation can also occur via activation of ILC2 cells, which produce IL-5 and IL-13 in  
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3 156 response to PGD2 and the epithelial-derived ‘alarmins’ IL-33, IL-25, and TSLP released after  
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5 157 epithelial damage by pollutants and microbes. Additional contributions might be from  
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7 158 macrophage-derived IL33 released following inflammasome activation. Whether these innate  
8  
9 159 and acquired T2-mediated immune mechanisms occur in COPD, the predominance of one over  
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11 160 another is more important in COPD versus asthma or whether there are alternative mechanisms  
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13 161 driving eosinophilic inflammation in COPD remains unclear.  
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### 19 163 ***Biological clustering to dissect heterogeneity of airways inflammation***

21 164 These eosinophilic versus neutrophilic associated inflammatory profiles represent extreme  
22  
23 165 phenotypes. However they are consistently reproducible and demonstrate phenotype stability  
24  
25 166 [[20](#), [2630](#), [38](#)]. The neutrophil and eosinophil-associated phenotypes also exhibit distinct  
26  
27 167 microbial ecology with  $\gamma$ P:F predominance in the neutrophilic phenotype [[8](#), [9](#), [3112](#), [13](#), [43](#)].  
28  
29 168 However, to describe extremes can be an over-simplification of a complex underlying biology.  
30  
31 169 To validate these phenotypes and to further inform the understanding of the heterogeneity of  
32  
33 170 COPD in stable state unbiased statistical approaches such as cluster analysis have been applied  
34  
35 171 to large clinical and biological datasets [[18](#), [32](#), [3328](#), [44](#), [45](#)]. Interestingly these have  
36  
37 172 underscored the importance of eosinophilic airway inflammation in asthma, COPD and the  
38  
39 173 asthma-COPD overlap syndrome (ACOS) [[32](#), [3444](#), [46](#)]. Combined data from asthma and  
40  
41 174 COPD revealed three biological clusters [[3244](#)]. Cluster 1 consisted of asthma subjects with  
42  
43 175 increase IL-5, IL-13 and CCL26 mediators and eosinophil predominance. Cluster 2 consisted  
44  
45 176 of an overlap between asthma and COPD with neutrophil predominance. Cluster 3 consisted  
46  
47 177 mainly of COPD patients with a mixed granulocytic airway inflammation. The differences seen  
48  
49 178 between neutrophilic COPD in cluster 2 and eosinophilic COPD in cluster 3 included the  
50  
51 179 presence of increased bacterial colonisation with an increased  $\gamma$ P:F ratio in the former and  
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3 180 increased CCL13 in the latter possibly explaining the observed airway inflammation  
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5 181 differences seen between these clusters (**Figure 4A**).

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10 183 Using a similar unbiased cluster analysis approach for COPD exacerbations four biological  
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12 184 clusters were identified and these validated the *a priori* aetiological groups: 'Pro-inflammatory'  
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14 185 bacterial-associated, 'Th1' viral-associated, 'Th2' eosinophilic-associated and a fourth group  
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17 186 that were termed 'pauci-inflammatory' as this was associated with limited changes in the  
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19 187 inflammatory profile (**Figure 4B**) [4335]. Disease severity was not different between these  
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22 188 biological clusters and the biomarkers were associated with their respective potential  
23  
24 189 aetiologies. In the pro-inflammatory bacterial-associated group the strongest discriminating  
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26 190 inflammatory mediator was sputum IL-1 $\beta$  with increased  $\gamma$ P:F consistent with bacterial  
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28 191 dysbiosis. The blood eosinophil count was the best predictor of sputum eosinophilic  
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30 192 inflammation (>3% eosinophils) at the time of the exacerbation in this study although the  
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33 193 correlations are typically weaker in stable disease [3547] Interestingly Bafadhel *et al* found  
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35 194 that patients experienced more bacterial exacerbations if their stable sputum samples contained  
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38 195 more bacteria and high  $\gamma$ P:F and more eosinophilic exacerbations if eosinophilic inflammation  
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40 196 was present in the stable state suggesting that the exacerbation event was an amplification of  
41  
42 197 the underlying phenotype [3345]. Thus these biomarkers in addition to directing therapy during  
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44  
45 198 the exacerbation event might identify subgroups to target therapy in stable state with the aim  
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47 199 of reducing future risk. The exception to this was a viral infection representing a new event  
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49 200 and a new inflammatory profile with increased blood and sputum concentrations of the  
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51 201 interferon-inducible chemokines CXCL10 and CXCL11.

