Single-cell RNA sequencing analysis of blood and nasal brushing from asthma patients receiving a single dose of lunsekimig (SAR443765), a novel, bispecific anti-TSLP/anti-IL-13 NANOBODY® molecule reveals significant impact on multiple pathological immune cell populations and downregulation of CCL26 expression in epithelial cell subpopulations

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Acknowledgments and disclosures

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Davide Lucchesi was an employee of Sanofi at the time of the study and is currently an employee of Nucleome Therapeutics.

Shantanu Bafna and Virginia Savova were employees of Sanofi at the time of the study.

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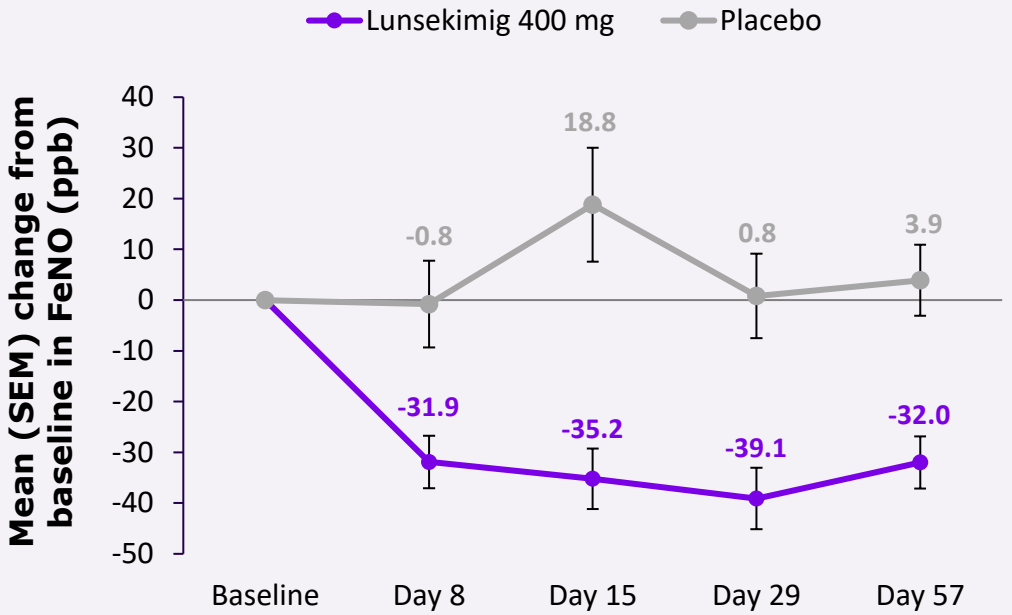
Lunsekimig, the first multi-targeting NANOBODY® molecule in asthma, has the potential for superior effect in asthma

- Lunsekimig (SAR443765) is a novel bispecific NANOBODY® molecule that blocks TSLP and IL-13[1,2]
- TSLP and IL-13 exert independent and synergistic effects on tissue inflammation[3,4]
- Multi-targeting approach with lunsekimig demonstrated improved potency compared to mono-targeting TSLP or IL-13 in preclinical models
- In a Phase 1b study, a single dose of lunsekimig led to a rapid and significant reduction in FeNO levels (a marker of airway inflammation) and improved lung function in participants with mild-to-moderate asthma with elevated baseline FeNO[1,2]
  - The reduction in FeNO appears to be greater than previous studies of monovalent agents[1,a]

Single dose of lunsekimig significantly reduced FeNO vs placebo[1,2]



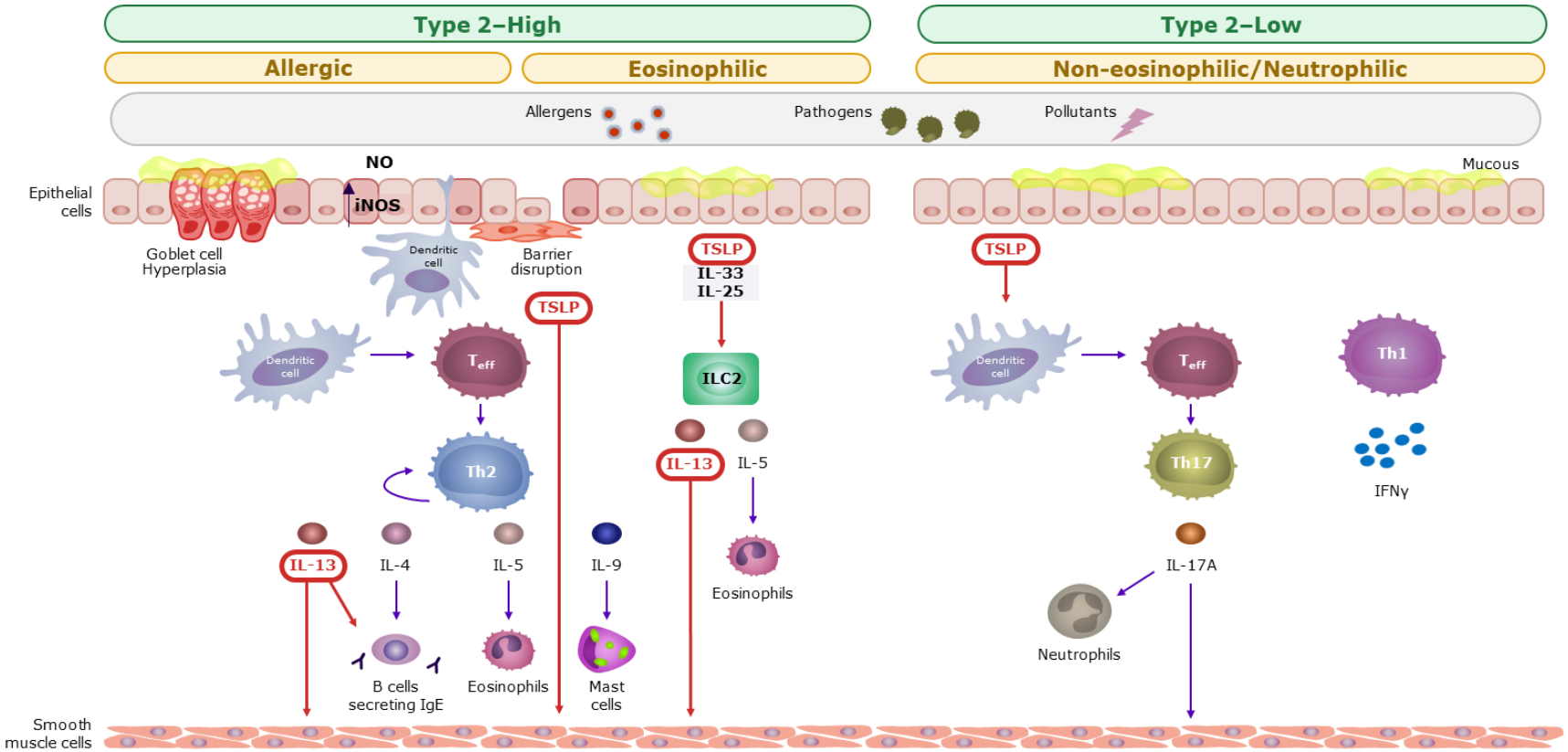
Note: NANOBODY® is a registered trademark of Sanofi. [a]No head-to-head comparisons were performed and patient populations and baseline characteristics may differ between studies. Estimates of FeNO change from baseline versus placebo were derived from published data.
FeNO, fractional exhaled nitric oxide; IL, interleukin; ppb, parts per billion; SEM, standard error of the mean; TSLP, thymic stromal lymphopoietin.
1. Deiteren A, et al. Presented at ATS 2023; May 19–24, 2023; Washington, DC, USA; 2. Deiteren A, et al. Presented at ERS 2023; September 9–13, 2023; Milan, Italy; 3. Doran E, et al. *Front Med (Lausanne)*. 2017;4:139; 4. Gauvreau GM, et al. *Expert Opin Ther Targets*. 2020;24(8):777-792.

