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CD122 Antagonism Prevented Lung and Esophageal Inflammation in a Murine Model of Eosinophilia and Reduced T cell Activity in Human Cells In Vitro

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BACKGROUND & OBJECTIVE

Eosinophilic esophagitis (EoE): Chronic, progressive, type 2 inflammatory disease characterized by esophageal dysfunction and Th2-mediated inflammation triggered by food and aeroallergens

IL-2 supports survival and expansion of CD4+ T cells and Type 2 innate lymphoid cells (ILC2s), capable of producing IL-5 and IL-13 responsible for eosinophil infiltration and tissue damage

- ILC2s are enriched in active EoE

IL-15 expression is elevated in EoE, correlates with eosinophilia, and induces cytotoxic mediators from CD8+ T and NK cells (e.g., IFN γ , granzyme B)

ANB033 (CD122 antagonist mAb)

- CD122 is the shared β subunit (IL2R β) of IL-15 and IL-2 receptors and represents an upstream therapeutic target
- Modulation of upstream immune signaling may influence broader inflammatory networks implicated in EoE
 - Therapies directed at downstream cell populations have not consistently improved dysphagia symptoms despite improved histologic responses¹

Mechanism of action (Figure 1):

- CD122 antagonism inhibits IL-15/IL-2 signaling, reducing activation, proliferation, and survival of CD122+ CD8+ T cells, CD4+ T cells, NK cells, and ILC2s
- Reduced activation of these immune cell populations is expected to decrease inflammatory cytokine production (IL-4, IL-5, IL-13, IFN γ) and mediators of cytotoxicity (perforin and granzymes)

