Pharmacological activation of LANCL2 ameliorates disease severity and decreases inflammation in a preclinical model of rheumatoid arthritis

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Abstract. Lanthionine synthetase C-like 2 (LANCL2) is a novel immunoregulatory therapeutic target for the treatment of autoimmune diseases. Activation of LANCL2 enhances Treg responses through engagement of immunometabolic pathways and interactions with IL2/CD25 signaling, while downregulating effector cell subsets. An impaired Treg compartment has been proposed as one of the key contributors to the pathogenesis of rheumatoid arthritis (RA). We have developed NIM-1324, an oral, once-daily, highly systemically distributed small-molecule first-in-class therapeutic that selectively activates LANCL2 with a benign safety profile. We evaluated the therapeutic efficacy of oral NIM-1324 in a collagen-induced arthritis (CIA) mouse model of RA. Oral daily treatment with NIM-1324 significantly reduced disease activity, including paw redness and swelling, and pathology compared to the vehicle group. Furthermore, activation of LANCL2 by NIM-1324 decreased systemic inflammation, reducing IL-17+ and IL-21+ CD4+ T cells plus TNF-producing myeloid cells while the Tregs compartment was enhanced. LANCL2 deficiency in mice resulted in increased disease severity, inflammatory pathology and frequency of inflammatory subsets, including Th17 and Th1 cells. In human peripheral blood mononuclear cells, NIM-1324 decreased secretion of inflammatory mediators. This data supports that the pharmacological activation of LANCL2 by NIM-1324 is a promising therapeutic approach for the treatment of RA.

Results. Activation of LANCL2 enhances Treg stability and suppressive functions while decreases differentiation of effector CD4+ T cells through engagement of immunometabolic signaling (**Fig. 1**). To evaluate the potential of the LANCL2 pathway in RA, we utilized a collagen-induced arthritis (CIA) model in C57Bl/6 mice. WT and Lancl2 whole body knock out (Lancl2-/-) mice were challenged with 200 µg of Type II chicken collagen in complete Freund's adjuvant (CFA) through two intradermic injections at the base of the tail. Loss of Lancl2 resulted in increased disease severity starting at day 21 post challenge (**Fig. 2**).

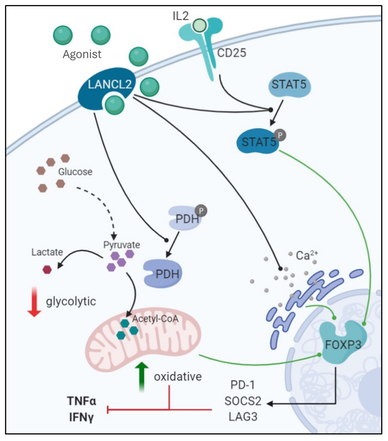

Figure 1. LANCL2 pathway and downstream effects in CD4+ T cells.

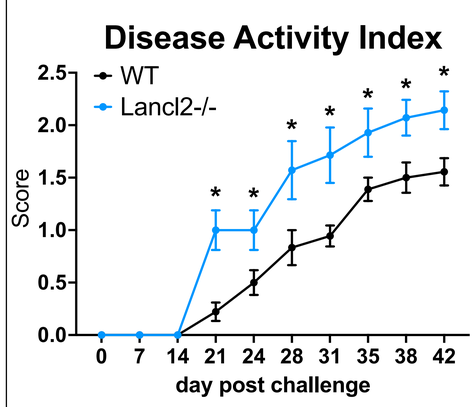

Figure 2. Clinical assessment of disease severity in WT and Lancl2-/- CIA mice (n=7-9) $P < 0.05$.

At the immunological level, Lancl2-/- mice displayed enlarged spleen compared to WT controls. Additionally, in the spleens, LANCL2 deficiency resulted in increased frequency of inflammatory subsets, including Th17 and Th1 cells, while CD4+ IL10+ regulatory cells were reduced (**Fig. 3**).

