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Semaphorin3A is a promising therapeutic tool for bronchial asthma

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The rational for this innovation:

For many years treatment for immune-mediated and or autoimmune diseases focused on improving suppression of pro-inflammatory processes. Products of T-helper 1 (Th1) cells such as TNF- α were the first to be targeted. In accordance with this approach, anti-IL-17 was also added to this arsenal of suppressive anti-inflammatory therapies intended to treat psoriasis and psoriatic arthritis. Although very beneficial in improving patients' prognosis and quality of life, these drugs are still do not achieve long-lasting remission for those with autoimmune skin diseases (1,2). In line with this, biological therapies for treating severe bronchial asthma were also introduced. Drugs such as omalizumab (monoclonal anti-IgE antibodies) or anti-IL-5 antibodies are widely used in patients with active disease despite all other classical treatments. Although steroid sparing and capable of reducing asthma severity,

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these drugs often do not prevent relapses (3), which is why a different approach, namely increasing regulatory functions such as those of T-regulatory (Treg), cells is needed. Th2 cell inflammation and Treg deficiency are key features of bronchial asthma. Patients with uncontrolled asthma have many fewer CD4⁺CTLA-4⁺ T cells. Modulating T cell responses with CTLA-4 Ig (the regulatory co-stimulation molecule on Treg cells) reduced immune mediated inflammation. This approach was the first to demonstrate the benefit of targeting inflammation by enhancing regulatory functions rather than by suppressing immune responses. Semaphorin3A (sema3A) is well-known for its regulatory functions at all stages of immune response and also as an angiogenic inhibitor. The administration of sema3A into a mouse model of allergic rhinitis was reported to reduce IL-4 and IL-17 levels as well as Th2 activation (4). By co-culturing sema3A with T cells, we demonstrated the enhancement of IL-10 and TGF- β expression of Treg cells. These successes encouraged us to evaluate the potential role of sema3A for bronchial asthma. Serum levels of sema3A were significantly lower in both severely and moderately asthmatic patients, as compared to healthy individuals (250, 409, and 636 ng/ml respectively; $p < 0.001$, and 0.01 respectively). Moreover, it was shown that sema3A expression on CD4⁺ CD25^{high} cells (Treg) during asthma exacerbations was significantly lower than that of normal controls [Mean Fluorescence Intensity (MFI); 2.6 vs. MFI; 3.5, $p < 0.006$]. The findings of decreased serum levels and lower Treg expression in severe asthmatics suggest that sema3A is crucial for regulating inflammation in asthma (5).

Description of this innovation and applications:

As it has high potential for regulating pro-inflammatory immune responses, sema3A was used in several mice models, including SLE, RA and allergic rhinitis. Sema3A effectively reduced inflammation and improved disease outcome in all. To assess the in-vivo effects of recombinant sema3A in bronchial asthma, it was injected into BALB/c-mice who were sensitized using ovalbumin (OVA) + adjuvant for 15 days followed by OVA aerosol

inhalation for five consecutive days. Four hours after air ways sensitization on each day - 15 of these mice were injected intraperitoneally with 50 µg of recombinant human sema3A-FR. The remaining 15 mice were injected with 50 µg purified vehicle (negative control). The mice were sacrificed five days later. Broncho-alveolar lavage (BAL) fluid was collected and lung biopsies were formalin-fixed and analyzed. Sema3A- treated mice had few mononuclear and neutrophil cells in the BAL while in the control mice, the fluid was enriched with mononuclear and neutrophil cells (P=0.0081). In sema3A- treated mice, only 20% of the bronchioles and arterioles were infiltrated by inflammatory cells as compared to 90% in the control group (P=0.0021). In addition, eosinophil infiltration was also significantly increased in the control group as compared with the sema3A- treated mice (P=0.033). Finally, in the control mice, angiogenesis was significantly increased in comparison with fewer micro-vessels in the lungs of sema3A- treated mice (P=0.0013) (See figure 1) (6).

