Lanthionine synthetase C-like receptors reduce eosinophilia, type 2 immune responses and lung inflammatory pathology in mouse models of asthma

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Airway hyperresponsiveness and asthma are associated with altered glucose metabolism with inhibition of anaerobic glycolysis reducing inflammation and lung eosinophilia. LANCL receptors are key focal points for metabolism in immune cells, controlling mitochondrial mass and oxidative pathways all while promoting immunoregulatory differentiation. Knockout of LANCL receptors leads to diminished Treg differentiation, altered epithelial cell metabolism and impaired phagocytic clearance, all of which can contribute to the chronicity of asthma. We developed an oral agonist for LANCL receptors and tested it within ovalbumin and house dust mite induced models of asthma in mice. Treatment in vitro with the agonist resulted in reduced differentiation of inflammatory and allergic CD4+ T cells and reduced production of inflammatory cytokines in macrophages. In the OVA model, oral treatment with an LANCL therapeutic, beginning on the second day of aerosol challenge significantly reduced neutrophilia and eosinophilia in the lung by flow cytometry and histology. LANCL treatment significantly reduced anti-OVA IgE titers and type 2 cytokines, IL-5 and IL-13. Reduction of eosinophilia and type 2 immune responses were validated in the house dust mite model. In both models, lung gene expression trends were consistent with improved pulmonary function and mitochondrial metabolism. Thus, activation of LANCL receptors is a feasible strategy for the treatment of asthma and pulmonary inflammation.

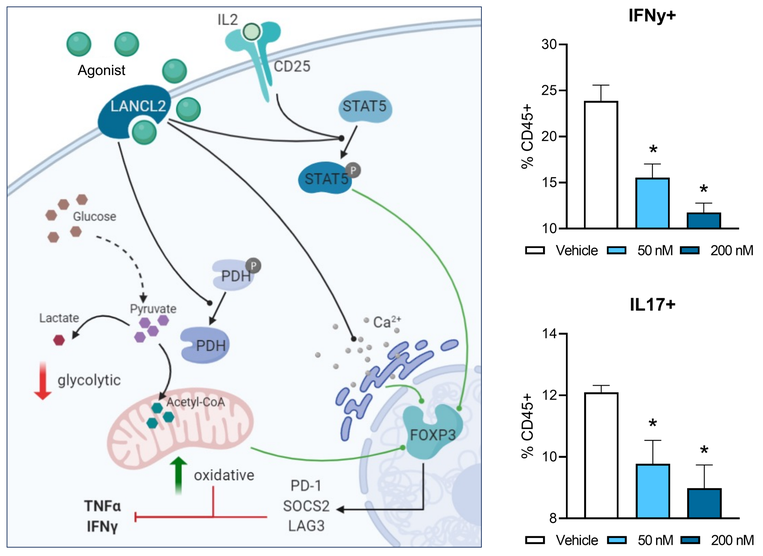

Figure 1. CD4+ T cell responses to LANCL2 activation.

When activated, LANCL2 results in downstream immunometabolic signaling that increases the differentiation of Tregs and decreases the differentiation of effector CD4+ T cells (**Fig. 1**). We designed a systemically distributed LANCL2 agonist (NIM-3301) that effectively modulates CD4+ T cell responses in vitro. At both 50 and 200 nM, NIM-3301 treatment results in the dose-dependent decrease of IFNγ+ and IL17+ CD4+ T cells after stimulation with PMA and ionomycin for 6 hours. An intron variant of LANCL2 was recently identified to be associated with the incidence of asthma in European Americans (Almoguera, 2016). Combined with our previous work on the relevance of LANCL2 in mucosal immune responses, including to influenza infection, we set out to determine the efficacy of LANCL2 activation on the prevention of inflammation in models of asthma.

Wild-type BALB/c mice were immunized with 10 µg of OVA in aluminum hydroxide (2 mg/mouse) by intraperitoneal injection on days 0 and 7. Daily from day 14 to 17, mice were exposed to OVA (6% w/v) by aerosolization for 25 minutes. Beginning on day 15, mice were treated with vehicle or 20 mg/kg of LANCL2 agonist by oral gavage once daily. Negative control mice that received immunization but aerosolization of only PBS were included for comparison. Lungs and blood were collected on day 18. Histologically, LANCL2 activation resulted in reduced perivascular, perialveolar and peribronchi eosinophilic infiltration, airway lumen narrowing and airway epithelial cell desquamation. Overall, type II immune responses were reduced in the lungs (**Fig. 2**). In addition to influencing the type II immune response to allergic stimuli, LANCL2 activation provides anti-inflammatory responses to viral stimulations in normal human bronchial epithelial (NHBE) cells after treatment ex vivo (**Fig. 3**).

