Sputum as a Diagnostic Tool for the Treatment of Asthma

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Background

Asthma is a heterogeneous disease with multiple clinical phenotypes and endotypes,[1] each with inflammatory etiology, triggers, and increasingly diverse treatment options.[2,3] It is increasingly accepted that a biomarker-based stratification is needed to match patients with targeted therapies for personalized treatment.[4,5] In this regard it is advantageous to have data from the lung itself rather than a surrogate tissue.[6]

Sputum provides a non-invasive and economical sampling of inflammatory cells with which to interrogate the inflammatory state of the lung.

Using flow cytometry we analyzed sputum cells from asthma and COPD patients and smokers to determine inflammatory cells and the expression levels of select biologic drug targets.

References

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(2) Kyriakopoulos, C.; Gogali, A.; Markozannes, G.; Kostikas, K. Biologic Agents Licensed for Severe Asthma: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Eur Respir Rev 2024, 33 (172), 230238. https://doi.org/10.1183/16000617.0238-2023.

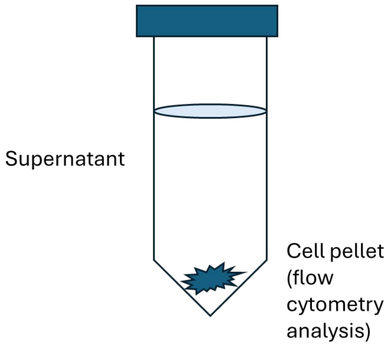
(3) Politis, J.; Bardin, P. G.; Leong, P. Contemporary Concise Review 2023: Asthma. Respirology 2024, 29 (8), 674–684. https://doi.org/10.1111/resp.14782.

(4) Quek, E.; Horn, N.; Siddiqui, S. Precision Medicine in Asthma: The Role of Biomarkers. Immunotargets Ther 2025, 14, 1479–1513. https://doi.org/10.2147/ITT.S532291.

(5) Agache, I.; Rogozea, L. Asthma Biomarkers: Do They Bring Precision Medicine Closer to the Clinic? Allergy Asthma Immunol Res 2017, 9 (6), 466. https://doi.org/10.4168/aair.2017.9.6.466.

(6) Porsbjerg, C. M.; Townend, J.; Bergeron, C.et al. Association between Pre-Biologic T2-Biomarker Combinations and Response to Biologics in Patients with Severe Asthma.

Sample processing



Sputum is dissociated to a single cell suspension and the cellular component stained with fluorescence labeled antibodies and analyzed using flow cytometry. Drug antibodies (dupilumab and benralizumab) were fluorescence labeled to interrogate their drug receptors.

Cellular Analysis Flow Cytometry

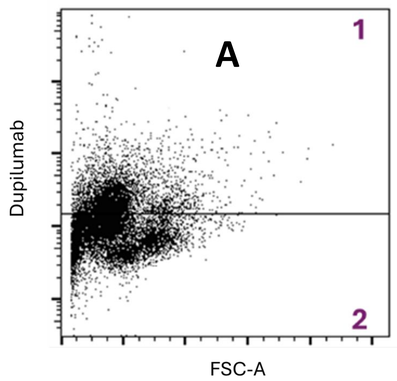
Antibody *	Feature or biomarker
Anti-CD45	Leucocytes (all)
Anti-CD206	Alveolar macrophages
Anti-CD3	T-cells (mature)
Anti-CD66b	granulocytes
Anti-CD19	B-cells
Anti-CD52	Eosinophils
Anti-CD123	Activated eosinophils
Anti-CD16	neutrophils
Dupilumab (Anti-CD124)	lymphocytes and granulocytes
Benralizumab (Anti-CD125)	lymphocytes and granulocytes

* Cells which strongly express the biomarker

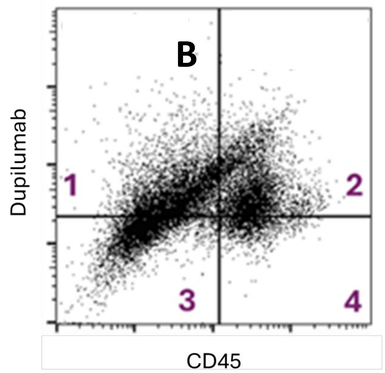
Results in select cases

We examined the expression of drug receptors (CD124 and CD125) in cells from the sputum of patients with asthma, COPD and smokers not diagnosed with asthma or COPD by flow cytometry. Dead cells were excluded using a viability stain. The antibody panel allowed differentiation of cell types and expression of drug receptors for dupilumab (Dup) and benralizumab (Ben). (**A**) In an asthma patient 25.5% of leukocytes expressed CD124. (**B**) In a COPD patient 25.5% of leukocytes expressed CD124. (**C**) In a COPD patient 16.5% of leukocytes expressed CD125. (**D**) In a smoker 31.5% of leukocytes expressed CD124. (**E**) In an asthma patient 11.2% of non-leukocytes expressed CD124. (**F**) In a smoker 41.5% of leukocytes expressed CD125.

