VSTM-1 Agonist mAb Therapy Reduces Granulocytic Inflammation And COPD

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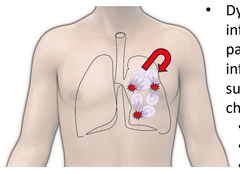
KEYSTONE SYMPOSIA

Background

There is a significant unmet need for novel therapeutics to treat patients with progressive inflammatory airway disorders such as chronic obstructive pulmonary disease (COPD) where sustained granulocytic inflammation promotes a gradual decline in lung function even during corticosteroid or β2-agonist intervention. VSTM-1 is a cell-surface inhibitory receptor highly expressed on granulocytes and pulmonary monocytes. VSTM-1 inhibitory signaling is induced when it binds to amphipathic alpha-helical damage-associated molecular pattern (DAMP) motifs on ligands such as cathelicidin and the S100 proteins. VSTM-1 thus functions as a regulator of myeloid cell-driven inflammatory cascades. This immunosuppressive function, combined with the strong expression profile of VSTM-1 on pulmonary myeloid cells—particularly neutrophils—coupled with the prominent role of neutrophils as inflammatory mediators of lung immunopathology, makes VSTM-1 a promising novel therapeutic target for COPD. We developed an agonist monoclonal antibody (mAb) against VSTM-1 to evaluate the therapeutic potential of VSTM-1 engagement and downstream immunosuppressive signaling under hyperinflammatory conditions. Augmentation of VSTM-1 signal transduction by an agonist mAb suppressed NETosis and the production of reactive oxygen species (ROS) in inflammatory granulocytes. Moreover, agonism of VSTM-1 by a therapeutic mAb regulated TNFα, IL-6, and IL-1β cytokine production in myeloid cells in response to danger stimuli. A limitation of testing the potential of VSTM-1 as a therapeutic target is that the receptor is not expressed in mice. We therefore used CRISPR/Cas technology to construct VSTM-1 knock-in C57BL/6 mice where human VSTM-1 is expressed under the neutrophil-specific mouse Ly6G promoter. In this novel system, engagement of VSTM-1 by an agonist mAb during LPS lung challenge reduced pulmonary neutrophilia and overall disease score. These preclinical data support targeting of VSTM-1 as a novel therapeutic intervention for chronic inflammatory diseases of the lung.

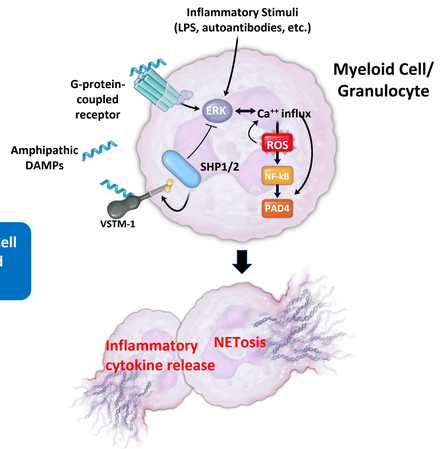
Granulocytic Inflammation Drives Pulmonary Inflammatory Disease

A Granulocytic Inflammation is a Driver of Pulmonary Disease

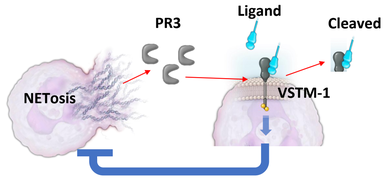


- Dysregulated neutrophil influx and activation drives pathology during inflammatory lung disorders such as COPD, ARDs, and chronic infection
  - Cytokine production
  - Reactive oxygen species
  - Degranulation and protease release

B VSTM-1 binds DAMPs to Regulate Granulocytic Inflammation



C NETotic proteinase 3 (PR3) Cleavage of VSTM-1 from the Cell Surface Raises the Threshold Necessary for Native Ligand Immunoregulatory Function