The hallmark of bronchial inflammation is considered to be T-cell-driven, involving all T cell phenotypes. The current study is the first to show that sema3A is very efficient in reducing infiltration of mononuclear cells, neutrophils and eosinophils in lung tissue inflammation and in BAL from OVA-sensitized mice. We assume that this effect is achieved by increasing local Treg functions, which subsequently reduces pro-inflammatory cytokines. It is also possible that sema3A inhibits IL-17 production, indirectly leading to the reduced influx of neutrophils. These findings are in agreement with in-vivo studies in which injection of recombinant sema3A effectively improved allergic rhinitis and atopic dermatitis in relevant mice models. In these models, use of sema3A improved clinical symptoms and tissue inflammation (7). Long-lasting airway inflammation may lead to structural changes termed remodeling. These changes consist of sub-epithelial layer thickening, airway smooth muscle hyperplasia and increased angiogenesis induced by the expression of angiogenic factors such as VEGF and angiopoietin. Though efficient in improving asthma symptoms, inhaled

corticosteroids and anti-leukotriens have limited effect on angiogenesis and remodeling. Of the reported mechanisms by which sema3A inhibits angiogenesis, the most important is its high efficacy in inhibiting VEGF activity as a result of activating inhibitory intracellular pathways that inhibit VEGF signal transduction (8).

Conclusion:

Our invention indicates that sema3A should possibly be considered a novel therapeutic agent for treating all phenotypes of bronchial asthma. It appears to affect Th2- induced inflammation and to reduce the influx of neutrophils. Future research should focus on studying potential routes of administration (intravenous or inhaled). Would it be approved for human asthma we should evaluate its safety and lack of toxicity. The beneficial effects of sema3A should include improvement in lung function and the possibility of achieving improved quality of life.

Conflict of Interest:

The authors have no conflict of interest in relation to this work.

Figure Legends:

Figure 1.

- A-** Comparison of the average grade of inflammatory cells in the BAL of vehicle versus sema3A treated mice based on 15 mice in each group. Error bars represent the standard error of the mean. Statistical significance was determined using the Mann–Whitney non-parametric test.
- B-** Percentage of bronchioles and blood vessels surrounded by inflammatory cells in high power field (HPF). The results are the mean value of 3-5 HPF, derived from 15 mice in each group.
- C-** Number of eosinophils per HPF in the inflammatory cell infiltrates in vehicle vs.sema3A treated mice. The results are the average of 3-5 HPF, derived from 15 mice in each group.
- D-** Quantification of the blood vessel concentrations across all histological slides in vehicle -treated and in sema3A- treated mice (15 in each group, $P=0.0013$).

Figure 2.

Semaphorin3A (Sema3A) is a potent therapeutic agent in OVA-induced mouse model of asthma.

It efficiently reduces neutrophil and eosinophil infiltration and angiogenesis in lung tissues and BAL.

References:

1. Bubbert-Roth A, Atzeni F, Masala IF, Caporali R, Montecucco C, Sarzi-Puttini P. TNF inhibitors in rheumatoid arthritis and spondyloarthritis: Are they the same? *Autoimmun Rev* 2018; 17: 24-28.
2. Thompson C, Davies R, Choy E. Anti cytokine therapy in chronic inflammatory arthritis. *Cytokine* 2016; 86: 92-99.
3. Busse WW. Biological treatments for severe asthma: where do we stand? *Curr Opin Allergy Clin Immunol* 2018; 18: 509-18
4. Xiang R, Xu Y, Zhang W, Kong YG, Tan L, Chen SM, Deng YQ, Tao ZZ. Semaphorin3A inhibits allergic inflammation by regulating immune responses in a mouse model of allergic rhinitis. *Int Forum Allergy Rhinol* 2018; (Epub ahead of print).
5. Cozacov R, Halasz K, Haj T, Vadasz Z. Semaphorin3A: Is a key player in the pathogenesis of asthma. *Clin Immunol* 2017; 184: 70-72.
6. Sabag D. Adi, Eiza N, Bejar J, Shefer H, Toledano S, Kessler O, Neufeld G, Toubi E, Vadasz Z. Semaphorin 3A is effective in reducing both inflammation and angiogenesis in a mouse model of bronchial asthma. *Front Immunol* 2019; ahead of print
7. Sawaki H, Nakamura F, Aihara M, Nagashima Y, komori-Yamaguchi J, Yamashita N, et al. Intranasal administration of semaphorin3A alleviates sneezing and nasal rubbing in a murine model of allergic rhinitis. *J Pharmacol Sci* 2011; 117: 34-44.
8. Acevedo LM, Barillas S, Weis SM, Gothert JR, Cheresh DA. Semaphorin3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor. *Blood* 2008; 111: 2674-2680.

