Supplement Table 1: Studies investigating T2 inflammatory mediators in COPD patients with higher eosinophil counts

T2 marker	Biological role in T2 inflammation	Method	Main findings	Ref
IL5	Eosinophil survival, maturation and trafficking [1]	Proteomics Proteomics	Sputum IL5 ↑ in BEC^{HIGH250} Sputum IL-5 ↑ in Sput$^{\geq3}$	[2] [3]
IL5RA	Eosinophil differentiation, recruitment, activation and survival	Gene Expr.	Sputum IL5RA ↑ in Sput$^{\geq3}$	[4]
IL-9	Eosinophil activation, promotes mast cell growth and differentiation and mediates IgE production [1]	Gene Expr.	Sputum IL9R ↑ in Sput$^{\geq3}$	[4]
IL-13	Mediator of eosinophil homeostasis and IgE production [1]	Gene Expr.	Sputum IL-13 ↑ in BEC^{HIGH250}	[5]
IL-33	Activation, migration, and recruitment of eosinophils, ILC2s, mast cells, T cells, dendritic cells. Inducer of T2 cytokines from innate immune cells [6]	Gene Expr.	Serum IL-33 ↑ in Sput$^{\geq3}$	[7]
CCL11	Eosinophilic chemotactic protein	Proteomics IHC	No difference in BAL CCL11 in BEC^{HIGH250} CCL11 associated with eosinophilia in resected lung	[2] [8]
CCL20	Chemoattractant of dendritic cells which orchestration T2 responses [2]	Proteomics	BAL CCL20 ↑ in BEC^{HIGH250}	[2]
CCL24	Eosinophil survival and trafficking	Proteomics IHC	BAL CCL24 ↑ in BEC^{HIGH250} CCL24 associated with eosinophilia in resected lung	[2] [8]
CCL26	Eosinophilic chemotactic protein	Gene Expr. Gene Expr.	Sputum CCL26 ↑ in Sput$^{\geq3}$ Sputum CCL26 ↑ in BEC^{HIGH250}	[4] [5]
CLC	Constituent of eosinophils and basophils [9]	Gene Expr. Gene Expr.	Sputum CLC ↑ in Sput$^{\geq3}$ Sputum CLC ↑ in Sput$^{\geq3}$	[10] [11]
CLCA1	Constituent of the IL-13 pathway and involved in goblet cell hyperplasis and mucus production [12]	Gene Expr. Gene Expr.	CLCA1 from BE, sputum and BAL ↑ in BEC^{HIGH250} Sputum CLCA1 ↑ in BEC^{HIGH250}	[12] [5]
CPA3	Highly specific to mast cells and basophils [13]	Gene Expr. Gene Expr. Gene Expr. Gene Expr.	Sputum CPA3 ↑ in Sput$^{\geq3}$ Sputum CPA3 ↑ in Sput$^{\geq3}$ and BEC^{HIGH300} Sputum CPA3 ↑ in Sput$^{\geq3}$ CPA3 associated with parenchymal eos in resected lung	[10] [13] [11] [14]
CST1	May promote eosinophilic inflammation by induction of IL-5 and CCL11 [15]	Gene Expr. Gene Expr. Gene Expr.	CST1 from BE correlation^{+} with BEC Sputum CST1 ↑ in Sput$^{\geq3}$ Sputum CST1 ↑ in BEC^{HIGH250}	[15] [10] [5]
DNASE1L3	Endonuclease which mediates DNA breakdown during cellular apoptosis [9]	Gene Expr. Gene Expr. Gene Expr.	Sputum DNASE1L3 ↑ in Sput$^{\geq3}$ Sputum DNASE1L3 ↑ in Sput$^{\geq3}$ Sputum DNASE1L3 ↑ in Sput$^{\geq3}$ and BEC^{HIGH300}	[10] [11] [13]
HDC	Catalyses the decarboxylation of histidine to histamine [16]	Gene Expr.	Sputum HDC ↑ in Sput$^{\geq3}$ and BEC^{HIGH300}	[13]
GATA2	Mast cell and basophil differentiation and maintenance [17]	Gene Expr.	Sputum GATA2 ↑ in Sput$^{\geq3}$	[13]
GATA3	Activates Th2 helper cells and ILC2 cells	IHC Gene Expr.	GATA3 associated with eosinophilia in resected lung GATA-3 inhibition = ↓ in sputum eos & IL-5	[8] [18]
TPSAB1	Mast cell related protease [19]	Gene Expr.	Sputum TPSAB1 ↑ in Sput$^{\geq3}$ and BEC^{HIGH300}	[13]
LTD$_4$	Released by eosinophils and mast cells, involved in smooth muscle	Mass spec	Sputum LTD$_4$ correlation^{+} with sputum eos	[20]