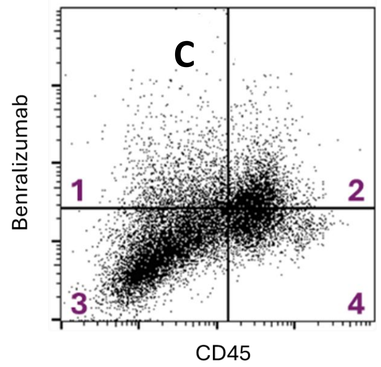
Flow cytometry



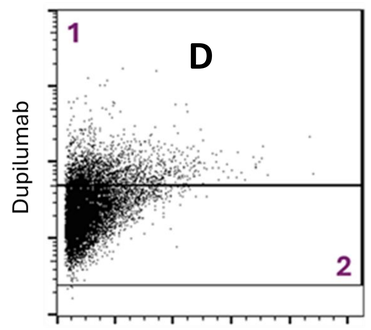
Dupilumab (Dup) receptor (CD124) expression in live leukocytes (CD45+) in sputum from an asthma patient. 1. Dup+. 2. Dup-. FSC = forward scatter. (FIG 8b)



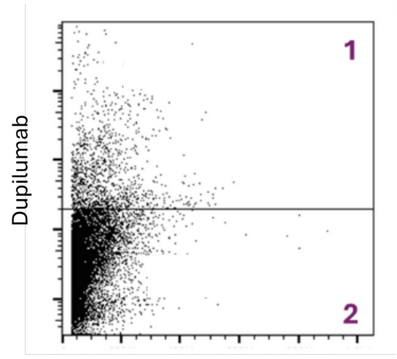
Dupilumab (Dup) receptor (CD124) expression in live cells in leukocytes (CD45) in sputum from a COPD patient. 1. Dup+ CD45-. 2. Dup+ CD45+, 3. Dup- CD45-, 4. Dup- CD45+. (FIG 9)



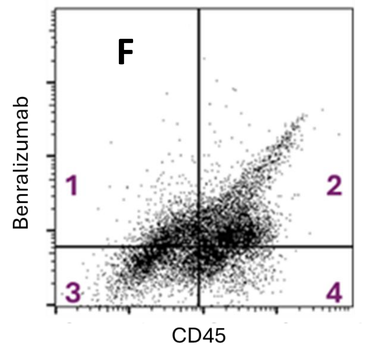
Benralizumab (Ben) receptor (CD125) expression in live cells stained with pan leukocyte (CD45) antibody in sputum from a COPD patient. 1. Ben+ CD45-. 2. Ben + CD45+, 3. Ben - CD45-, 4. Ben - CD45+ (FIG 11).



Dupilumab (Dup) receptor (CD124) expression in live leukocytes (CD45+) in sputum from a smoker. 1. Dup+. 2. Dup-. FSC = forward scatter. . (FIG 10)



Dupilumab (Dup) receptor (CD124) expression in live non-leukocytes (CD45-) in sputum from a smoker. 1. Dup+. 2. Dup-. FSC = forward scatter. . (FIG 10)



Benralizumab (Ben) receptor (CD125) expression in live cells stained with pan leukocyte (CD45) antibody in sputum from a smoker. 1. Ben+ CD45-. 2. Ben + CD45+, 3. Ben - CD45-, 4. Ben - CD45+ (FIG 11).

Conclusions

We analyzed sputum samples from patients with asthma and COPD by flow cytometry using fluorescence labeled antibodies targeting cell membrane biomarkers including those that are drug targets for dupilumab and benralizumab. We used drugs labeled with fluorescent antibodies to interrogate cells for the expression of the drug receptors. The expression of drug receptors (CD124 and CD125) was measured in asthma, COPD and smokers (not diagnosed with asthma or COPD). As expected leukocytes expressed the drug receptors more than non-leukocytes in a small sample set.

Further work

Further work will use machine learning to identify cell populations including those expressing select drug receptors in a larger group of patient samples.