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56 203 *Airway damage and remodelling- emphysema and small airway obliteration*  
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3 204 Airway inflammation in COPD contributes to airway damage, remodelling, loss of small  
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5 205 airways and emphysema (tissue damage with permanent dilatation distal to the terminal  
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7 206 bronchiole). Chronic airflow obstruction is due to a combination of emphysema and small  
8  
9 207 airway obliteration. Small airways are the major site of airway obstruction in COPD [48]. This  
10  
11 208 small airways obliteration is due to a combination of remodelling and accumulation of  
12  
13 209 inflammatory exudates within the airway lumen, both of which increase with disease severity  
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15 210 [36, 3748, 49]. Remodelling changes observed in COPD include disruption and loss of  
16  
17 211 epithelial cilia, squamous metaplasia of the respiratory epithelium, goblet cell hyperplasia and  
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19 212 mucous gland enlargement, bronchiolar smooth muscle hypertrophy, airway wall fibrosis and  
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21 213 inflammatory cell infiltration [36, 3748, 49].  
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28 214  
29 215 Computed tomography (CT) and micro CT has demonstrated a reduction in the luminal area  
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31 216 of terminal bronchioles in COPD, but also substantial loss of terminal airways [38-4050-52].  
32  
33 217 This is consistent with the view that the inflammation and remodelling of the small airways  
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35 218 largely as a consequence of inflammation leads to destruction of the terminal followed by  
36  
37 219 respiratory bronchioles to form centrilobular lesions. This in turn can result in destruction of  
38  
39 220 entire lung lobules which coalesce to form bullous emphysema. Thus narrowing and  
40  
41 221 consequent disappearance of small conducting airways can explain the increased peripheral  
42  
43 222 airway resistance reported in COPD prior to the development of emphysema [38-4050-52].  
44  
45 223 The distribution of emphysema can be centrilobular or panacinar. It is uncertain if these  
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47 224 represent a spectrum with panacinar a consequence of centrilobular emphysema or if they  
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49 225 represent distinct conditions. Panacinar is observed in individuals with alpha-1-anti-trypsin  
50  
51 226 deficiency perhaps suggesting this form of emphysema might be largely a consequence of the  
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53 227 imbalance between protease and anti-protease activity whereas centrilobular is largely due to  
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55 228 loss of and remodelling of small airways caused by persistent airway inflammation.  
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3 229 Quantitative CT has demonstrated that gas trapping due to small airway disease moreover than  
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6 230 emphysema is related to lung function impairment [41, 4253]. These mechanisms of small  
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8 231 airway obliteration and emphysema are important when considering anti-inflammatory therapy  
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10 232 as only the remaining inflamed airways can be targeted in contrast to the airways and alveoli  
11  
12 233 that are already destroyed in patients with COPD.  
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### 17 235 **Airway inflammation in COPD- progress to precision medicine**

18  
19 236 Increasing knowledge of disease pathology and inflammatory phenotypes will inform our  
20  
21 237 understanding of COPD and enable phenotype-specific clinical management beyond the first  
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23  
24 238 line bronchodilator therapy for COPD.  
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### 28 240 ***Eosinophilic COPD- corticosteroids***

