Effect of RLS-0071 (pegtarazimod) on airway neutrophilia following inhaled endotoxin challenge assessed by ChipCytometry

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Background

- **Airway neutrophilia** is associated with several lung diseases, including acute lung injury, severe asthma, and chronic obstructive pulmonary disease.
- **Pegtarazimod**, a novel dual-targeting anti-inflammatory peptide, has been shown to reduce airway neutrophilia in a human endotoxin challenge model.[1]
- **ChipCytometry** allows quantification of airway cells including intracellular staining of effector molecules at a single cell level.[2]

Aim

Investigation of the effects of pegtarazimod on airway neutrophil counts and function following inhaled endotoxin challenge by ChipCytometry.

Methods

Study design: Pegtarazimod at two doses (low-dose: 10 mg·kg-1; high-dose: 120 mg·kg^{-1} loading dose followed by two doses of 40 mg·kg^{-1}) versus placebo was given in a randomized, double-blind, proof-of-mechanism study following inhaled lipopolysaccharide (LPS) challenge in 30 healthy participants (NCT05351671). Induced sputum was collected before, 6 and 24 hours after LPS challenge.

ChipCytometry: Sputum cells (2.5x10^5) were loaded on ChipCytometry-specific chips. Cell surface markers (CD45, CD66b, CD14, CD3, CD11a, CD11b, CD89, CD15) and intracellular effector molecules (myeloperoxidase (MPO), neutrophil elastase (NE), eosinophil peroxidase (EPX)) were stained with corresponding antibodies and then analyzed by ChipCytometry.

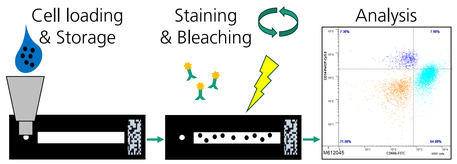

Figure 1. ChipCytometry method.

Results

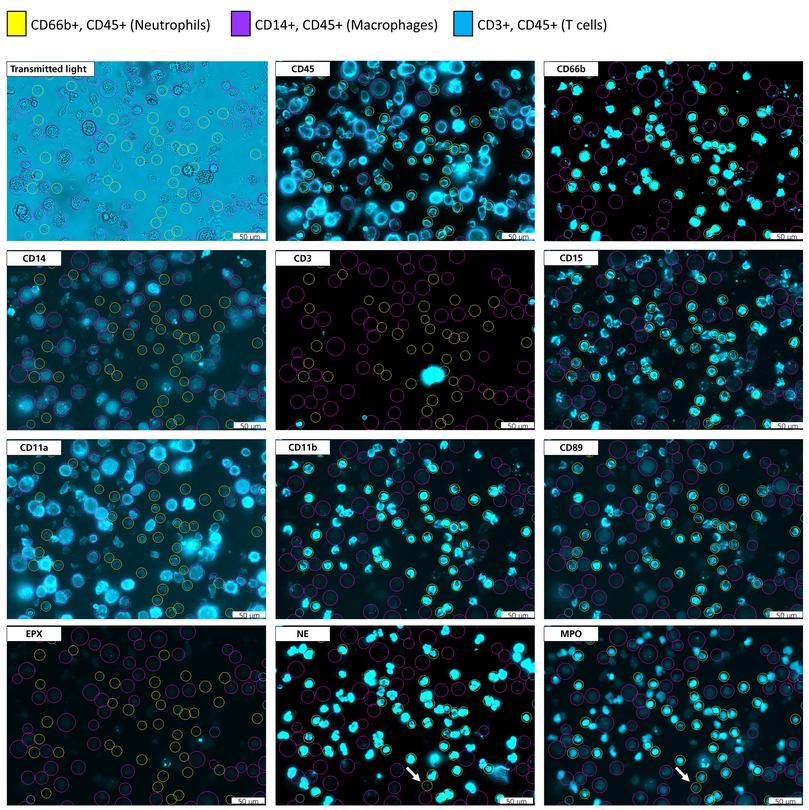

Figure 2. Representative images for surface and intracellular marker staining of induced sputum cells analyzed by ChipCytometry. Each image shows the same position of one baseline chip loaded with induced sputum cells. Arranged from left to right in rows, one image each is depicted for transmitted light, cell surface antibody staining of CD45, CD66b, CD14, CD3, CD15, CD11a, CD11b, and CD89, and intracellular antibody staining of EPX, NE and MPO (Scale = 50 µm). Neutrophils (CD66b$^+$, CD45$^+$) are encircled in yellow, Macrophages (CD14$^+$, CD45$^+$) in purple, and T cells (CD3$^+$, CD45$^+$) in blue.

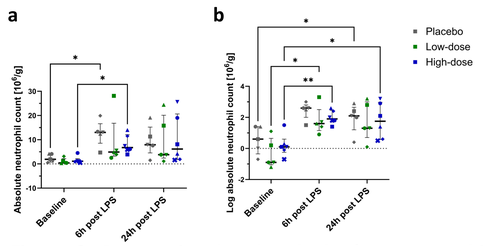

Figure 3. Pegtarazimod reduced absolute neutrophil counts in sputum at 6h post LPS. (a) Values and (b) log-transformed values are given as median with interquartile range counts for the placebo (grey, n=5), low-dose pegtarazimod (green, n=5) and high-dose pegtarazimod (blue, n=6) group at pre-challenge baseline, 6h post and 24h post LPS. Each symbol represents an individual participant. Two-way ANOVA followed by Tukey's multiple comparison test. *p < 0.05, **p < 0.01.