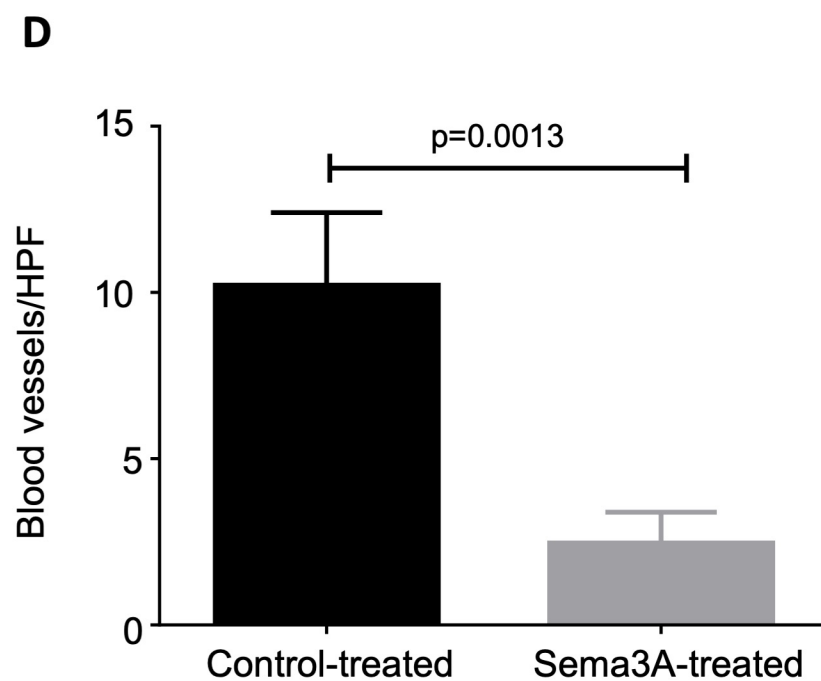
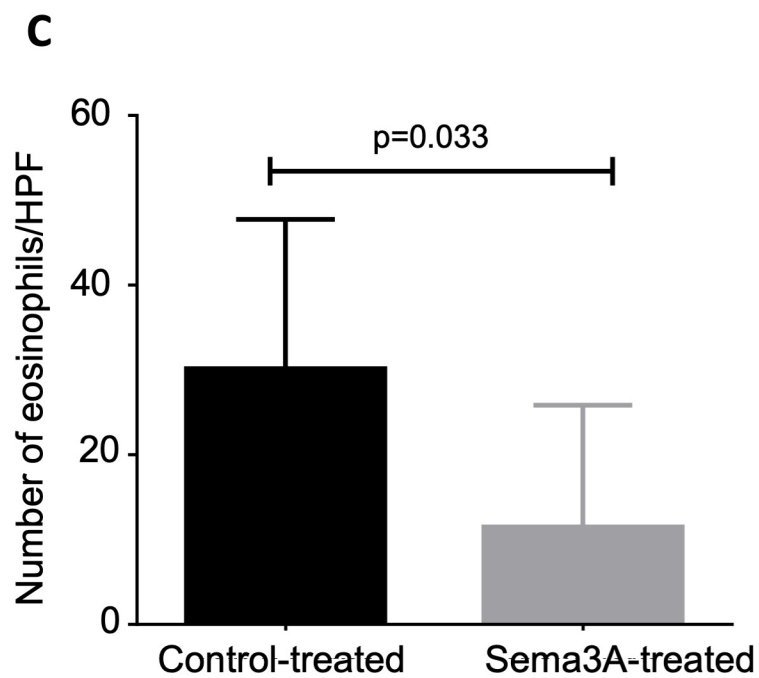