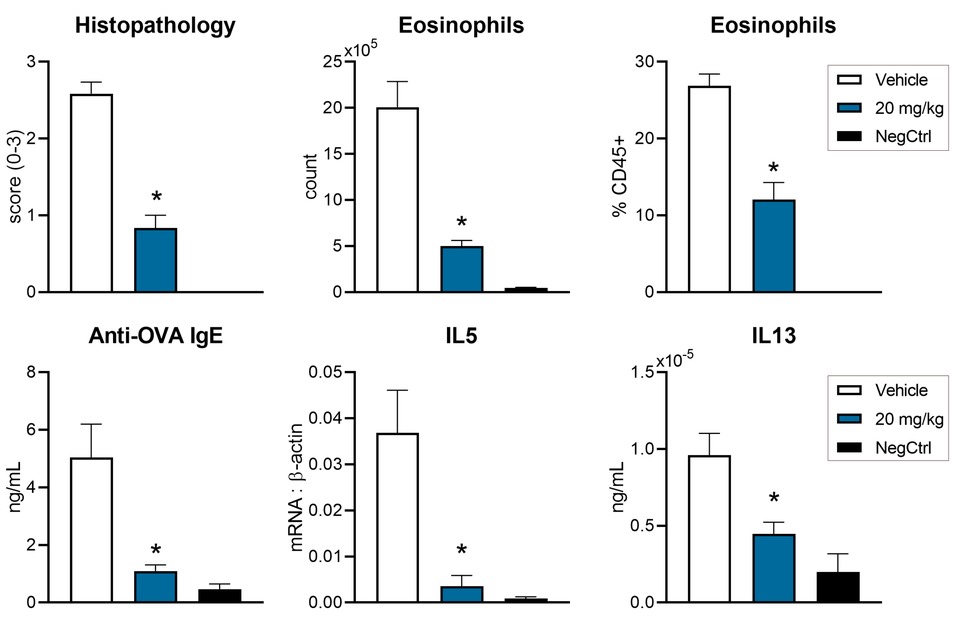

Figure 2. Immune responses to LANCL2 activation in an OVA-induced model of asthma after 3 days of treatment (n = 6).

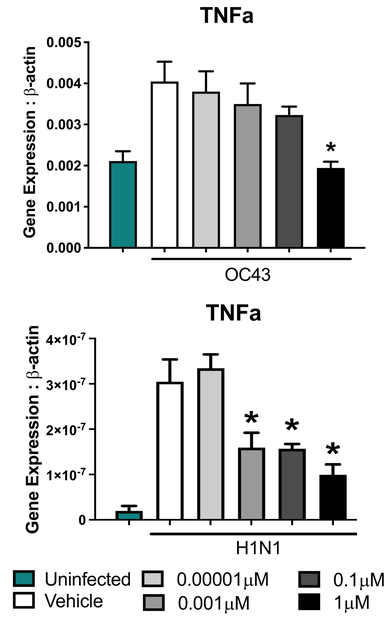

Figure 3. NHBE cells treated with LANCL agonist (n = 3).

Mice were immunized with 100 µg of house dust mite (HDM) in complete Freund's adjuvant on day 0. One week following sensitization, mice were administered 10 µg HDM daily by intranasal instillation from Monday to Friday for 3 consecutive weeks. Beginning on week 3, mice were treated with vehicle or 20 mg/kg of LANCL2 agonist by oral gavage once daily. Negative control mice that received immunization but instillation of only saline were included for comparison. One day after the final instillation, lungs were harvested and processed for flow cytometry. LANCL2 activation reduced lung eosinophils and neutrophils as well as IL5+ and IL4+ immune cells (**Fig. 4**).