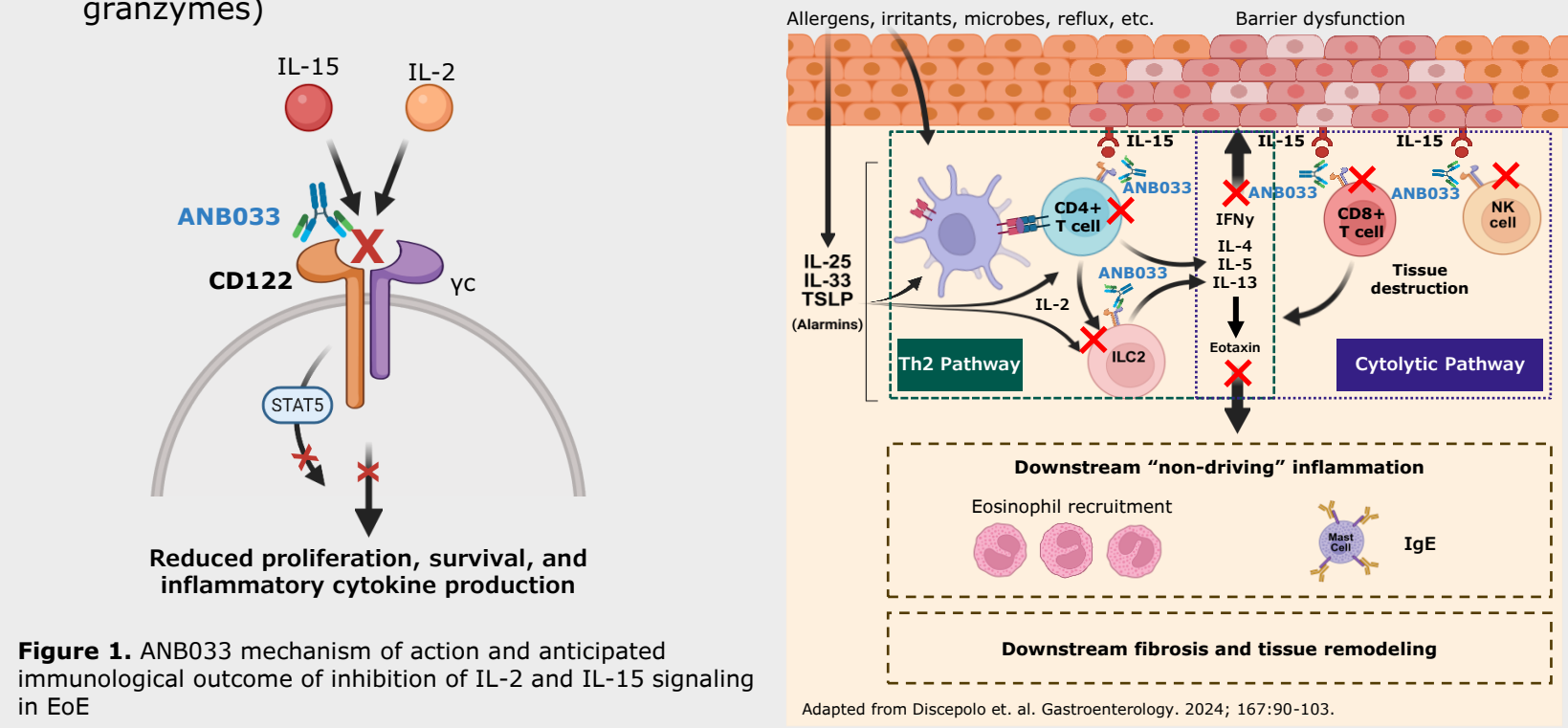


Figure 1. ANB033 mechanism of action and anticipated immunological outcome of inhibition of IL-2 and IL-15 signaling in EoE

OBJECTIVE

To evaluate the role of CD122 antagonism with ANB033 in preventing inflammation associated with EoE in a murine model of eosinophilia and human peripheral blood mononuclear cells (PBMCs) in vitro

METHODS

Secondary analysis of publicly available scRNA-seq data (SCP1242)

scRNA-seq data from EoE patient-derived esophageal biopsies¹ were analyzed for CD122 (IL2RB) expression, immune cell composition, and related pathways (Fig.2)

In vitro evaluation of EoE patient-derived PBMCs

Cytokine-induced proliferation: PBMCs (n=4) were stimulated with IL-2 and IL-15 for 6 days and measured for cytokine-induced cell proliferation via flow cytometry. Ki67+ cells (%) were normalized to isotype control

TCR-induced cytokine secretion: PBMCs (n=4) were stimulated with anti-CD3/CD28 for 3 days and supernatant was assayed for TCR-induced cytokine production. Cytokine concentrations were measured by MSD and normalized to stimulation alone, and statistical analysis was performed using a one-way ANOVA with a Dunnett's Test for 3 simultaneous comparisons (*p<0.05, **p<0.01, ***p<.001, ****p<0.0001)

In vitro evaluation of cytokine production in healthy donor ILC2s

ILC2s were isolated from peripheral blood using negative selection and stimulated with IL-33 and IL-2 in the presence of ANB033 or isotype control for 3 days. Th2 cytokines were measured in supernatant using MSD

Generation of ANB033 surrogate antibody

To enable murine in vivo studies, an antibody reactive with murine CD122 was selected based on epitope similar to ANB033, then engineered to have a comparable affinity to and the same isotype as ANB033

Murine model of allergen-induced eosinophilia with *Aspergillus fumigatus*

Balb/c mice (N=20) were challenged intranasally, 3x/week for 3 weeks with saline or *A. fumigatus*. Test articles were administered to treatment groups 24h prior to the first *A. fumigatus* challenge and dosed 2x/week through Day 21. Esophageal and lung tissue, bronchoalveolar fluid (BALF) and plasma samples were collected. Statistical analysis was performed using a one-way ANOVA with a Dunnett's Test for 5 simultaneous comparisons (*p<0.05, **p<0.01, ***p<.001, ****p<0.0001)

RESULTS

Marked Infiltration of CD122-Expressing CD4+ T cells, CD8+ T cells, NK cells and ILC2s, and their Respective Inflammatory Mediators in EoE Tissue

Single cell RNAseq from EoE patient esophageal biopsies were characterized by:

- Dense infiltration of CD122-expressing CD4+ T cells, CD8+ T cells, and NK cells
- Broad IL-15 expression across structural cells (epithelial cells, myeloid cells, and lymphocytes)