Study aims

- ***Hypothesis:*** *Lunsekimig modulates signaling pathways associated with TSLP and IL-13 in Type 2-high asthma both in the blood and in the respiratory tract*

Study aims

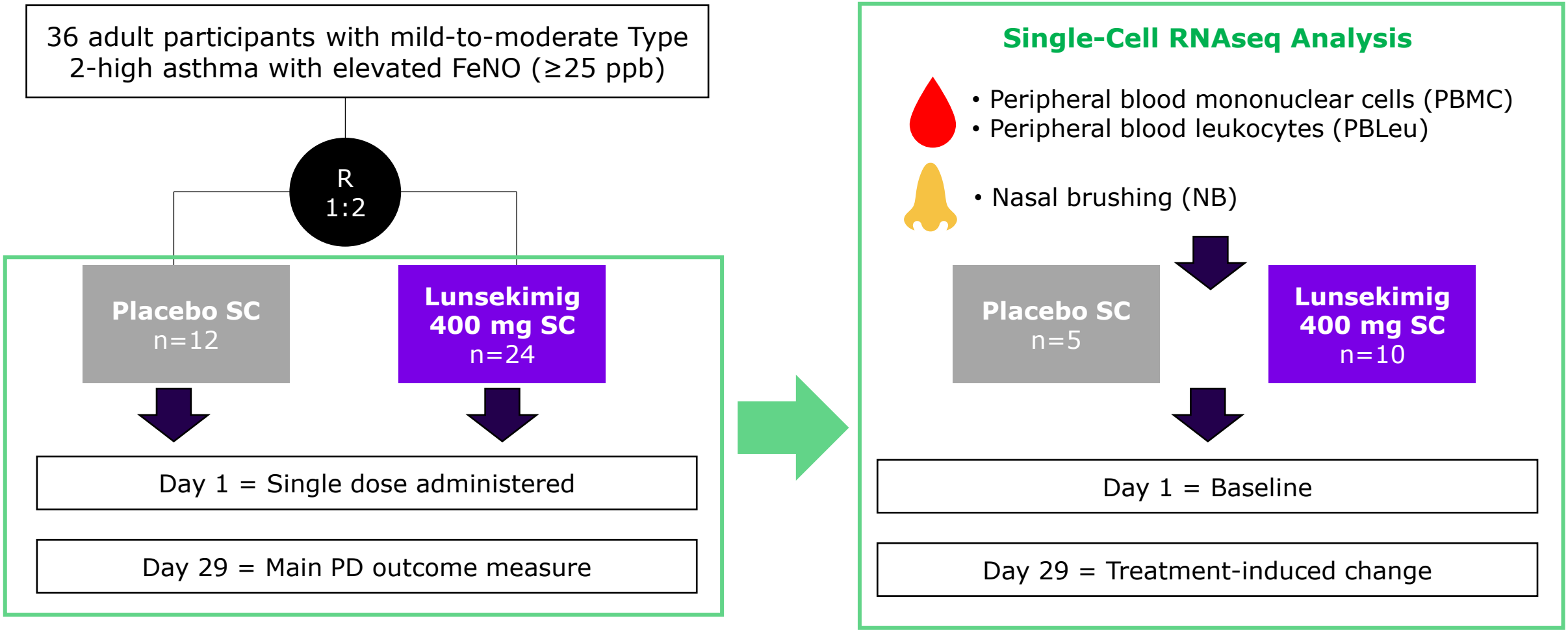
- Elaborate on the **MOA of lunsekimig** using single-cell RNAseq analysis
- Explore markers that **correlate with pharmacodynamic response** (eg, FeNO)



Note: Figure was adapted from: 1. Gauvreau GM, et al. *Expert Opin Ther Targets*. 2020;24(8):777-792; 2. Brusselle GG, Koppelman GH. *N Engl J Med.* 2022;386(2):157-171.
FeNO, fractional exhaled nitric oxide; IFNγ, interferon gamma; IgE, immunoglobulin E; IL, interleukin; ILC, innate lymphoid cell; iNOS, inducible nitric oxide synthase; MOA, mechanism of action; NO, nitric oxide; RNAseq, RNA sequencing; T_{eff}, effector T cell; Th, helper T cell; TSLP, thymic stromal lymphopoietin.

Study design and single-cell RNAseq analysis

Proof of mechanism (Phase 1b) in asthma (NCT05366764)[1,2]