29  
30 241 Corticosteroids have been used in the treatment of COPD for more than 40 years with moderate  
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32  
33 242 overall benefit in terms of improvement in lung function, health status, 6 minute walk distance  
34  
35 243 and exacerbation frequency [1]. More recently a differential response in patients has been seen  
36  
37 244 based on eosinophil count. An elevated sputum eosinophil count is associated with a greater  
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39  
40 245 response to both inhaled and oral corticosteroids in stable disease [43, 4454-56], whilst blood  
41  
42 246 eosinophil count can be used to predict response to corticosteroid response in stable [45, 4657-  
43  
44 247 59] and acute COPD [4760-62] and titration of corticosteroids directed by sputum eosinophil  
45  
46 248 counts reduces hospital admissions [4863]. Importantly most of these studies have recruited  
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48  
49 249 COPD subjects with frequent exacerbations and thus whether findings can be generalised to all  
50  
51 250 COPD subjects is uncertain. Additionally it is unclear if the clinical benefits, such as lung  
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53 251 function and health status, with corticosteroids are independent of the reduction of  
54  
55 252 exacerbations. In contrast non-T2 pathways such as IL-17 activation as determined by the  
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57  
58 253 epithelial IL-17A response transcriptome signature are associated with a decreased response to  
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3 254 corticosteroids [4964]. Whether the benefit from corticosteroids in COPD associated with  
4  
5 255 eosinophilic inflammation is restricted to its effects upon the eosinophil or due to other broader  
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8 256 anti-inflammatory effects is uncertain. GOLD now includes the blood eosinophil count as a  
9  
10 257 biomarker to direct the use of ICS in COPD patients with frequent exacerbations [1]. Benefits  
11  
12 258 in response to roflumilast are also possibly due to attenuation of eosinophilic inflammation  
13  
14  
15 259 [5065].  
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17 260

### 19 261 *Eosinophilic COPD- T2 targeted therapies*

21 262 Evidence for targeting T2-mediated inflammation using biologics has revolutionised clinical  
22  
23  
24 263 practice in severe asthma [30, 5142, 66]. As described above significant eosinophilic  
25  
26 264 inflammation does exist in COPD, albeit in a smaller proportion of patients than in asthma.  
27  
28 265 However, the findings from the phase 2 and 3 trials of T2-directed therapies for COPD  
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30  
31 266 summarised in **Table 1** have been disappointing compared to asthma [42, 5266, 67].  
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33 267

35 268 In the first anti-IL5R biologic (benralizumab) trial in COPD while a reduction in eosinophilic  
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37 269 inflammation was observed, the primary outcome annual rate of acute exacerbations was not  
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39  
40 270 met, which included all patients with COPD, irrespective of baseline eosinophil count [5368].  
41  
42 271 Importantly the sample size was small to study exacerbations and was underpowered to observe  
43  
44 272 small effects. Secondary outcomes showed an improvement in FEV<sub>1</sub> in those receiving  
45  
46 273 benralizumab but no difference was observed in health status. In a pre-specified post hoc  
47  
48 274 analysis improvements in exacerbation frequency, lung function and health status were related  
49  
50 275 to the intensity of baseline blood and sputum eosinophil count. In the yet to be fully reported  
51  
52 276 phase 3 trials of benralizumab in COPD the primary outcome of exacerbations in those with  
53  
54 277 increased blood eosinophil count ( $\geq 220$  cells/ $\mu$ L) was also not met [5469]. In a small single  
55  
56 278 centre study mepolizumab reduced sputum eosinophil count, but did not improve lung function  
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3 279 or health status [557068]. In two phase 3 trials of mepolizumab in COPD (METREX and  
4  
5 280 METREO) there were small reductions in moderate or severe exacerbations in the eosinophilic  
6  
7 281 sub-group ( $\geq 150$  cells/ $\mu\text{L}$ ), which was statistically significant in the METREX (18% reduction)  
8  
9  
10 282 but not in METREO [5671]. In a *post hoc* analysis there was no reduction in exacerbation  
11  
12 283 events treated with antibiotics alone in those receiving mepolizumab versus placebo but the  
13  
14 284 reduction in exacerbations treated with oral corticosteroids with or without antibiotics was  
15  
16 285 ~35% in those with blood eosinophil counts  $>300$  eosinophils/ $\mu\text{L}$ . No improvements in lung  
17  
18 286 function and health status in those receiving mepolizumab versus placebo were observed.  
19  
20  
21 287  
22  
23  
24 288 Importantly, both the mepolizumab and benralizumab studies suggest that the effect size is  
25  
26 289 smaller than that seen in severe asthma (**Figure 5**) although, like asthma, the magnitude of  
27  
28 290 benefit is directly related to the intensity of eosinophilic inflammation [5772]. The sub-  
29  
30 291 population of COPD patients most likely to respond to anti-IL-5(R) therapy remains unclear,  
31  
32  
33 292 although it is most likely those with a greater disease burden and higher degree of eosinophilic  
34  
35 293 inflammation. Importantly in those with a low blood eosinophil count there was a suggestion  
36  
37 294 of a poorer outcome following treatment with anti-IL5(R) which was not observed in asthma.  
38  
39  
40 295 Whether this reflects a role for the eosinophil in host defence in COPD or the importance of  
41  
42 296 IL-5 in IgA B cell differentiation [5873] as a possible reason for this adverse effect in the low  
43  
44 297 eosinophil group and an attenuated response in those with the same degree of eosinophilic  
45  
46 298 inflammation as asthma or because the eosinophil is less important in COPD needs to be further  
47  
48 299 explored. However, a small *post hoc* study of the effects of benralizumab upon the airway  
49  
50 300 microbiome from samples obtained in the phase 2a study suggest that benralizumab does not  
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52  
53 301 have an adverse effect on the bacterial load or composition [5974].  
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3 303 Other T2-directed therapies have been tested in COPD or are ongoing. GATA 3 inhibition  
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5 304 reduces the sputum eosinophil count in COPD but like ant-IL5 did not affect clinical endpoints  
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7  
8 305 [[6075](#)]. A single trial of an anti-IL-13 (Lebrikizumab) has been tested in COPD. The full result  
9  
10 306 of the study is yet to be published but the press release reported that COPD exacerbations were  
11  
12 307 not reduced in those receiving lebrikizumab versus placebo ([NCT02546700](#)). In phase 3 studies  
13  
14  
15 308 for asthma, anti-IL-13 [[5166](#)] failed to meet their primary outcome for reduction in  
16  
17 309 exacerbations, whereas in contrast anti-IL4R $\alpha$  substantially reduced exacerbations. Whether  
18  
19 310 anti-IL4R $\alpha$  has efficacy in COPD is currently being tested. The role of the DAMPs thymic  
20  
21 311 stromal lymphopoietin (TSLP) and IL33 are also being tested in COPD. DP2 antagonism in  
22  
23 312 COPD reduced the intensity of eosinophilic inflammation [[6176](#)]. Whether DP2 antagonists  
24  
25 313 are beneficial in a subgroup of COPD patients with underlying eosinophilic inflammation  
26  
27 314 requires future studies.  
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### 316 ***Specific pro-inflammatory and pro-neutrophilic cytokines and chemokines in COPD***