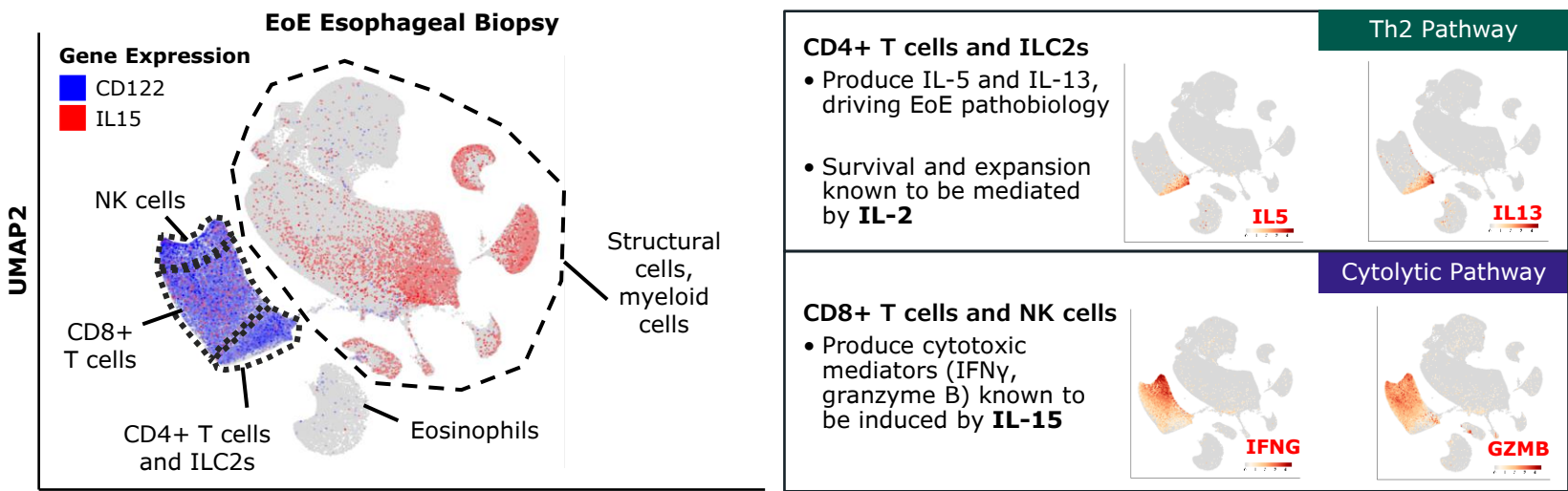


Figure 2. UMAP of single-cell RNA-seq data from EoE esophageal biopsies, colored and scaled by expression of gene pairs: high expression (red or blue), no expression (gray). Inflammatory mediator gene expression (IL5, IL13, IFN γ , and GZMB) colored and scaled: high gene expression (red), no expression (gray)

ANB033 Significantly Reduced Cytokine-induced Proliferation and TCR-induced Cytokine Secretion in EoE Patient-Derived PBMCs, and Type 2 Cytokine Production in Healthy Donor ILC2s