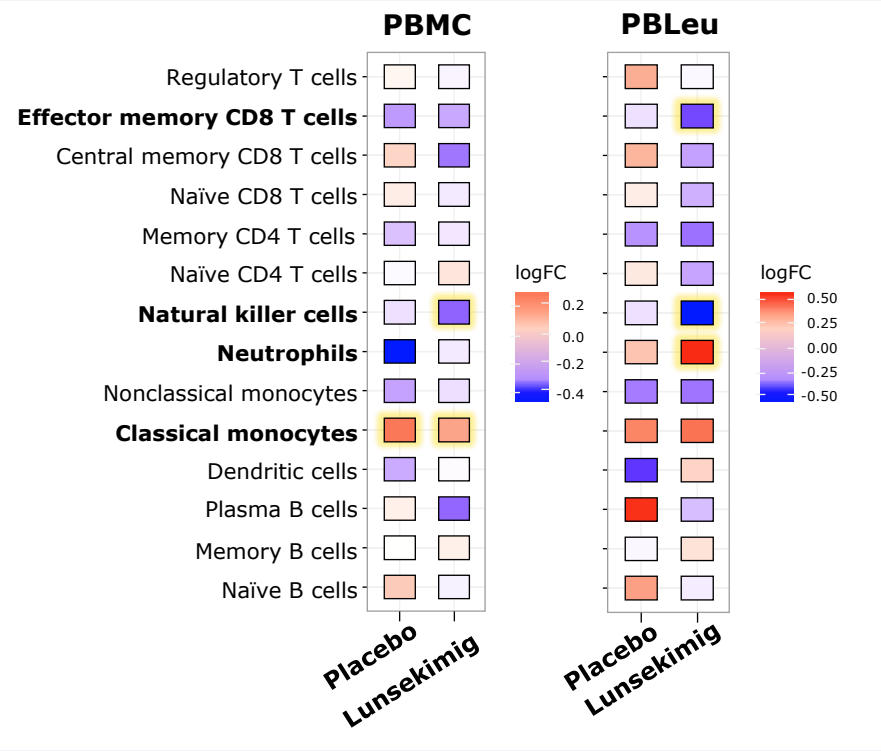


FeNO, fractional exhaled nitric oxide; PD, pharmacodynamic; R, randomization; SC, subcutaneous; RNAseq, RNA sequencing.

1. Deiteren A, et al. Presented at ATS 2023; May 19–24, 2023; Washington, DC, USA; 2. Deiteren A, et al. Presented at ERS 2023; September 9–13, 2023; Milan, Italy.

Natural killer cells and CD8 effector memory T cells were decreased with lunsekimig

scRNAseq analysis (Day 29 vs baseline): Peripheral blood monocytes (PBMC) and total leukocytes (PBLeu)



Lunsekimig significantly decreased the ratio of:

- Natural killer cells
- CD8 effector memory T cells

Lunsekimig and placebo significantly increased the ratio of:

- Classical monocytes
- Neutrophils

Clinical hematology samples

Lunsekimig decreased the number of:

- Eosinophils
- Basophils
- Lymphocytes

Lunsekimig did not affect the number of:

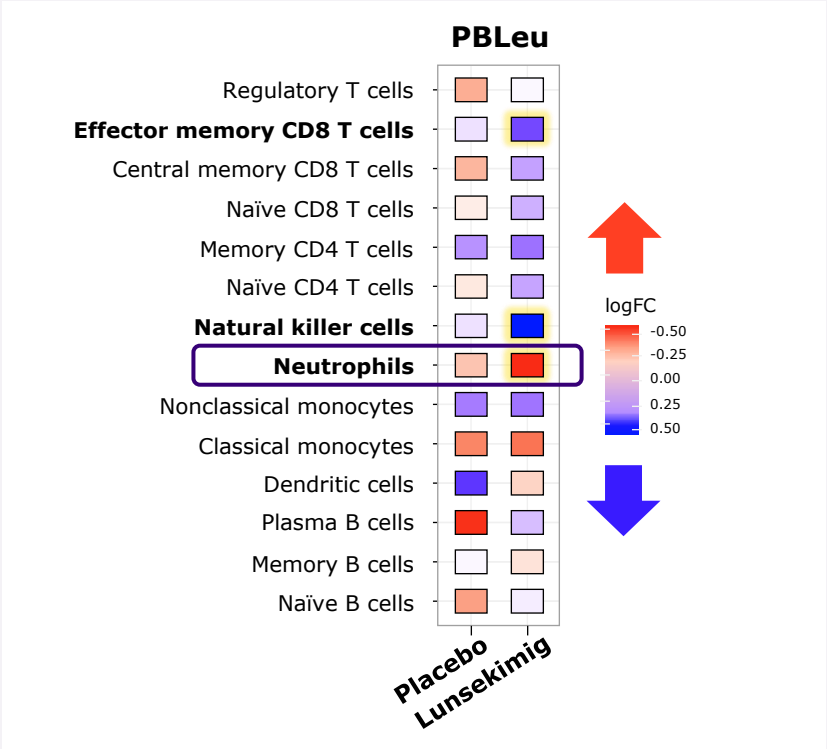
- Monocytes
- Neutrophils

The change in cell type ratios with lunsekimig seemed to be driven by reductions in eosinophils, basophils, and lymphocytes, with no change in the absolute number of monocytes and neutrophils

Note: Significance cutoffs: p-value ≤0.05. Yellow glowing frame indicates p-value ≤0.05. Changes in the proportion of cell types observed are relative, not total.
CD, cluster of differentiation; logFC, log fold change; PBMC, peripheral blood mononuclear cells; scRNAseq, single-cell RNA sequencing.

Enrichment analysis showed downregulation of inflammatory pathway signaling in neutrophils with lunsekimig

Proportion of neutrophils in blood increased with lunsekimig at Day 29 vs baseline[a]



Neutrophil pathway module score[b]

Downregulation with statistical significance

Upregulation with statistical significance

No change in delta score with statistical significance

No change in delta score and not statistically significant

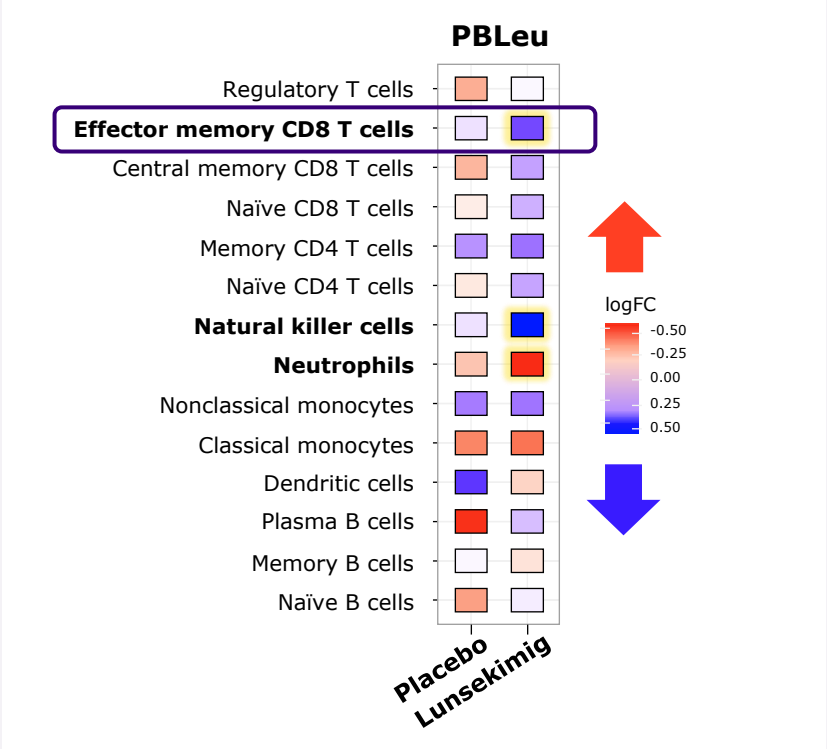
Pathway Module	Placebo Day 29 vs Baseline	Lunsekimig Day 29 vs Baseline
IL-4	**Δscore=0.001 p≤0.0001**	**Δscore=-0.003 p≤0.0001**
IL-13	Δscore=0.000 p=0.29	Δscore=-0.000 p=1
IFNα	**Δscore=0.000 p≤0.0001**	**Δscore=-0.006 p≤0.0001**
IFNγ	**Δscore=0.000 p≤0.0001**	**Δscore=-0.005 p≤0.0001**