317 While the main inflammatory pathway in COPD is neutrophilic in nature, studies targeting  
318 neutrophilic inflammation have been disappointing to date (**Table 2**). The chemokine CXCL8  
319 (IL-8) is known to attract and activate neutrophils during an inflammatory response via the  
320 CXC chemokine receptor 1 (CXCR1) and CXCR2. In a small study a monoclonal antibody  
321 targeting IL-8 in COPD showed improved dyspnoea measured using the transitional dyspnoea  
322 index [[6277](#)]. Anti-CXCR2 demonstrated small improvements in lung function particularly in  
323 those who were current smokers but did reduce exacerbations and led to increased infection  
324 rates in longer-term follow-up [[63](#), [6478](#), [79](#)]. Anti-TNF (infliximab) in COPD showed no  
325 improvements in health status, lung function, symptoms nor exacerbation frequency [[65-6780-](#)  
326 [82](#)]. Importantly, increased adverse events were noted in those receiving infliximab, including  
327 cancer and pneumonia [[6782](#)]. Targeting IL-17 with biological therapy has also been  
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3 328 ineffective in COPD [683]. The inflammasome has been targeted with two independent anti-  
4  
5 329 IL-1R1 biologics [69, 7084, 85]. In both trials there were neither benefits nor increased adverse  
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7  
8 330 events in those COPD subjects that received the biologic versus placebo.

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11  
12 332 Thus targeting neutrophilic inflammation, the inflammasome, TNF and IL17 have been  
13  
14 333 ineffective in COPD and in some cases have increased risk of infection. This suggests that  
15  
16 334 intrinsic activation of these pathways driving an auto-inflammatory process is probably less  
17  
18 335 important than their activation secondary to persistent airway colonisation and infection. It  
19  
20 336 remains a possibility that targeting auto-immunity with B-cell targeted biologics could be  
21  
22 337 beneficial in COPD. However, it is more likely that targeting bacterial dysbiosis in stable state  
23  
24 338 and infection at exacerbation events will be more efficacious and will consequently impact  
25  
26 339 upon airway inflammation. Indeed benefits with long-term anti-microbials such as  
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28 340 azithromycin might exert their effects largely upon the airway ecology and then ameliorate  
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30 341 airway inflammation rather than having substantial direct anti-inflammatory effects [71, 7286,  
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32 342 87].