D Therapeutic Intervention with a High Affinity Agonist mAb

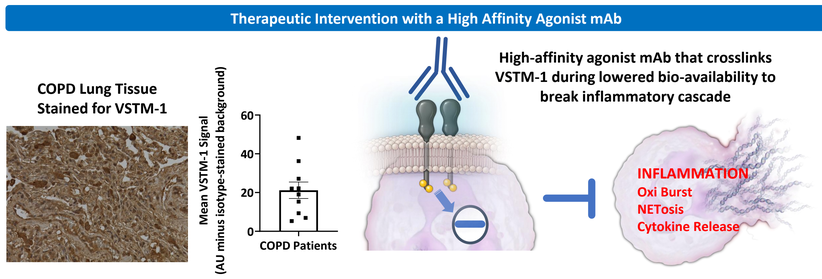


Figure 1. A) Schematic of granulocyte-mediated pathology during pulmonary inflammatory disease. **B)** Schematic of VSTM-1 regulatory mechanism. VSTM-1 binds soluble amphipathic damage-associated molecular pattern (DAMP) motifs that are released during inflammatory insult. Ligand binding results in recruitment of SHP1/2 phosphatase, which dampens ERK-mediated signaling and results in suppression of reactive oxygen species (ROS) generation, NF-kB activity, and (in neutrophils) PAD4-mediated NETosis. **C)** Illustration of VSTM-1 function and regulation during hyper-inflammatory processes. **D)** (Left) VSTM-1 staining of FFPE lung tissue from COPD patients. Quantitation performed by staining patient matched samples with anti-VSTM-1 or isotype control, deconvoluting images, then subtracting the background (isotype) signal from the VSTM-1 signal. N = 10 COPD patients. (Right) Illustration of anti-VSTM-1 agonist mAb therapeutic strategy.

VSTM-1 Is Highly Expressed on Granulocytes And Some Monocytes

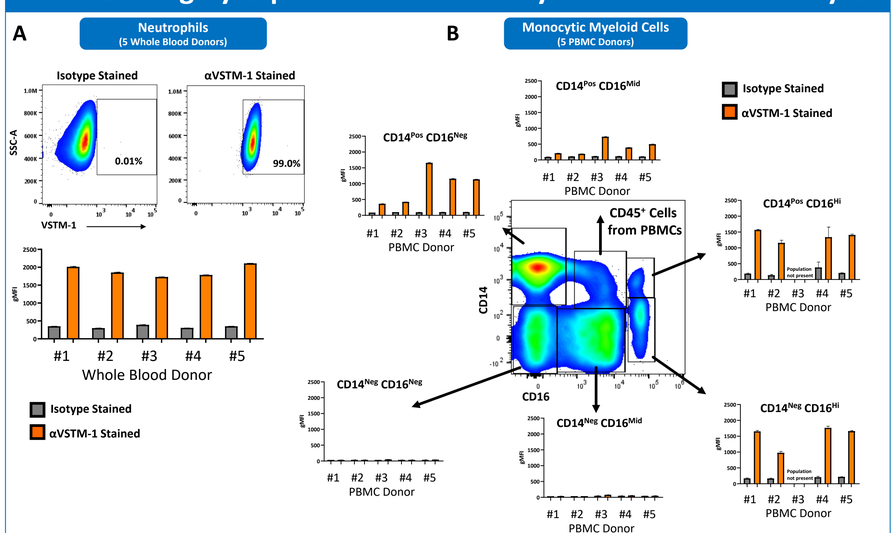


Figure 2. A) VSTM-1 cell surface expression on CD66b+ neutrophils isolated from fresh whole blood or **B)** monocytic subpopulations from cryopreserved PBMCs. Each paired bar graph represents VSTM-1 (orange) or isotype control (gray) staining of cells from an individual donor.