IL-4, IFNα, IFNγ pathway signaling in neutrophils appears to be downregulated by lunsekimig

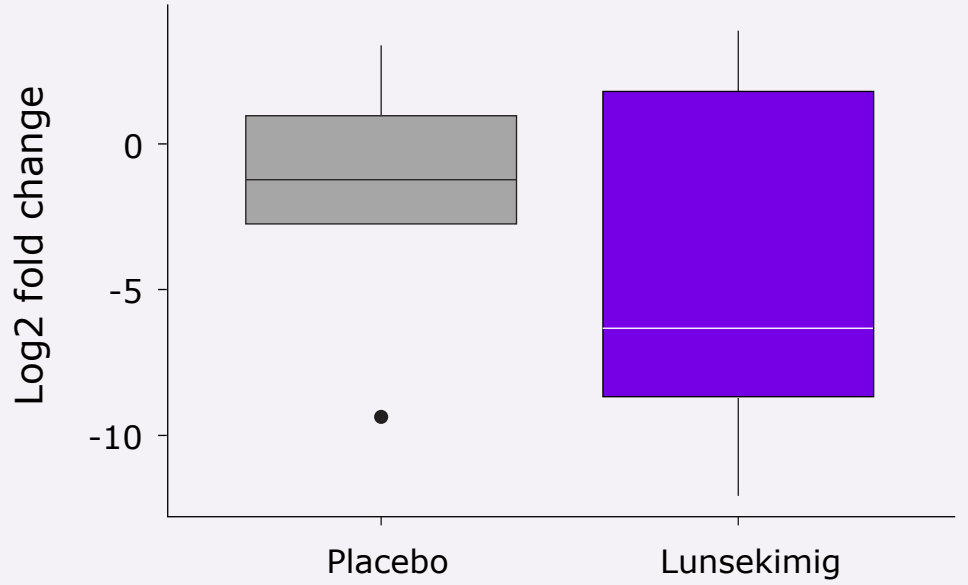
[a]Significance cutoffs: p-value ≤0.05. Yellow glowing frame indicates p-value ≤0.05.
[b]The delta score is the difference between Day 29 and Day 1 scores. Multiple genes comprise a pathway. The score is calculated as an average expression of the genes of that pathway on single-cell level for each cell type subtracted by the aggregated expression of control gene sets. Kolmogorov–Smirnov test is performed to compare the aggregate scores between Day 29 and Day 1 in the two treatment groups to check if they are significantly different.
CD, cluster of differentiation; IFNα, interferon alpha; IFNγ, interferon gamma; IL, interleukin; logFC, log fold change; PBLeu, peripheral blood leukocytes.

Hemoglobin-β, a gene associated with lower FEV_1 in asthma,[1] was significantly decreased in CD8 effector memory T cells with lunsekimig

- Proportion of CD8 effector memory T cells in blood decreased with lunsekimig at Day 29 vs baseline[a]



- HBB gene expression in CD8 effector memory T cells at Day 29 vs baseline



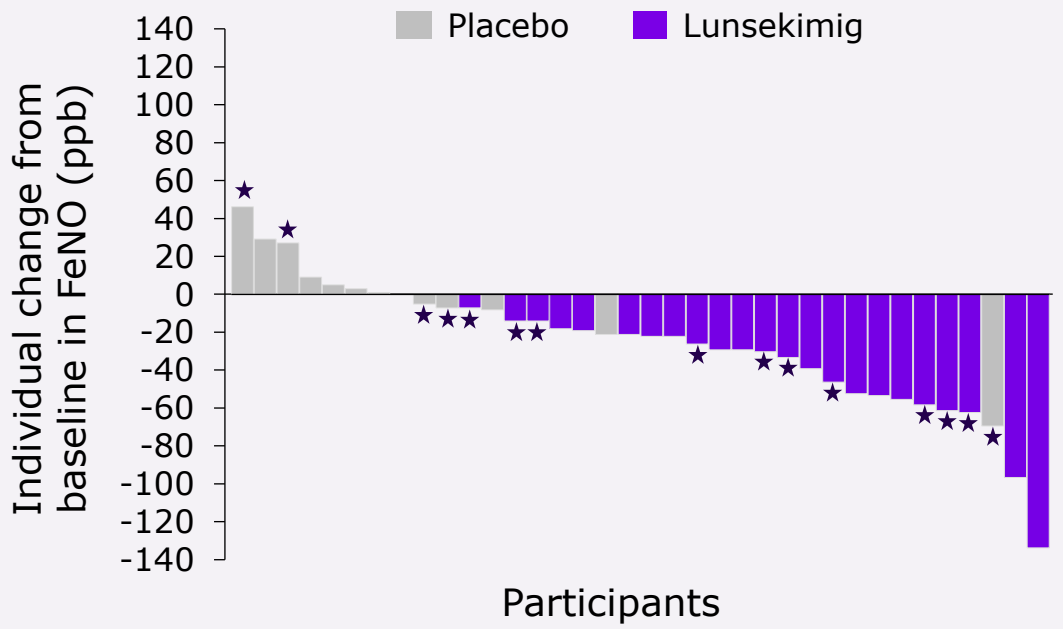
Adjusted p<0.05

- *HBB from CD8 effector memory T cells was significantly downregulated at Day 29 with lunsekimig[b]*