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#### 39 40 344 **Future Directions**

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42 345 Our current understanding of the role of different inflammatory phenotypes in COPD  
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44 346 demonstrate that the identification of eosinophilic COPD has value in directing the use of  
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46 347 corticosteroids in COPD. This fits with the concept of a ‘treatable trait’ [7388]. This suggests  
47  
48 348 that in some COPD sufferers targeting T2-immunity beyond corticosteroids might have value.  
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50 349 However as described above it is not straightforward to extrapolate findings in asthma to COPD  
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52 350 and the response to T2-targeted therapies is likely to be different and will need to be carefully  
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54 351 tested for each mechanism. Notwithstanding this limitation it would seem likely that this  
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56 352 approach will uncover further effective therapies for eosinophilic COPD patients. The impact  
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3 353 on the airway ecology and potential risk of promoting airway infection as observed with non-  
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5 354 T2 targeted anti-inflammatory therapies needs to be carefully studied. However eosinophilic  
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8 355 associated inflammation remains a minority of patients with COPD, meaning therapies to target  
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10 356 other pathways are a priority. Targeting neutrophilic and inflammasome mediated  
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12 357 inflammation in COPD does not seem to be an attractive strategy and more attention should be  
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15 358 focussed upon trying to normalise the airway ecology either through novel anti-microbials or  
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17 359 alternative strategies such as vaccines and phage therapy [74, 7589, 90].  
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361 The multi-dimensional phenotyping strategy also suggests that the impact of the airway  
362 inflammation might have led to airway and alveoli loss which is then not amenable to anti-  
363 inflammatory therapy. This suggests that again in contrast to asthma the degree to which the  
364 COPD is reversible in response to anti-inflammatory therapy in established disease is limited.  
365 This will require a paradigm shift in identifying disease early and having biomarkers that are  
366 predictive of high risk of progression in order to intervene early and change the natural history  
367 of the disease. This would be similar to approaches for inflammatory joint diseases and other  
368 chronic inflammatory conditions. Genome-wide association studies have revealed multiple  
369 genes that are associated with lung function and implicated some genes involved in tissue repair  
370 and immunity. Together these genes have formed a genetic risk score for COPD. This risk  
371 score needs to be extended to identify genetic risk of disease progression or under-development  
372 of full lung function with altered lung function trajectories [7691, 92] and increased likelihood  
373 of response to treatment. To date the clinical impact of COPD genetic studies has been limited.  
374 However, the genetic risk score together with early disease biomarkers of changes in small  
375 airway disease such as oscillometry and imaging which have been extensively validated in the  
376 asthma study ATLANTIS [7793] could identify at risk groups. The longitudinal study of  
377 airway inflammation and airway ecology in these at risk groups with 'early' COPD [78] would

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3 378 help to define mechanism for disease onset and progression such as whether changes in  
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5 379 bacterial dysbiosis trigger inflammation and airway damage or a ~~a~~-consequence of these  
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8 380 features-. Improved adoption of current biomarkers into clinical practice and the development  
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10 381 of new simple, safe, repeatable and preferably near-patient biomarkers will provide insights of  
11  
12 382 the inflammatory profile in the patient and their airway microenvironment. This will mean that  
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14 383 the tests could be done serially to help with clinical decision making in stable state but also  
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17 384 predict exacerbation events [79] prior to their onset. Breathomics is a particularly attractive  
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19 385 approach with early findings suggesting this could be applied to measure airway and systemic  
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21 386 inflammation as well as microbial dysbiosis with pathogen- and inflammatory profile-specific  
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24 387 breath signatures beginning to be described [8094-96]. Urine biomarkers of systemic  
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26 388 inflammation are more distant from the lung but with the development of home monitoring  
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28 389 strategies for multiple inflammatory mediators coupled to artificial intelligence algorithms to  
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31 390 provide risk stratification of future events could become part of clinical care [8197, 98].  
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## 35 392 **Conclusion**