	contractility, vascular leak and mucus production [20]			
LTE_4	Released by eosinophils and mast cells, involved in smooth muscle contractility, causing vascular leak and mucus production [20]	Mass spec	Sputum LTE_4 correlation$^+$ with sputum eos	[20]
5-HETE	Biosynthesis of lipoxins, which have anti-inflammatory properties [20]	Mass spec	Sputum 5-HETE correlation$^+$ with sputum eos	[20]
5-OXO-ETE	Biosynthesis of lipoxins, which have anti-inflammatory properties [20]	Mass spec	Sputum 5-OXO-HETE correlation$^+$ with sputum eos	[20]
PGD_2	Eosinophil chemotaxis and activation [21]	Mass spec	Sputum PGD_2 correlation$^+$ with sputum eos	[20]
PGE_2	May inhibit eosinophil action [21]	Mass spec	Sputum PGE_2 correlation$^+$ with sputum eos	[20]

↑ = Increased; ↓ = Reduced

BAL = bronchoalveolar lavage; BE = bronchial epithelial cell; BEC = blood eosinophil count; $BEC^{HIGH250}$ = COPD patients with a blood eosinophil count >250 cells / uL; $BEC^{HIGH300}$ = COPD patients with a blood eosinophil count ≥300 cells / uL; Correlation$^+$ = positive correlation; Eos = eosinophil; Gene Expr. = gene expression; IHC = immunohistochemistry; Mass spec = mass spectrometry; $Sput^{\geq 3}$ = COPD patients with a sputum eosinophil count ≥3%.