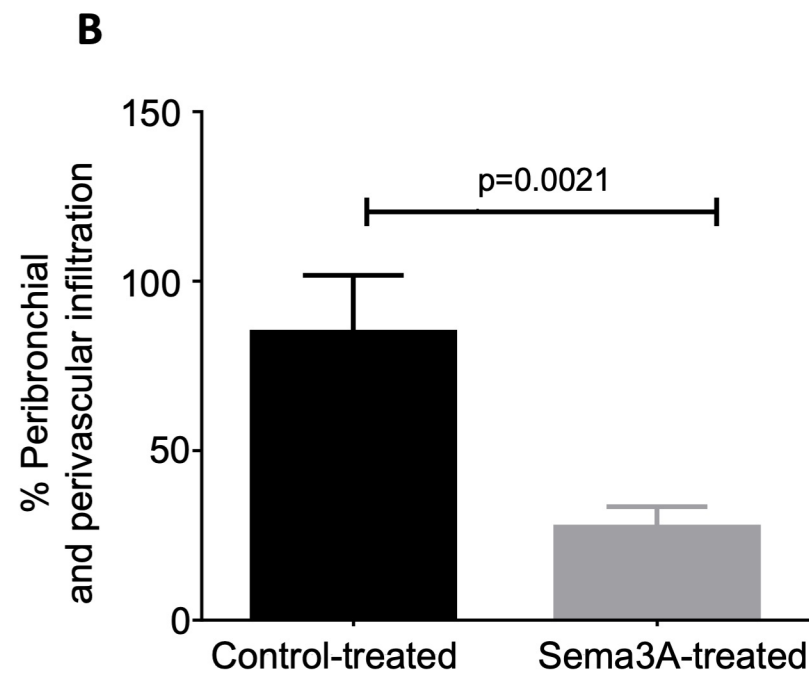
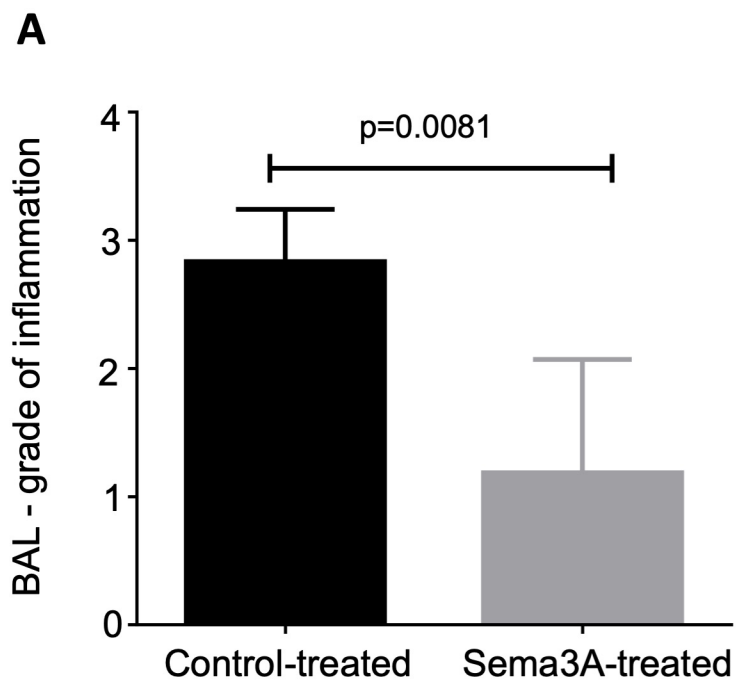