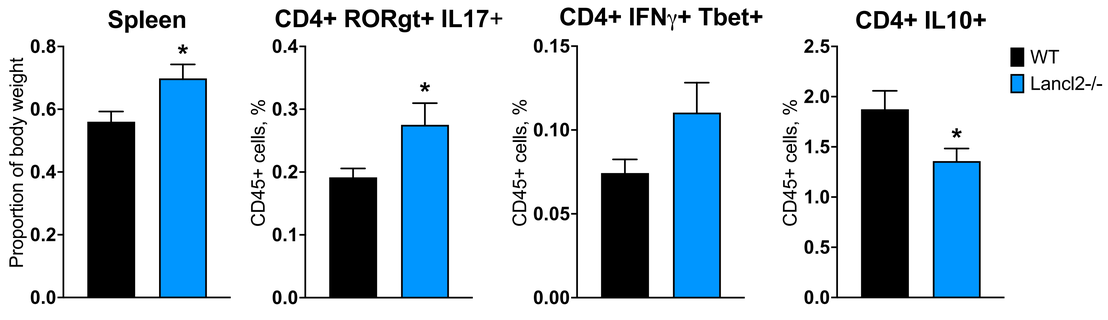

Figure 3. Immune responses of Lancl2 deficiency in spleen of CIA mice (n=7-9) $P < 0.05$.

To assess the local immunological effects of the LANCL2 pathway in RA, draining lymph nodes of CIA mice were also collected. Lancl2-/- mice also presented enlarged inguinal lymph nodes (ILN) relative to the WT group. Additionally, *ex vivo* culture of ILN-derived cells from WT and Lancl2-/- CIA mice displayed upregulated proportion of IL17+ CD4+ T cells in both the presence and absence of collagen associated with Lancl2 deficiency (**Fig. 4**). These results support the role of LANCL2 in RA and highlight the therapeutic potential of pharmacological activation of this pathway for the treatment of this chronic autoimmune disorder.

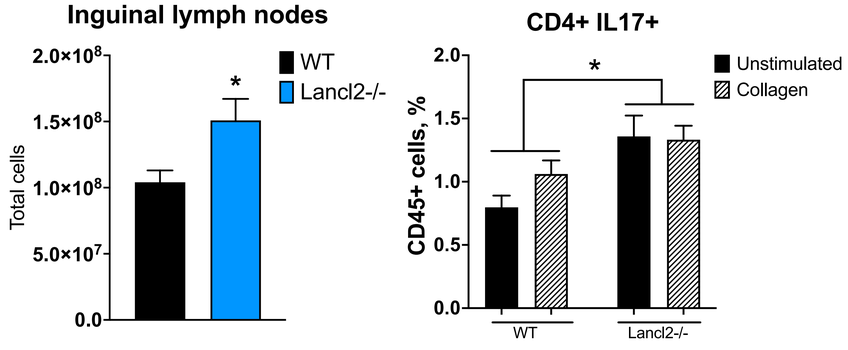

Figure 4. *Ex vivo* culture of ILN-derived cells from WT and Lancl2-/- CIA mice (n=6-9) $P < 0.05$.

NIM-1324 is an oral, once-daily, highly systemically distributed LANCL2-targeting therapeutic in clinical development for rheumatic diseases. NIM-1324 has two open INDs (RA and systemic lupus erythematosus (SLE)), is well-tolerated and has completed Phase I clinical testing in humans reporting no-dose limiting-toxicities for up to a dose of 1500 mg (**Fig. 5**). We evaluated the pharmacological potential of oral NIM-1324 in the mouse model of CIA. Mice were treated with 20 mg/kg/d of NIM-1324 starting at the onset of symptoms (2 weeks post challenge). Oral NIM-1324 ameliorated disease severity with an almost 2-fold change at 6 weeks post disease induction (**Fig. 5**).

NIM-1324	
Distribution	Systemic, highly bioavailable
Type	LANCL2-targeting small molecule
Mechanism of action (MoA)	Novel multipronged MoA that enhances regulatory responses upon engagement of immunometabolic pathways and Treg linage signaling
Route of administration	Oral
Systemic relevance	Can directly modulate the systemic immune system
Development stage	In clinical development for Rheumatic Diseases (completed Phase I study)
Safety profile	Well tolerated, with a benign safety profile and no reported toxicities

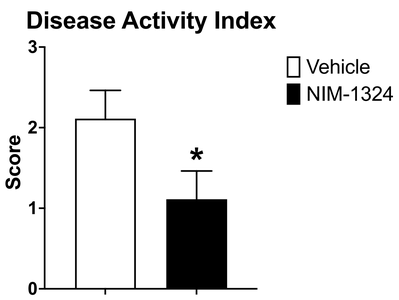

Figure 5. Oral NIM-1324 treatment in CIA mice (n = 9) $P < 0.05$.