References

1. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 2001: 34: 50s-59s.
2. Kolsum U, Damera G, Pham TH, Southworth T, Mason S, Karur P, Newbold P, Singh D. Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. *J Allergy Clin Immunol* 2017: 140(4): 1181-1184 e1187.
3. Bafadhel M, Saha S, Siva R, McCormick M, Monteiro W, Rugman P, Dodson P, Pavord ID, Newbold P, Brightling CE. Sputum IL-5 concentration is associated with a sputum eosinophilia and attenuated by corticosteroid therapy in COPD. *Respiration* 2009: 78(3): 256-262.
4. Singh D, Bassi M, Balzano D, Lucci G, Emirova A, Anna Nandeuil M, Jellema G, Afolabi EK, Leaker B, Kornmann O, Michael Beeh K, Watz H, Govoni M. COPD patients with chronic bronchitis and higher sputum eosinophil counts show increased type-2 and PDE4 gene expression in sputum. *J Cell Mol Med* 2021: 25(2): 905-918.
5. Higham A, Beech A, Wolosianka S, Jackson N, Long G, Kolsum U, Southworth T, Pham TH, Sridhar S, McCrae C, Newbold P, Singh D. Type 2 inflammation in eosinophilic chronic obstructive pulmonary disease. *Allergy* 2021: 76(6): 1861-1864.
6. Donovan C, Hansbro PM. IL-33 in Chronic Respiratory Disease: From Preclinical to Clinical Studies. *ACS Pharmacol Transl Sci* 2020: 3(1): 56-62.
7. Tworek D, Majewski S, Szewczyk K, Kiszalkiewicz J, Kurmanowska Z, Gorski P, Brzezianska-Lasota E, Kuna P, Antczak A. The association between airway eosinophilic inflammation and IL-33 in stable non-atopic COPD. *Respir Res* 2018: 19(1): 108.
8. Jogdand P, Siddhuraj P, Mori M, Sanden C, Jonsson J, Walls AF, Kearley J, Humbles AA, Kolbeck R, Bjermer L, Newbold P, Erjefalt JS. Eosinophils, basophils and type 2 immune microenvironments in COPD-affected lung tissue. *Eur Respir J* 2020: 55(5).
9. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, Cowan DC, Taylor DR, Cowan JO, Gibson PG. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. *J Allergy Clin Immunol* 2014: 133(4): 997-1007.
10. Baines KJ, Negewo NA, Gibson PG, Fu JJ, Simpson JL, Wark PAB, Fricker M, McDonald VM. A Sputum 6 Gene Expression Signature Predicts Inflammatory Phenotypes and Future Exacerbations of COPD. *Int J Chron Obstruct Pulmon Dis* 2020: 15: 1577-1590.
11. Southworth T, Jevnikar Z, McCrae C, Singh D. A sputum 6-gene signature predicts airway inflammation endotypes and exacerbation frequency in chronic obstructive pulmonary disease. *Biomark Med* 2022: 16(4): 277-289.
12. Long G, Kolsum U, Higham A, Sridhar S, Pham T-H, Southworth T, Newbold P, Singh D. Blood eosinophils in COPD: relationship with CLCA1 and mucus production. *European Respiratory Journal* 2018: 52(suppl 62): PA4396.
13. Winter NA, Gibson PG, McDonald VM, Fricker M. Sputum Gene Expression Reveals Dysregulation of Mast Cells and Basophils in Eosinophilic COPD. *Int J Chron Obstruct Pulmon Dis* 2021: 16: 2165-2179.
14. Siddhuraj P, Jonsson J, Alyamani M, Prabhala P, Magnusson M, Lindstedt S, Erjefalt JS. Dynamically upregulated mast cell CPA3 patterns in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Front Immunol* 2022: 13: 924244.
15. George L, Taylor AR, Esteve-Codina A, Soler Artigas M, Thun GA, Bates S, Pavlidis S, Wagers S, Boland A, Prasse A, Boschetto P, Parr DG, Nowinski A, Barta I, Hohlfeld J, Greulich T, van den Berge M, Hiemstra PS, Timens W, Hinks T, Wenzel S, Siddiqui S, Richardson M, Venge P, Heath S, Gut I, Tobin MD, Edwards L, Riley JH, Djukanovic R, Auffray C, De-Meulder B, Erik-Dahlen S, Adcock IM, Chung KF, Ziegler-Heitbrock L, Sterk PJ, Singh D, Brightling CE, U B, the Ev Ast. Blood eosinophil count and airway epithelial transcriptome relationships in COPD versus asthma. *Allergy* 2020: 75(2): 370-380.

16. Barcik W, Pugin B, Bresco MS, Westermann P, Rinaldi A, Groeger D, Van Elst D, Sokolowska M, Krawczyk K, Frei R, Ferstl R, Wawrzyniak M, Altunbulakli C, Akdis CA, O'Mahony L. Bacterial secretion of histamine within the gut influences immune responses within the lung. *Allergy* 2019: 74(5): 899-909.
17. Li Y, Qi X, Liu B, Huang H. The STAT5-GATA2 pathway is critical in basophil and mast cell differentiation and maintenance. *J Immunol* 2015: 194(9): 4328-4338.
18. Greulich T, Hohlfeld JM, Neuser P, Lueer K, Klemmer A, Schade-Brittinger C, Harnisch S, Garn H, Renz H, Homburg U, Renz J, Kirsten A, Pedersen F, Muller M, Vogelmeier CF, Watz H. A GATA3-specific DNAzyme attenuates sputum eosinophilia in eosinophilic COPD patients: a feasibility randomized clinical trial. *Respir Res* 2018: 19(1): 55.
19. Caughey GH. Mast cell proteases as pharmacological targets. *Eur J Pharmacol* 2016: 778: 44-55.
20. Celejewska-Wojcik N, Kania A, Gorka K, Nastalek P, Wojcik K, Gielicz A, Mastalerz L, Sanak M, Sladek K. Eicosanoids and Eosinophilic Inflammation of Airways in Stable COPD. *Int J Chron Obstruct Pulmon Dis* 2021: 16: 1415-1424.
21. Peinhaupt M, Sturm EM, Heinemann A. Prostaglandins and Their Receptors in Eosinophil Function and As Therapeutic Targets. *Front Med (Lausanne)* 2017: 4: 104.