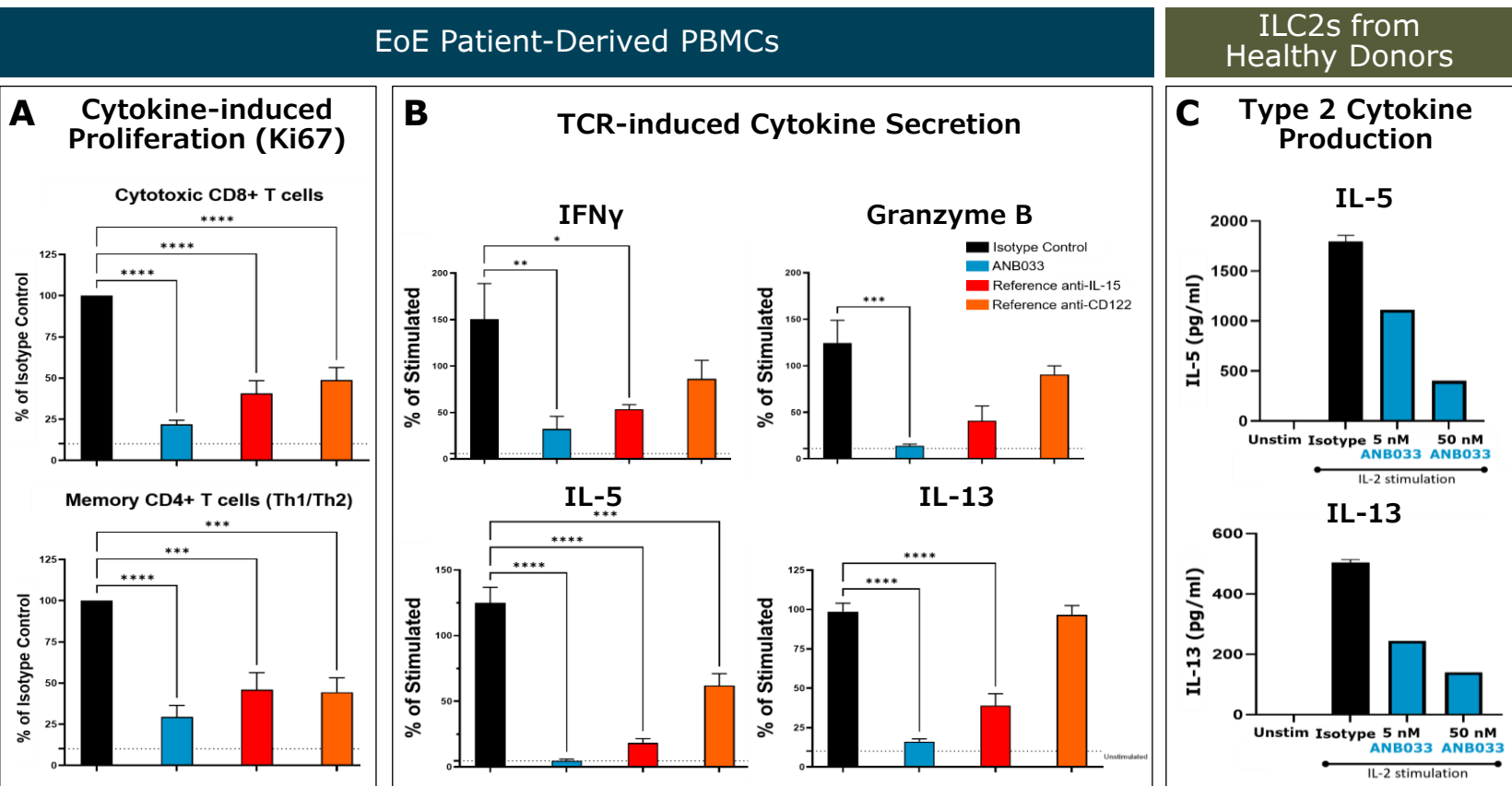


Figure 3. Evaluation of ANB033 compared to isotype control and other reference anti-CD122 and anti-IL-15 antibodies on IL-15- and IL-2-mediated proliferation (A) and TCR-induced cytokine secretion (B) in EoE patient-derived PBMCs, and type 2 cytokine production in ILC2s isolated from healthy donor peripheral blood (C).

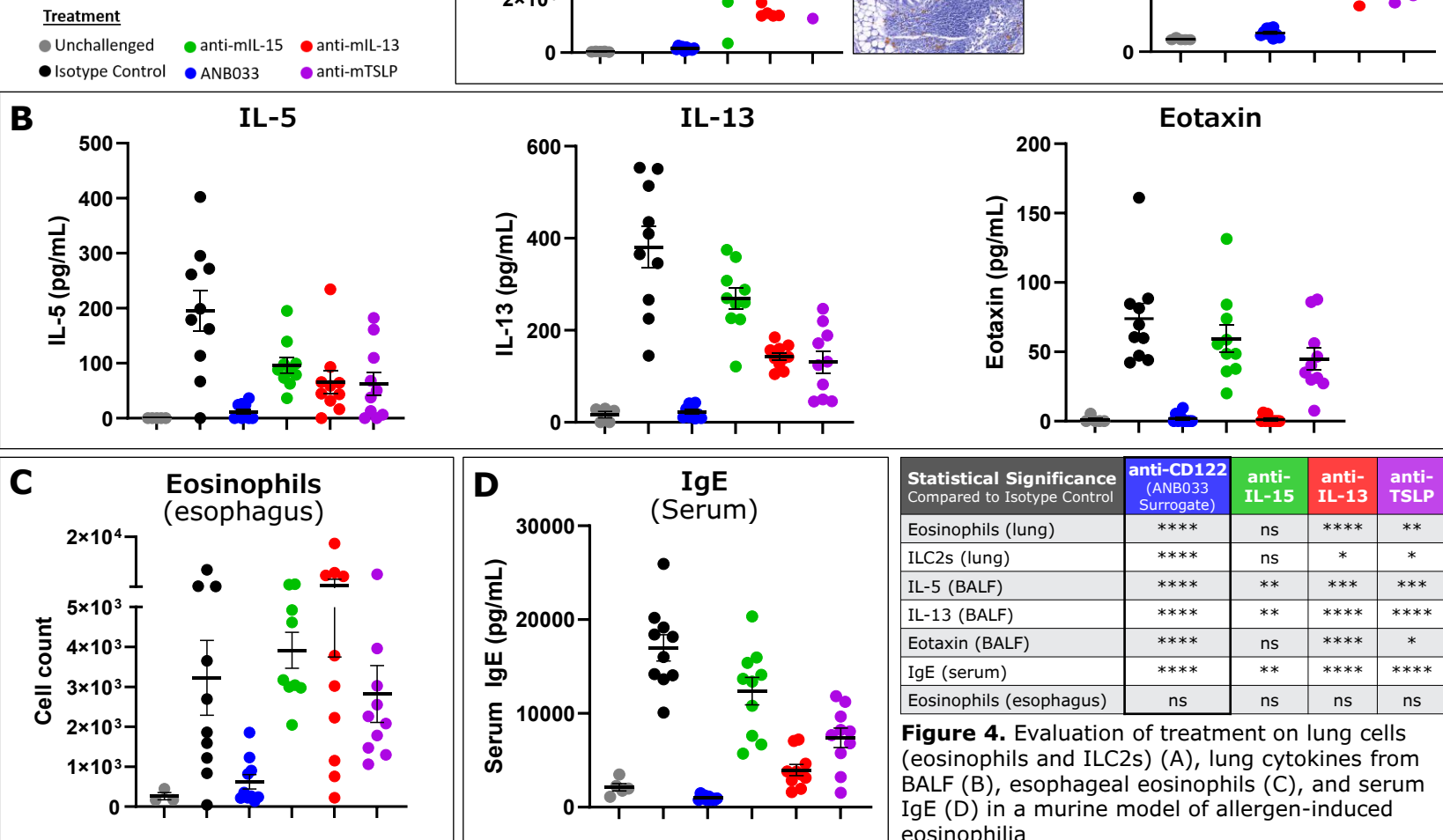
- Compared to isotype control and other reference anti-CD122 and anti-IL-15 antibodies in EoE patient-derived PBMCs, ANB033 demonstrated:
 - Greater reduction of IL-15- and IL-2-mediated proliferation of cytotoxic CD8+ and memory CD4+ Th1/Th2 T cells (Fig. 3A)
 - Greater reduction of Th1 (IFN γ , granzyme B) and Th2 (IL-5, IL-13) cytokine secretion by PBMCs stimulated with anti-CD3/CD28 (Fig. 3B)
- In healthy donor ILC2s, ANB033 reduced production of type 2 cytokines (IL-5 and IL-13) known to be drivers of EoE pathogenesis (Fig. 3C)