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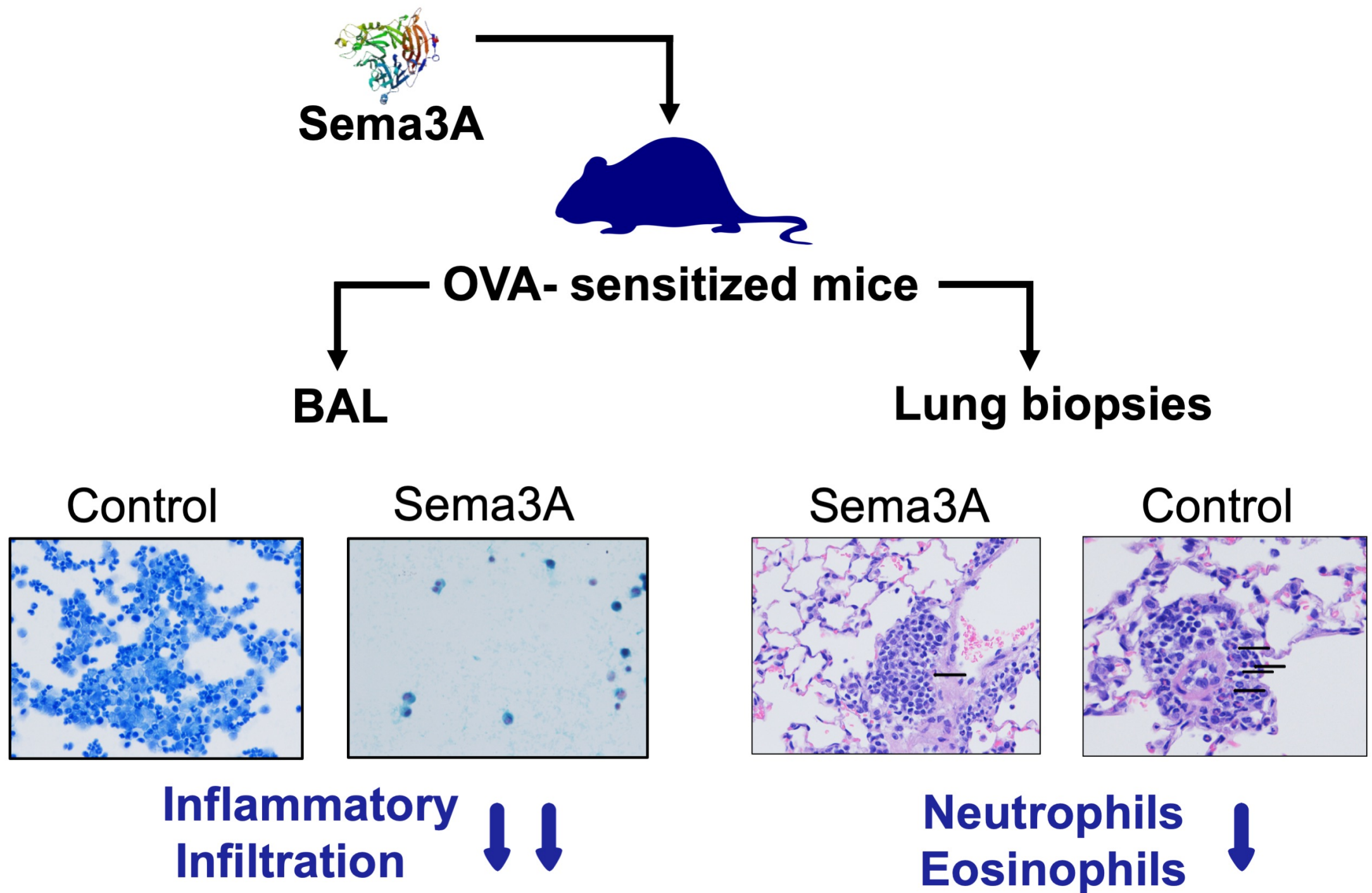


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