Generation Of An Anti-VSTM-1 Agonist Monoclonal Antibody

A Top mAb Candidate Affinity Data

mAb	Kd(nM)	Kd (M)	ka (1/Ms)	kdis (1/s)	Full R^2
αVSTM-1.IgG1	0.67	6.69E-10	2.18E+05	1.46E-04	0.9812

B Top mAb Candidate Cell Binding Data

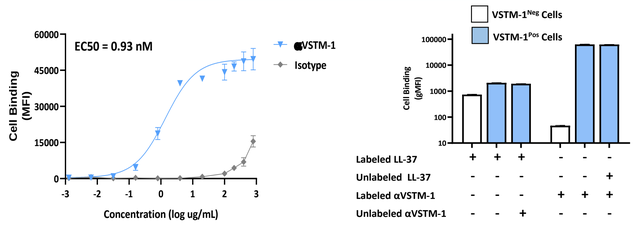


Figure 3. A) Binding kinetics and affinity of top anti-VSTM-1 mAb candidate as measured by Octet™ (Sartorius). **B)** (left) Cell surface binding or (right) competition binding between equimolar concentrations of recombinant LL-37 or αVSTM-1 mAb on VSTM-1-transduced or empty vector HEK293T cells.

VSTM-1 Agonist mAb Suppresses Inflammatory Cytokine Production

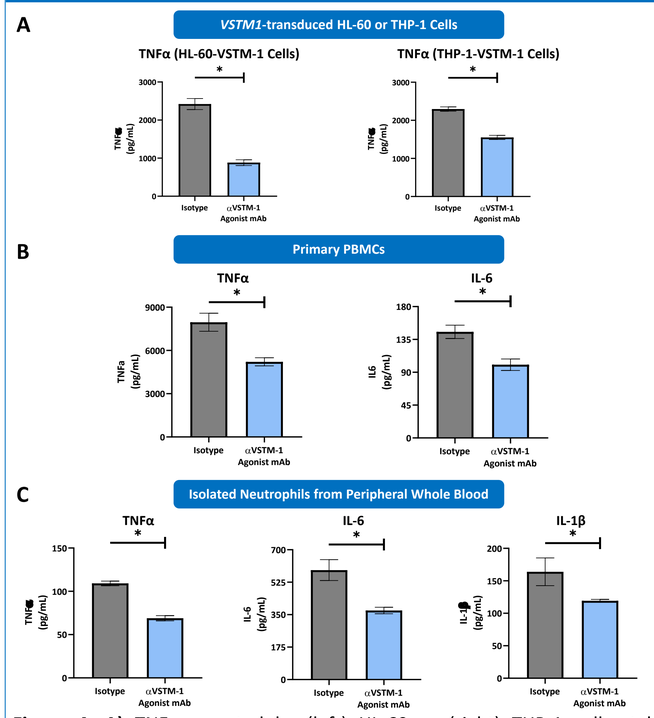


Figure 4. A) TNFα secreted by (left) HL-60 or (right) THP-1 cells stably transduced with *VSTM1* and activated with 20 ng/mL LPS + 50 ng/mL IFNγ for 24 hrs. **B)** Cytokines secreted by αCD3/28 activated PBMCs or **C)** cytokines secreted by LPS activated blood neutrophils *ex vivo*. All cells treated with 5 ug/mL coated mAb. N = 4-5 technical replicates