37 393 In conclusion, airway inflammation is a consistent feature of COPD and is implicated in the  
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40 394 pathogenesis and progression of COPD. Inflammation in COPD is heterogeneous underscoring  
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42 395 the need for a precision medicine approach [82]. Corticosteroids are most effective in those  
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44 396 with eosinophilic inflammation. Anti-IL5 biologics have been disappointing in COPD versus  
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47 397 asthma suggesting that the role of the eosinophil is different in COPD. However, the response  
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49 398 to corticosteroids and partial response to anti-IL5 in this group does suggest that it is a tractable  
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51 399 phenotype and further studies of mechanism and alternative interventions are warranted.  
52  
53 400 Therapies targeting neutrophilic inflammation and the inflammasome have been ineffective  
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56 401 and in some cases increased risk of infection suggesting that their activation might be a  
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58 402 consequence of bacterial colonisation and dysbiosis. Underscoring the need to focus on  
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3 403 bacterial dysbiosis as a target to then secondarily attenuate airway inflammation. Therefore to  
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5 404 realise anti-inflammatory precision medicine in COPD we need to stop chasing rainbows and  
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7 405 improve the characterisation of the disease to reflect the complexity of the multi-dimensional  
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9 406 mechanisms driving COPD in individual patients.  
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**Box 1: Key Points**

- COPD results from an abnormal inflammatory response which is highly heterogeneous in nature
- Eosinophilic COPD is responsive to corticosteroids and identifies those most likely to respond to T2 targeted biological therapy
- Treatments to target neutrophilic inflammation have failed to show efficacy
- Neutrophilic inflammation is likely to be a response to changes in microbial ecology

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410 **Table 1. Randomised placebo-controlled trials of anti-T2 therapies in COPD**

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Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Benralizumab; anti-IL-5R 100mg every 4 weeks (3 doses) then every 8 weeks (5 doses), 56 week N= 82 <a href="#">[5368]</a>	↔ Moderate-to-severe exacerbations	↑ FEV1 in intervention group ↔ health status ↓ Blood and sputum eosinophils
Benralizumab (TERRANOVA); anti-IL5R (NCT02155660) 10, 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=2255 <a href="#">[5469]</a>	↔ Exacerbations	Not yet reported
Benralizumab (GALATHEA); anti-IL5R (NCT02138916) 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=1656 <a href="#">[5469]</a>	↔ Exacerbations	Not yet reported
Mepolizumab; anti-IL-5 (NCT01463644) 750mg/month, for 6 months N= 18 <a href="#">[5570]</a>	↓ Sputum eosinophils	↓ Blood eosinophil ↔ FEV1, CAT, CRQ, exacerbations
Mepolizumab; anti-IL-5 (METREX) (NCT02105961) 100mg or 300mg every 4 weeks, 52 weeks N= 1070 <a href="#">[5671]</a>	↓ Exacerbations in pre-specified (n= 462) eosinophilic group	↑ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Mepolizumab; anti-IL-5 (METREO) (NCT02105948) 100mg or 300mg every 4 weeks, 52 weeks N= 674 <a href="#">[5671]</a>	↔ Exacerbations	↔ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Anti-GATA3 Inhaled 10 mg SB010 BID 28 days, N=23 <a href="#">[6075]</a>	Feasibility study	↓ Sputum eosinophil ↔ FEV1, FENO, symptoms

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413

414 **Table 2. Randomised placebo-controlled trials of anti-neutrophil, TNF and**  
 415 **inflammasome targeted therapies in COPD**