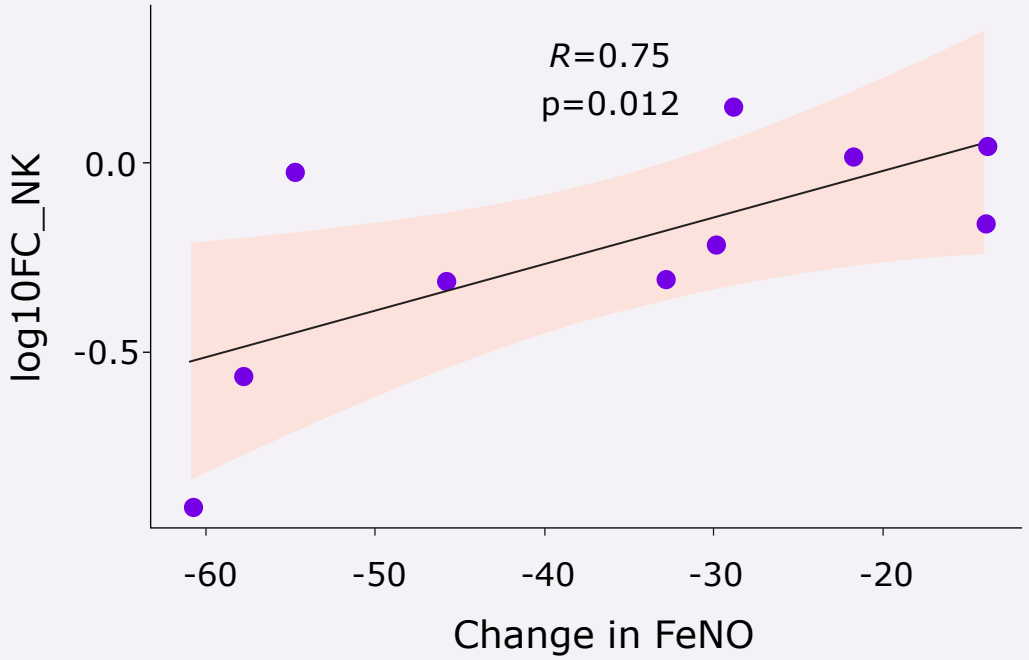
[a]Significance cutoffs: p-value ≤0.05. Yellow glowing frame indicates p-value ≤0.05.
[b]Multiplicity correction was based on an adjusted p-value of <0.05.
CD, cluster of differentiation; FEV_1, forced expiratory volume in 1 second; HBB, Hemoglobin-β; logFC, log fold change; PBLeu, peripheral blood leukocytes.
1. Marozkina N, et al. *Sci Rep*. 2021;11(1):15498.

Significant association between relative reduction in NK cells and reduction seen in FeNO at Day 29 with lunsekimig

- Key secondary endpoint from Phase 1b trial: *Change from baseline in FeNO levels by participant at Day 29[a]*



- Correlation between fold change in NK cell proportion in blood and change in FeNO by Day 29 *(lunsekimig treatment only)*

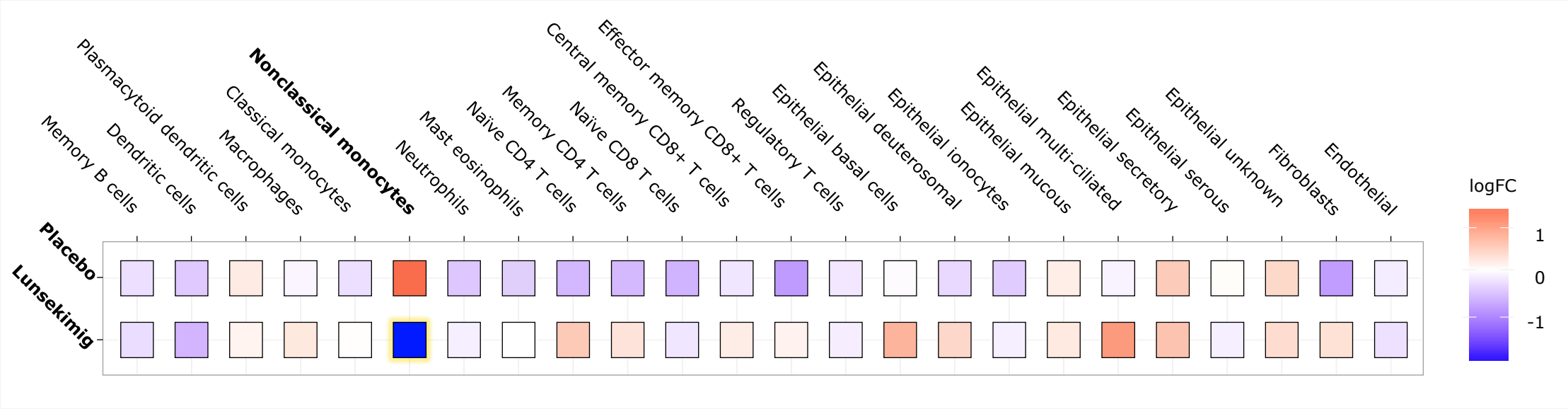


- *FeNO levels were reduced from baseline at Day 29 in nearly all participants who received lunsekimig*
- *While a significant correlation was seen between NK cell reduction and FeNO reduction after lunsekimig treatment, a similar relationship was not seen with placebo*

[a]Star symbol indicates participants who had corresponding scRNAseq data.
FeNO, fractional exhaled nitric oxide; NK, natural killer; ppb, parts per billion; scRNAseq, single-cell RNA sequencing.

Significant differences seen in proportion of nonclassical monocytes in upper respiratory tract with lunsekimig

- scRNAseq analysis (Day 29 vs baseline): Nasal brushings



Lunsekimig significantly decreased the ratios of:

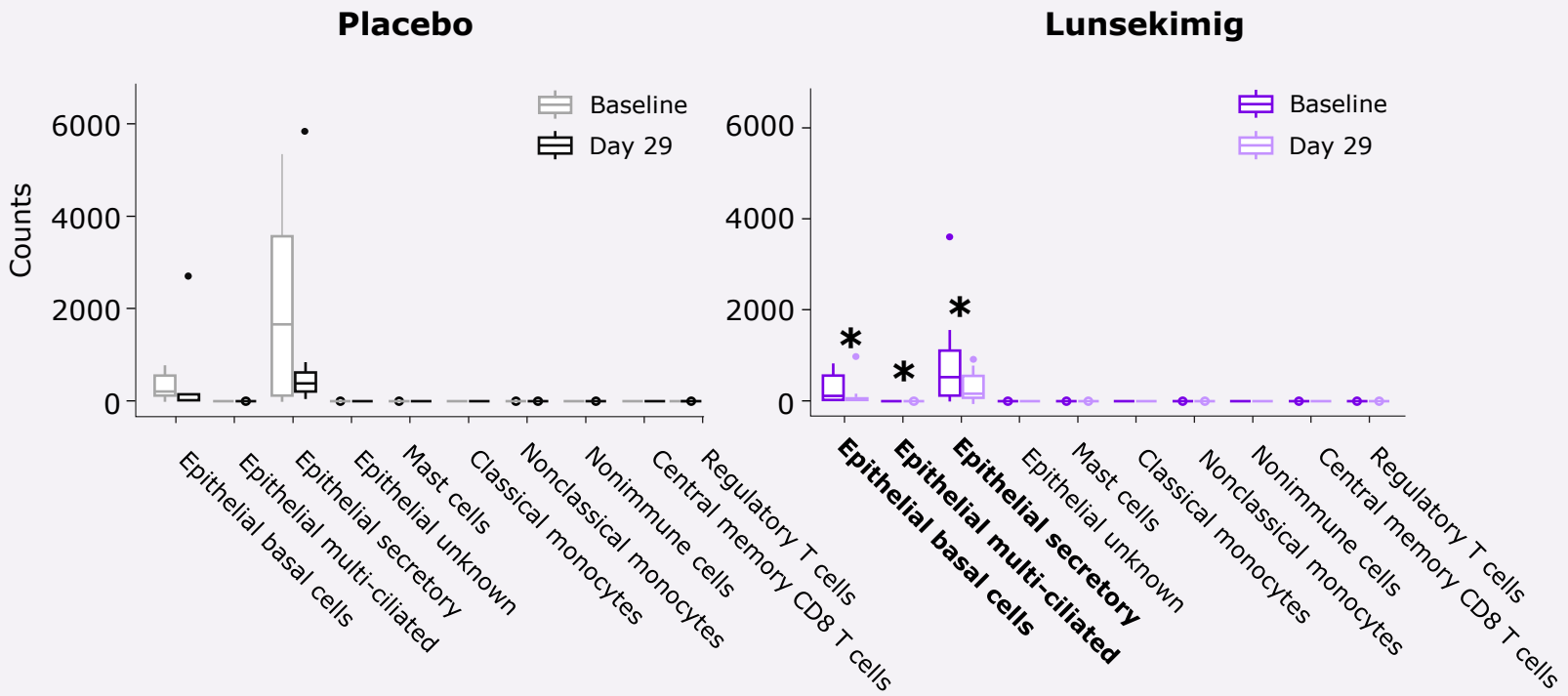
- Nonclassical monocytes

No significant change with placebo for any cell types

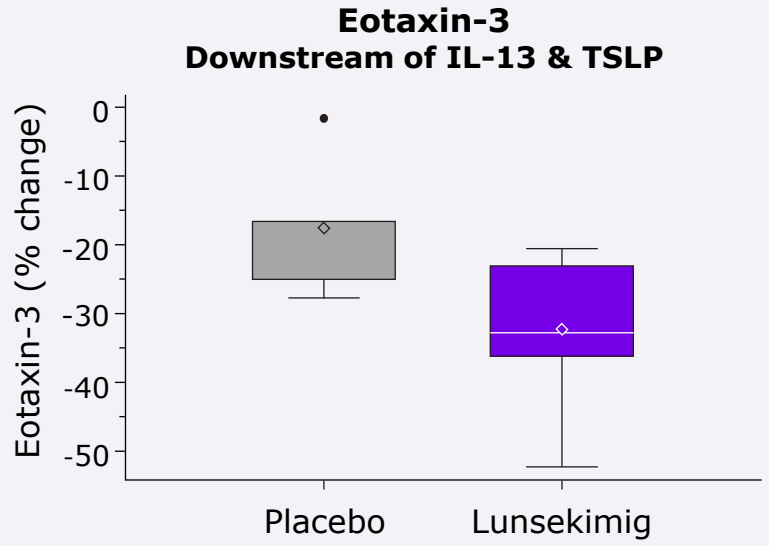
Note: Significance cutoffs: p-value ≤0.05. Yellow glowing frame indicates p-value ≤0.05.
CD, cluster of differentiation; logFC, log fold change; scRNAseq, single-cell RNA sequencing.

Eotaxin-3/CCL26 expression was significantly reduced in nasal multi-ciliated, secretory, and basal epithelium with lunsekimig

- scRNAseq analysis of eotaxin-3 in nasal brushings (n=15)



- Percent change from baseline to Day 29 in plasma eotaxin-3 (n=15)



- *Lunsekimig appeared to downregulate eotaxin-3 expression in respiratory epithelial cell subtypes*
- *Reduced blood levels of eotaxin-3 were found after lunsekimig*

*p<0.05.
CCL, C-C motif chemokine ligand; CD, cluster of differentiation; IL, interleukin; scRNAseq, single-cell RNA sequencing; TSLP, thymic stromal lymphopoietin.

Conclusions

- Lunsekimig is a novel, bispecific, anti-TSLP/anti-IL-13 NANOBODY® molecule that rapidly reduced FeNO and improved lung function in mild-to-moderate asthma in a Phase 1b study[1,2]

- scRNAseq results suggest that a single dose of lunsekimig in participants with Type 2-high asthma suppressed disease-relevant signaling pathways in both the blood and the upper respiratory tract, supporting the Phase 1b clinical findings

- In the blood, lunsekimig modulated cell type ratios by reducing the proportion of specific T-cell subsets as well as natural killer cells, the latter of which correlates with the observed FeNO reduction

- In the respiratory epithelium, lunsekimig significantly reduced the proportion of nonclassical monocytes and downregulated chemoattraction of eosinophils, underscoring its potential suppressive effects in Type 2 inflammation

FeNO, fractional exhaled nitric oxide; IL, interleukin; scRNAseq, single-cell RNA sequencing; TSLP, thymic stromal lymphopoietin.
1. Deiteren A, et al. Presented at ATS 2023; May 19–24, 2023; Washington, DC, USA; 2. Deiteren A, et al. Presented at ERS 2023; September 9–13, 2023; Milan, Italy.