VSTM-1 Agonist mAb Suppresses ROS and NETosis

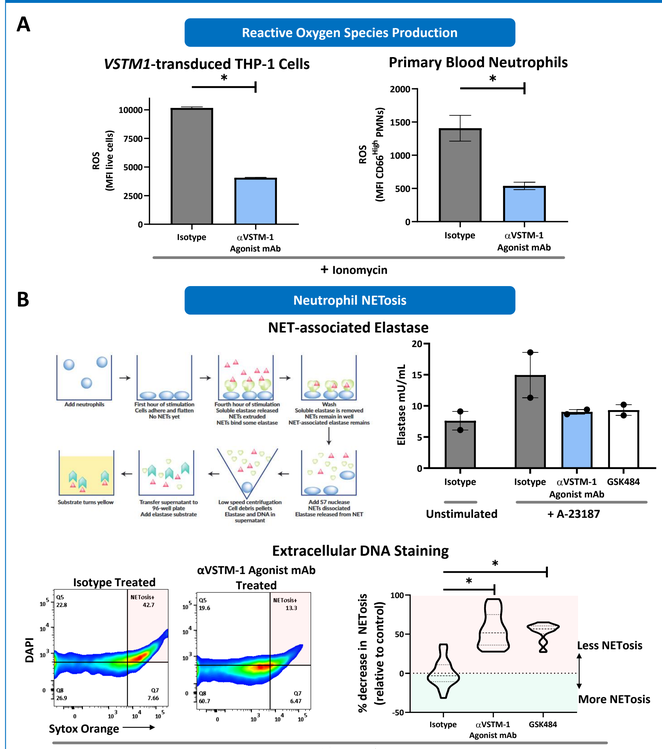
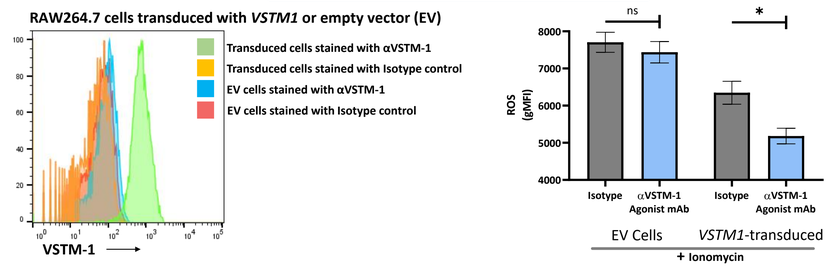


Figure 5. A) Reactive oxygen species (ROS) or **B)** NETosis in the indicated cell type after Ca^{++} ionophore stimulation in the presence of 5 ug/mL coated mAb or 10 uM GSK484 (PAD4 enzyme inhibitor). N = 2-4 technical replicates or (for DNA staining) 6 donor samples.

Generation of VSTM-1-expressing Knock-in Mice

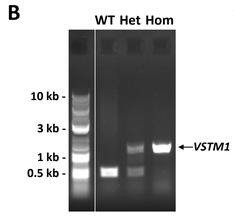
A Verification of VSTM-1 Functionality in Mouse Myeloid Cells



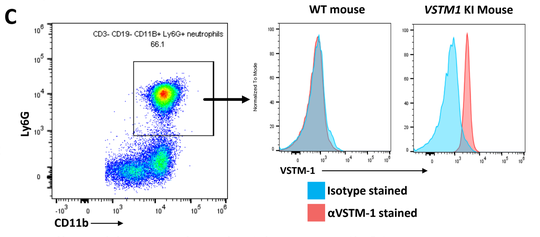
Knock In of *VSTM1* in C57Bl/6 Mice via CRISPR/Cas9

- Ly6G expression is restricted to murine granulocytes
- Human *VSTM1* was knocked-in directly downstream of the *Ly6g* promoter in C57BL/6 mice, engendering VSTM-1 expression in murine neutrophils

B



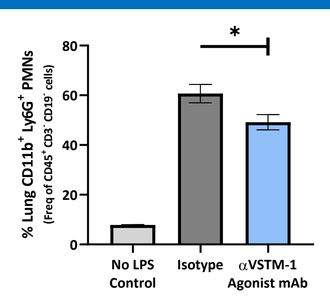
C



No VSTM-1 expression on mouse:
- $CD4^+$ T cells
- $CD8^+$ T cells
- $CD19^+$ B cells
- $CD11b^+$ $Ly6G^-$ Myeloid cells

Figure 7. A) RAW264.7 mouse cells stably transduced with *VSTM1* (left) express VSTM-1 on the cell surface and (right) respond to anti-VSTM-1 agonist mAb ROS suppression when under ionomycin stimulation. **B)** PCR amplification of human *VSTM1* in tail snips from wild type (WT), heterozygous (Het), or homozygous (Hom) knock-in mice. **C)** Cell surface expression of VSTM-1 on circulating neutrophils from WT or *VSTM1* Het mice.