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Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Anti-IL8; IL-8 (NCT00035828) 800mg loading dose, 400mg/month for 3 months, 5 month follow-up N= 109 [6277]	↓ Severity of dyspnoea as measured by transition dyspnoea index	↔ Health status, lung function, 6-min walk test, rescue use of albuterol
Anti-CXCR2 50mg BD OR 80mg BD, 4 weeks [6378]	Safety and tolerability	↓ Blood neutrophil counts
AntiCXCR2 Dose 10mg, 30mg or 50mg, 6 months [6479]	↑ FEV1 at 6 months	↔ Time to first exacerbation ↓ absolute and percent sputum neutrophil counts ↔ SGRQ score ↑ Rate of respiratory infection
Infliximab; anti-TNF (NCT00244192) 5mg/kg, for 8 weeks N= 22 [6580]	↔ Sputum inflammatory cells	↔ FEV1, SGRQ
Etanercept; anti-TNF (NCT 00789997) 50mg, for 90 days N= 81 [6681]	↔ FEV1 over 14 days from exacerbation onset	↔ 90 day treatment failure, dyspnoea, health status
Infliximab; TNF (NCT00056264) 3mg/kg or 5mg/kg, 44 weeks. N= 157 [6782]	↔ CRQ	↔ FEV1, 6 mins walk test, TDI ↑ Malignancy, pneumonia
CNTO 6785(61); anti-IL-17 (NCT01966549) 6mg/kg every two weeks for 4 weeks then every 4 weeks for remaining 8 weeks N= 186 [6883]	↔ pre-BD % predicted FEV1	↔ Post-BD % predicted FEV1 ↔ SGRQ-C ↔ frequency of AECOPD ↔ weekly usage of rescue medication
MEDI 8968; anti-IL-1 (NCT01448850) 300 mg every 4 weeks, 52 weeks N= 160 [6984]	↔ Moderate-to-severe exacerbations	↔ SGRQ-C
Canakinumab/ IL-1 (NCT00581945) 1x 1mg/kg, 2x 3mg/kg, , 42x 6mg/kg, 45 weeks [7085]	Changes from baseline in FEV1, FVC No statistical analysis provided for changes in FEV1, FVC from baseline	Serious adverse events  No statistical analysis provided

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3 **418 Figure Legends**  
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8 **420 Figure 1.** COPD is a heterogeneous complex disease as a consequence of complex host-  
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10 421 environment interactions due to multiple environmental exposures over time the host's  
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12 422 underlying susceptibility and various host responses at the protein-to-cell and tissue-to-organ  
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14 423 scales leading onto the clinical presentation of daily symptoms and exacerbations.  
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19 **425 Figure 2.** Sampling approaches to study inflammation in COPD illustrating how these  
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21 426 approaches in concert provide insights into the host airway and systemic inflammatory  
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23 427 response and the local airway ecology  
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28 **429 Figure 3.** Cytokine networks in a) Neutrophil-associated inflammasome mediated COPD and  
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30 430 b) eosinophil-associated T2-mediated COPD illustrating the immunological responses to the  
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32 431 multiple environmental stimuli.  
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37 **433 Figure 4.** a) Biological cluster analysis of COPD exacerbations derived from multiplex of  
38  
39 434 sputum mediators revealing 4 clusters: T2-mediated eosinophilic inflammation, T1-mediated  
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41 435 viral associated, Inflammasome mediated bacteria associated neutrophil associated and pauci-  
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43 436 inflammatory without evidence of increased airway inflammation. Ellipsoid size is reflective  
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45 of the number of patients in each cluster. b) Principal component analysis of biological clusters  
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47 437 derived from subjects with asthma and COPD illustrating that the viral, bacterial and  
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49 438 eosinophilic clusters are present in asthma and COPD exacerbations with different proportions  
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51 439 represented in each cluster for each disease.  
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442 **Figure 5.** Forest-plot of the effect of mepolizumab versus placebo in severe asthma derived  
443 from the MENSA trial and in COPD from the METREX and METREO trials illustrating the  
444 greater reduction in exacerbations in asthma versus COPD for the same blood eosinophil counts

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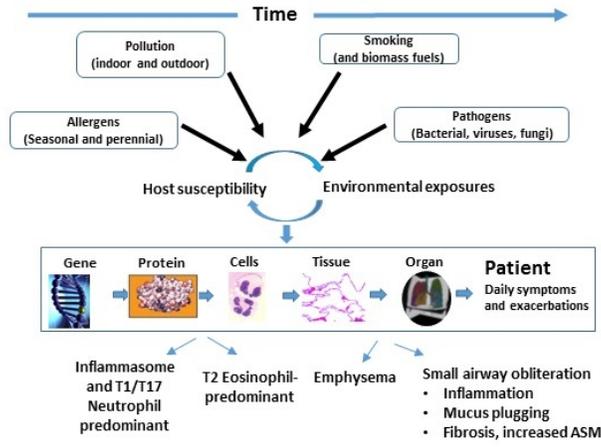
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Figure 1



190x275mm (96 x 96 DPI)