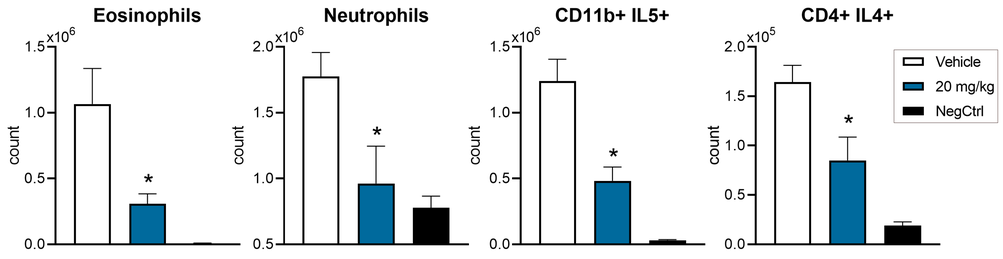

Figure 4. Efficacy of LANCL2 activation in a HDM-induced model of asthma (n = 7).

LANCL2 activation was also effective in reducing inflammation in models of chronic pulmonary disease (**Fig. 5**). In a bleomycin model of idiopathic pulmonary fibrosis, LANCL2 activation reduced lung neutrophils and CD4+ IL5+ cells after 1 week of treatment. Meanwhile, in an elastase model of COPD, LANCL2 activation reduced inflammatory macrophages and CD4+ IL17+ cells in lung following 2 weeks of treatment.

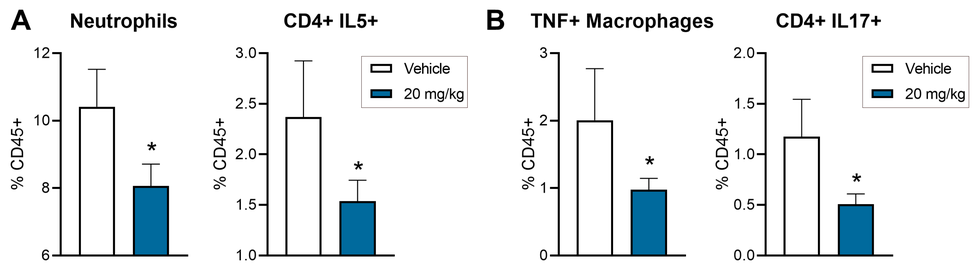

Figure 5. Efficacy of LANCL2 activation in IPF (A) and COPD (B) models (n = 7).

Publicly available datasets from asthma patients were analyzed for the expression levels of LANCL2 associated genes (**Fig. 6**). Datasets included analysis of asthma patients experience frequent exacerbations and those without (GSE211158), CD4+ T cell differences between asthma patients and healthy controls (GSE 217904), and airway brushing sample differences between hyperresponsive asthma patients and control (GSE241016). Notably, genes associated with the amelioration of cellular stress were largely downregulated along with anti-inflammatory immune signaling genes. Meanwhile, metabolic genes were moderately upregulated across all three datasets.

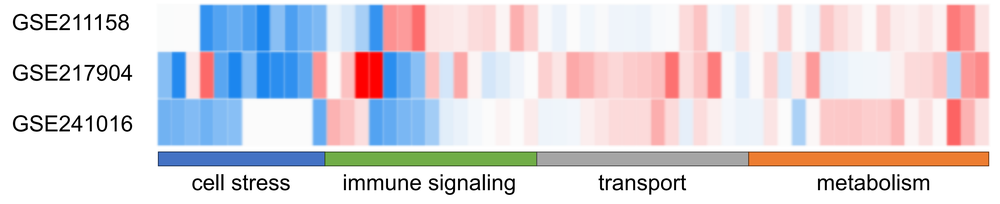

Figure 6. LANCL2-associated genes in human asthma datasets.

Conclusions & Acknowledgements

LANCL2 is a promising target for pulmonary inflammation. Asthma patients refractory to inhaled corticosteroids or with non-type 2 disease are underserved by the current therapeutic pipeline. LANCL2 is a novel mechanism that has the capacity to control neutrophilia and eosinophilia while restoring immune homeostasis through Tregs. Further, potential increases in mitochondrial metabolism within airway epithelial cells can prevent oxidative stress, cell death and production of chemokines.

We are currently developing orally active, systemically distributed LANCL2 compounds for a variety of autoimmune and inflammatory conditions. We have plans to initiate IND-enabling studies in 2024 for an allergy and asthma program.