RESULTS

ANB033 Surrogate Antibody Prevented Lung and Esophageal Inflammation in a Murine Model of Allergen-induced Eosinophilia

In a murine model of allergen-induced eosinophilia, ANB033 surrogate:

- Reduced expansion of lung eosinophils and ILC2s, and esophageal eosinophils
- Reduced induction of lung IL-5, IL-13, and eotaxin, and serum IgE



Statistical Significance Compared to Isotype Control	anti-CD122 (ANB033 Surrogate)	anti-IL-15	anti-IL-13	anti-TSLP
Eosinophils (lung)	****	ns	****	**
ILC2s (lung)	****	ns	*	*
IL-5 (BALF)	****	**	****	****
IL-13 (BALF)	****	**	****	****
Eotaxin (BALF)	****	ns	****	*
IgE (serum)	****	**	****	****
Eosinophils (esophagus)	ns	ns	ns	ns

Figure 4. Evaluation of treatment on lung cells (eosinophils and ILC2s) (A), lung cytokines from BALF (B), esophageal eosinophils (C), and serum IgE (D) in a murine model of allergen-induced eosinophilia

CONCLUSION

- Single cell RNAseq from EoE patient esophageal biopsies showed dense infiltration of CD122+ immune cells involved in both Th2 and cytolytic pathways, suggesting that IL-15 and IL-2 also play a role in EoE pathobiology
- In vitro, ANB033 demonstrated:
 - Greater reduction of cytokine-induced proliferation and TCR-induced cytokine secretion in EoE patient-derived PBMCs compared to other reference anti-CD122 or anti-IL-15 antibodies
 - Greater reduction of IL-5 and IL-13 production from healthy donor ILC2s vs isotype control
- An ANB033 surrogate antibody prevented allergen-induced lung and esophageal inflammation in mice, reducing lung eosinophils, ILC2s, cytokines, serum IgE, and esophageal eosinophils – confirming that inhibiting upstream CD122+ immune cells may reduce downstream eosinophils and other mediators not directly targeted by ANB033
- These data support the continued evaluation of ANB033 in a Ph1b study in EoE (ISRCTN90323813)

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- ANB033 is investigational and has not been approved by FDA or any other regulatory authority for any use. Safety and efficacy have not been established. Findings from in vitro and animal models may not predict clinical activity or safety in humans

REFERENCES

- Rothenberg ME, et al. *N Engl J Med* 2024;390:2252-2263. 2. Ding J, et al. *Nature Commun.* 2024 Apr 18;15(1):3344