THANK YOU

BACKUP

Significant correlations were observed between change in FeNO and fold change in cell type proportion between baseline and Day 29

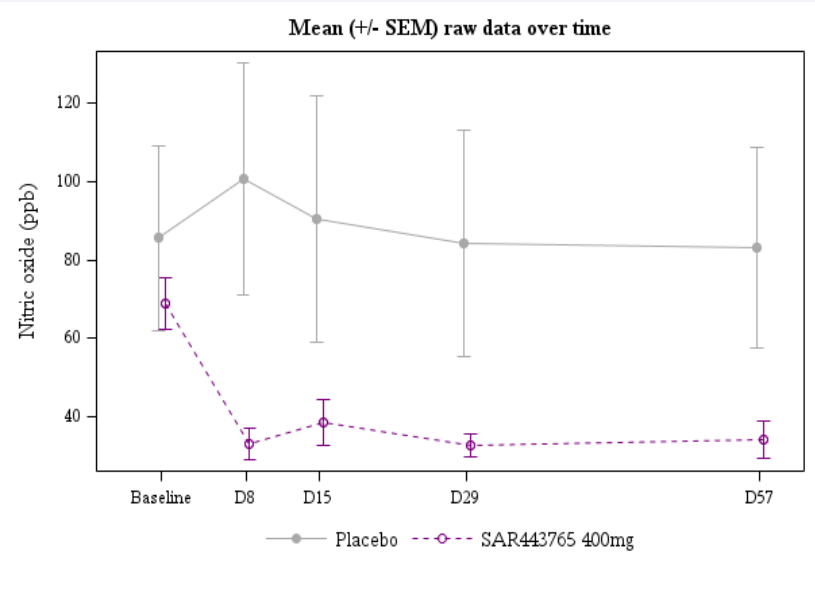
Sample Type	Treatment Group	Cell Type	Correlation	p-Value
Nasal brushing	Lunsekimig only	Mast cells and eosinophils	-0.6626	0.0368
Peripheral blood leukocytes	Lunsekimig only	Natural killer cells	0.6606	0.0440
Peripheral blood mononuclear cells	Lunsekimig only	Regulatory T cells	0.5225	0.0443

- *Correlation was observed **only** among **lunsekimig-treated** samples*
  - ***Positive correlation**: cell proportion fold change decrease from baseline to Day 29 associated with decrease in FeNO*
  - ***Negative correlation**: cell proportion fold change increase from baseline to Day 29 associated with decrease in FeNO*

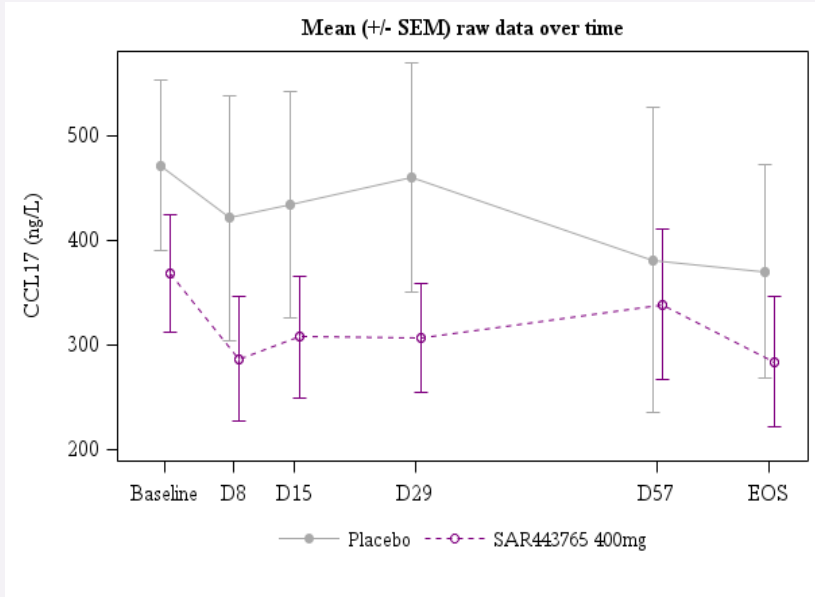
FeNO, fractional exhaled nitric oxide.

Trends of biomarkers for participants with scRNAseq profile

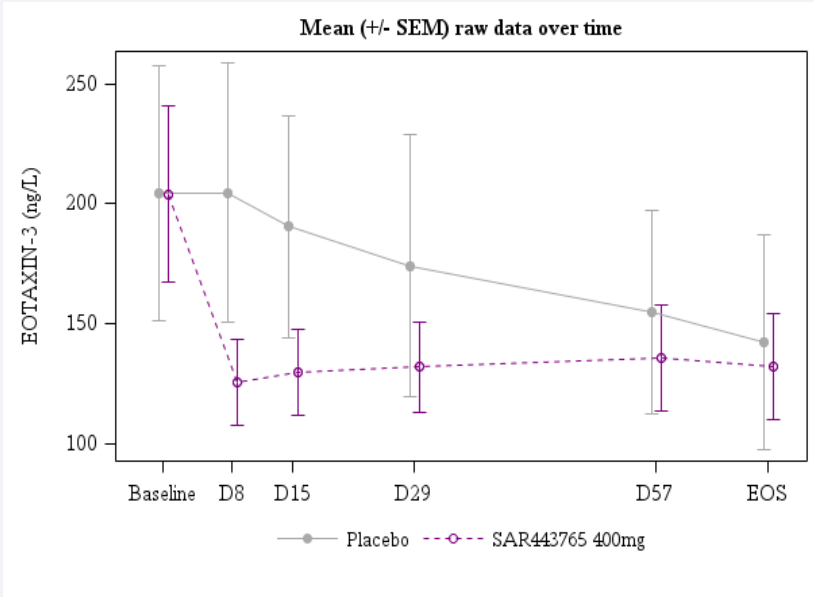
- Change from baseline in **nitric oxide** levels by participant through Day 57 (lunsekimig vs placebo)



- Change from baseline in **CCL17** levels by participant through end of study (lunsekimig vs placebo)



- Change from baseline in **eotaxin-3** levels by participant through end of study (lunsekimig vs placebo)