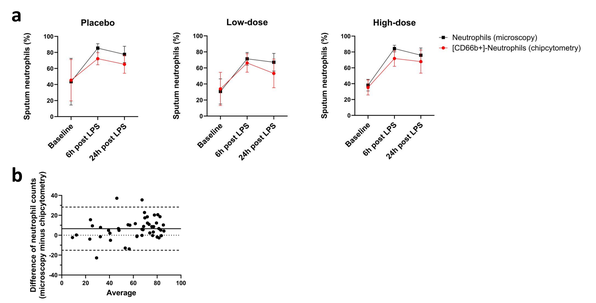

Figure 4. Method comparison. (a) Neutrophil counts obtained via light microscopy (black) versus CD66b-based immunophenotyping via ChipCytometry (red). Values are given as mean with standard deviation. (b) Bland-Altman plot of relative neutrophil populations showing the mean bias (6.6 ± 11.1 %) as black line.

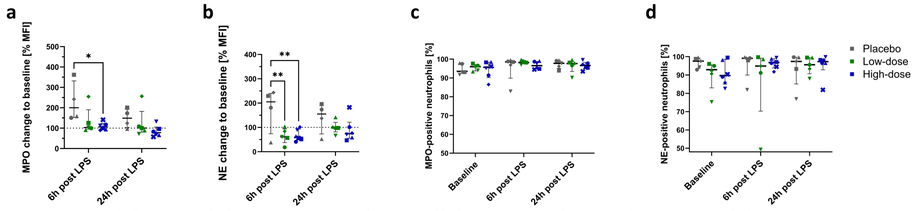

Figure 5. Pegtarazimod decreased the expression of intracellular MPO and NE within neutrophils in sputum. Intracellular (a) MPO and (b) NE within neutrophils, and percentage of (c) MPO- and (d) NE-positive neutrophils among total neutrophils for the placebo (grey, n=4), low-dose pegtarazimod (green, n=5) and high-dose pegtarazimod (blue, n=6) group at 6h post LPS and 24h post LPS. Values are given as median with interquartile range. Each symbol represents an individual participant. Dashed line = values at baseline set as 100%. Two-way ANOVA followed by Tukey's multiple comparison test. *p < 0.05, **p < 0.01.

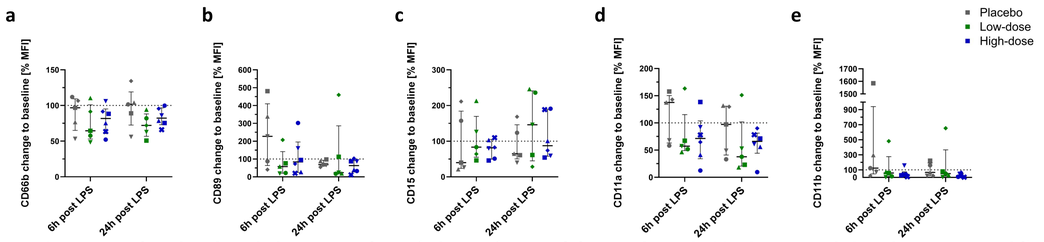

Figure 6. Pegtarazimod reduced the expression of the surface activity markers CD89, CD11a and CD11b on neutrophils in sputum. Changes in expression of neutrophilic cell surface activity markers for the placebo (grey, n=5), low-dose pegtarazimod (green, n=5) and high-dose pegtarazimod (blue, n=6) group at 6h post LPS and 24h post LPS. Values are given as median with interquartile range. Each symbol represents an individual participant. Dashed line = values at baseline set as 100%. Two-way ANOVA followed by Tukey's multiple comparison test.

Conclusions

- ChipCytometry data confirm and extend previous proof-of-mechanism that pegtarazimod decreases neutrophil numbers in induced sputum and modulates neutrophil activation and adhesion.
- Consistent with previous findings of reduced MPO and NE concentrations in sputum supernatant[1], we found decreased intracellular staining of MPO and NE in neutrophils.
- Pegtarazimod demonstrates potential as a novel anti-inflammatory treatment for neutrophil-dominated lung diseases by reducing neutrophil activity and infiltration into the lungs.

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References

[1] Cunnion et al. (2024): RLS-0071, a novel anti-inflammatory agent, significantly reduced inflammatory biomarkers in a randomised human evaluation of mechanisms and safety study. *ERJ open research* 10 (4). DOI: 10.1183/23120541.01006-2023.

[2] Carstensen, Holz, Hohlfeld and Müller (2021): Quantitative analysis of endotoxin-induced inflammation in human lung cells by ChipCytometry. *Cytometry A* 99 (10). DOI: 10.1002/cyto.a.24352.

Abbreviations

CD = cluster of differentiation, EPX = Eosinophil peroxidase, LPS = lipopolysaccharide, MFI = Mean fluorescence intensity, MPO = Myeloperoxidase, NE = Neutrophil elastase.