VSTM-1 Agonist mAb Treatment Reduced Granulocytic COPD in *VSTM1* Knock-in Mice



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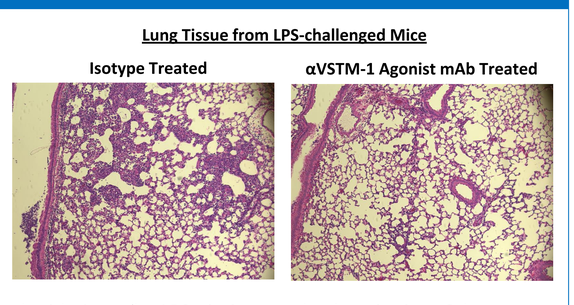
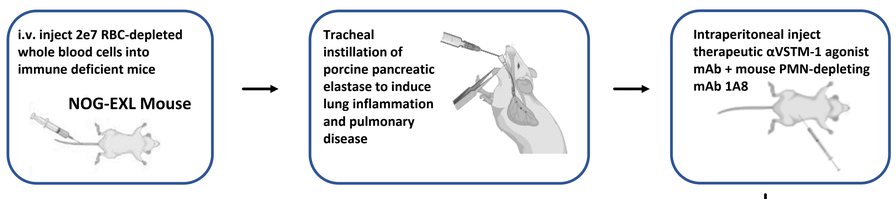


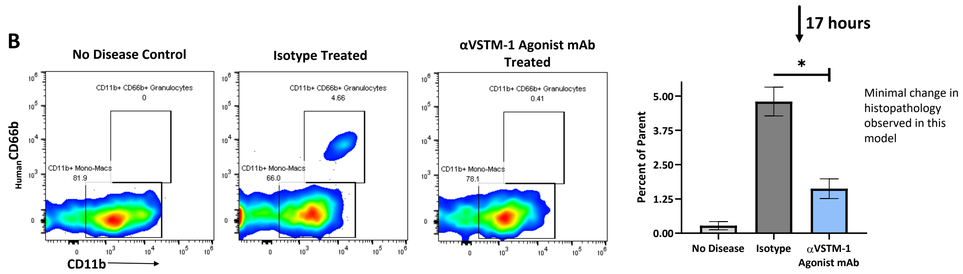
Figure 9. *VSTM1* knock-in mice were challenged with 1 mg/kg LPS via intratracheal aspiration. 8 hours post-challenge mice were treated with 10 mg/kg mAb via intraperitoneal injection. 24 hours post-challenge, mouse lungs were harvested for **A)** neutrophil influx and **B)** pathology by H&E staining. N = 5 mice per treatment group

VSTM-1 Agonist mAb Treatment Reduced Granulocytic COPD in a Human Myeloid Cell Replete Model of Disease

A



B



Minimal change in histopathology observed in this model

Figure 6. A) Schematic of pulmonary disease modeling in human myeloid cell–replete mice. **B)** Representative scatterplots and quantification of human neutrophil accumulation in the lungs of elastase challenged mice after treatment with 10 mg/kg mAb. N = 6 mice

VSTM-1 Agonist mAb Summary

- VSTM-1 is a myeloid cell restricted immune inhibitory receptor that is highly expressed on granulocytes
- An anti-VSTM-1 agonist mAb generated for therapeutic intervention of granulocytic inflammatory disorders of the lung suppressed ROS, NETosis, and inflammatory cytokines
- $_{Human}$VSTM-1 expressing syngeneic mice were generated and characterized
- Pilot *in vivo* lung disease models indicate that anti-VSTM-1 agonist mAb treatment can reduce pulmonary pathology
- Ongoing Efforts:
  - Optimizing the modality of VSTM-1 agonist mAb for maximal activity and therapeutic index
  - Expansion of *in vivo* modeling to more chronic models of inflammatory disease
  - Benchmarking and studies of combo treatments to investigate potential synergy with current clinical therapeutics