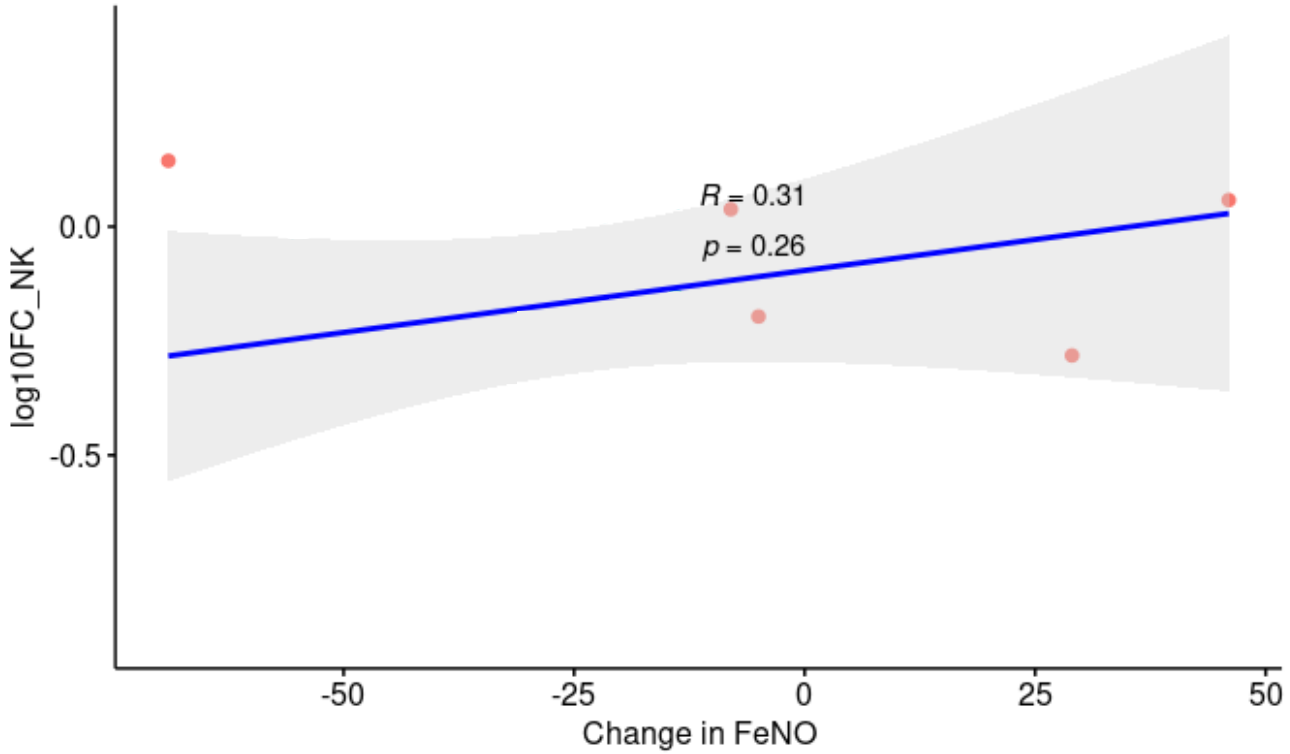


- *Data from the scRNAseq cohort (n=15) followed the same trend as the full set of 36 participants for FeNO, FEV_1, and other markers (no significant differences were observed)*

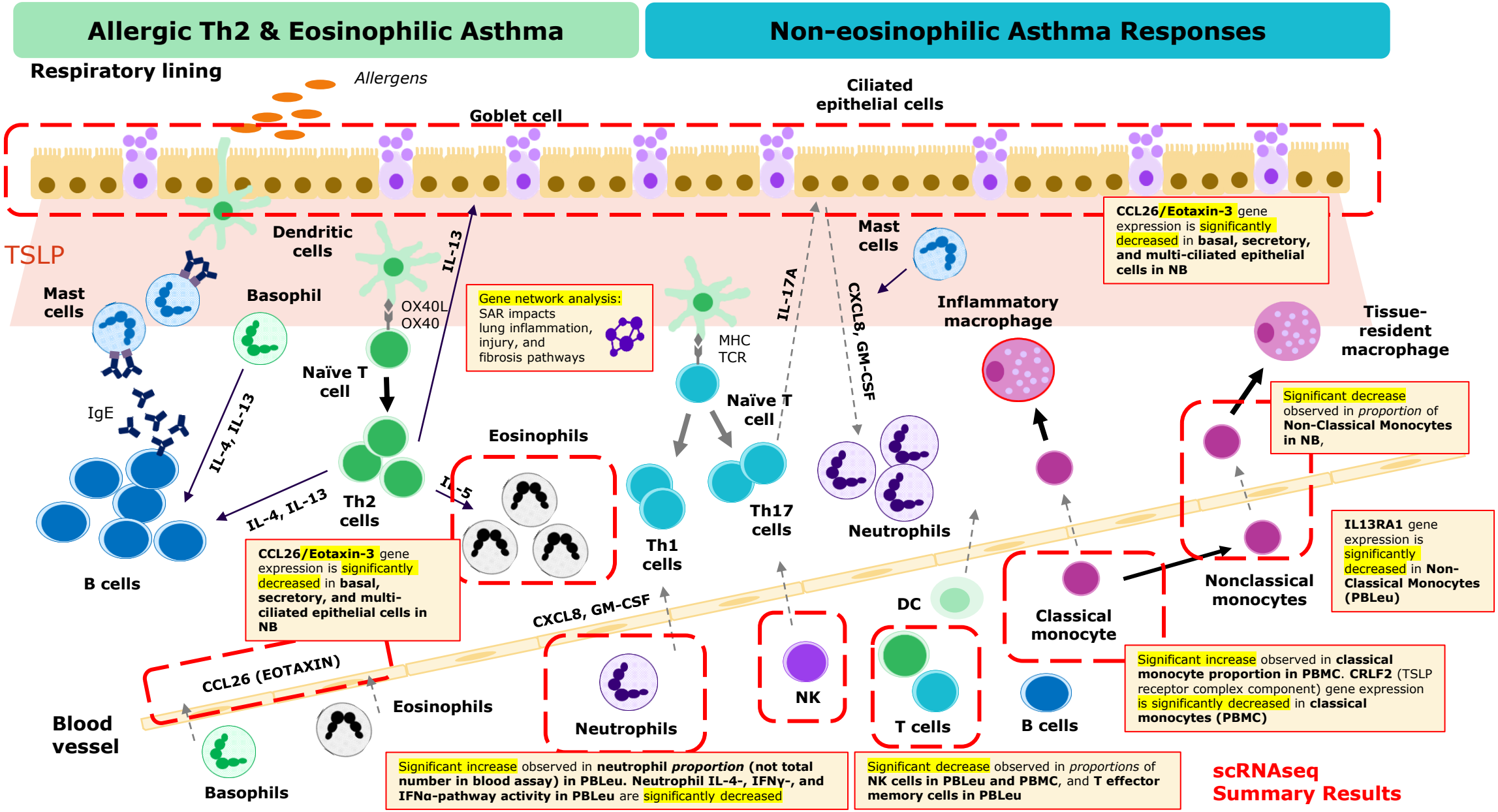
CCL, C-C motif chemokine ligand; EOS, end of study; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; ppb, parts per billion; scRNAseq, single-cell RNA sequencing; SEM, standard error of the mean.

No significant correlation was observed in reduction in natural killer cell proportion in peripheral blood leukocytes and reduction in FeNO at Day 29 with placebo

- Correlation between fold change in natural killer cell proportion in peripheral blood leukocytes and change in FeNO by Day 29 (placebo treatment only)



FeNO, fractional exhaled nitric oxide; NK, natural killer.



Note: Figure adapted from: 1. Gauvreau GM, et al. *Expert Opin Ther Targets*. 2020;24(8):777-792; 2. Rua R, McGavern DB. *J Leukoc Biol*. 2015;98(3):319-332; 3. Marsh SA, et al. *Cytometry A*. 2017;91(11):1055-1058.