Figure 2

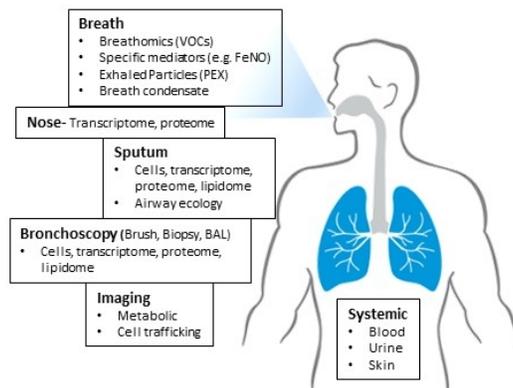


Figure 2

190x275mm (96 x 96 DPI)

Figure 3A

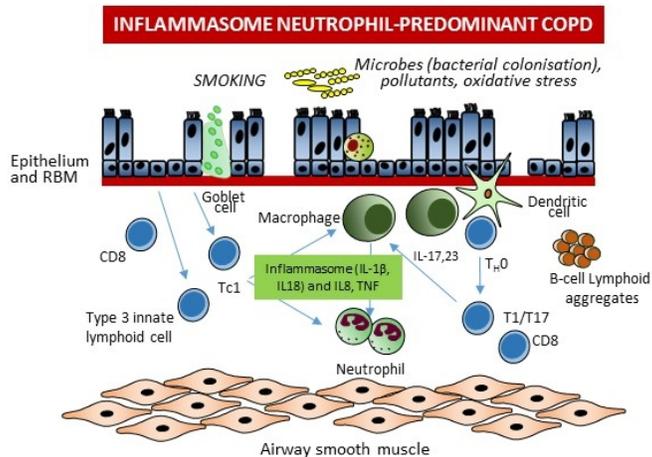
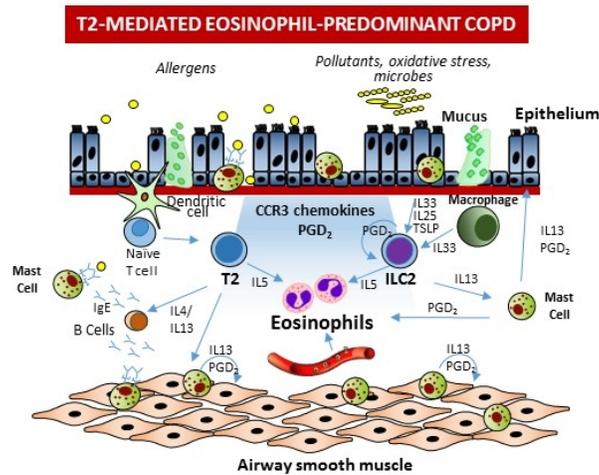


Figure 3B



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Figure 4A

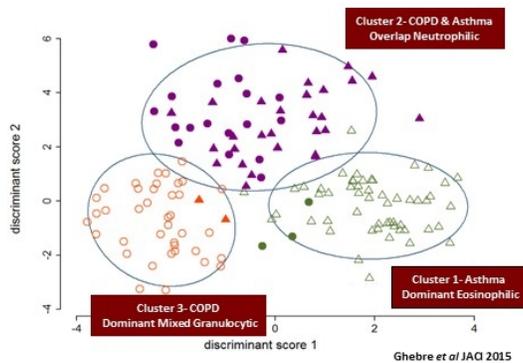
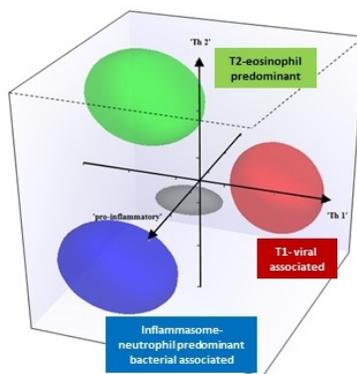


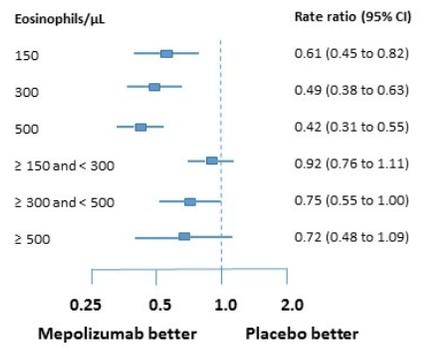
Figure 4B



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Figure 5



190x275mm (96 x 96 DPI)