Decreased disease severity in NIM-1324-treated mice correlated with decreased induction of inflammatory subsets in the spleen, while regulatory responses were enhanced. Indeed, oral NIM-1324 reduced the frequency of IL17+ and IL21+ CD4+ T cells, while Treg cells were increased (**Fig. 6**)

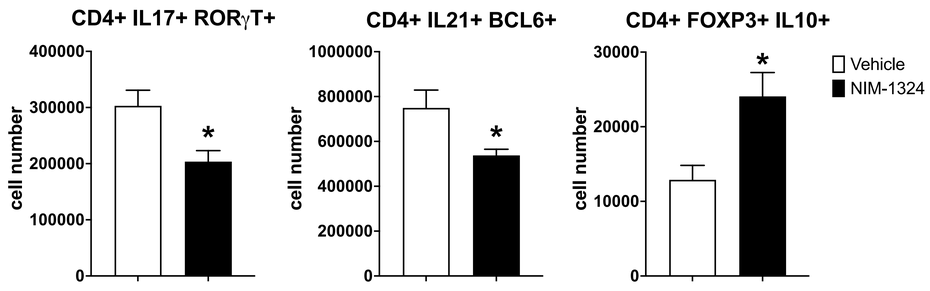

Figure 6. Immune responses of NIM-1324 in spleen of CIA mice (n = 9) $P < 0.05$.

The translational potential of NIM-1324 and the activation of the LANCL2 pathway was assessed in human PBMCs *ex vivo*. NIM-1324 treatment significantly decreased the production of the proinflammatory cytokines IL-6 and IL-8 across three distinctive stimulations (PMA-ionomycin, the TLR7 agonist gardiquimod (GDQ) and the TLR9 agonist CpG oligodeoxynucleotides), outperforming the positive control, hydroxychloroquine (**Fig. 7**).

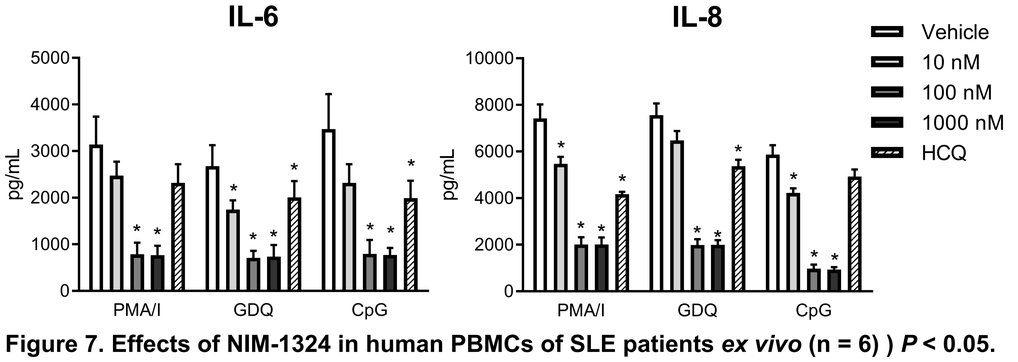

Figure 7. Effects of NIM-1324 in human PBMCs of SLE patients *ex vivo* (n = 6)) $P < 0.05$.

Conclusions & Acknowledgements

LANCL2 is a novel therapeutic target for the treatment of RA. LANCL2 is a strong immunoregulatory receptor that enhances Treg stability and function upon engagement of immunometabolic mechanisms and Treg signaling. Oral NIM-1324 reduces disease severity and systemic inflammation in CIA mice, while decreasing inflammatory responses and upregulating Treg cells.

NIM-1324 has an open IND in RA, has successfully completed Phase I clinical testing, and we have plans to initiate Phase II clinical studies in RA patients.