Pharmacological activation of LANCL receptors has three-fold benefit in metabolic dysfunction-associated steatohepatitis

AJ Leber[1], R Hontecillas[1], N Tubau Juni[1], SN Fitch[1] and J Bassaganya-Riera[1]

[1]NIMML Institute 1800 Kraft Dr. Suite 100, Blacksburg, 24060 Virginia (USA);
www.nimml.org; ajleber@nimml.org

The LANCL family of receptors includes LANCL2, a membrane receptor associated with glycemic control and regulatory immune responses, and LANCL3, a mitochondrial receptor that is significantly downregulated in multiple inflammatory diseases. In metabolic dysfunction-associated steatohepatitis (MASH), LANCL3 expression is reduced more than two-fold in cholangiocytes, hepatic stellate cells and Kupffer cells relative to healthy control. Based on the mechanistic overlap between the LANCL receptors and MASH, we assessed loss of function and pharmacological activation in MASH relevant *in vitro* and *in vivo* systems. Loss of LANCL2 or LANCL3 resulted in increased production of IL-17 and TNF from CD4+ T cells. Production of COL1A1 in hepatic stellate cells increased three-fold after TGF-β stimulation in the KO relative to WT. *In vivo*, liver weight increased and glucose tolerance decreased in KO mice relative WT after high fat diet feeding for 12 weeks. In both carbon tetrachloride and choline deficient L-amino acid defined (CDAA) models of MASH, LANCL activation resulted in lower liver weight, triglycerides and fibrosis through measures of total, perivascular and bridging collagen. Treatment also resulted in a significant reduction of Th17 cells and TNF+ macrophages in the models of MASH. This data suggests that LANCL receptors are promising therapeutic mechanisms for MASH and other metabolic diseases through an ability to directly modulate the three hallmarks of MASH pathogenesis.

LANCL receptors are downregulated in a variety of inflammatory diseases including a greater than 20-fold suppression in expression in human cirrhotic liver. LANCL2 and its natural ligand, abscisic acid are important for glycemic control with defined roles in skeletal muscle, adipocytes and immune cells. The loss of LANCL2 results in impaired glucose tolerance, accelerated weight gain and increased fasting blood glucose levels (**Fig. 1**). In a HFD model, these changes are paired with increased blood NEFA and IL-6 levels along with increased inflammatory cells, like TNF+ macrophages and Th1 cells, in adipose tissue.

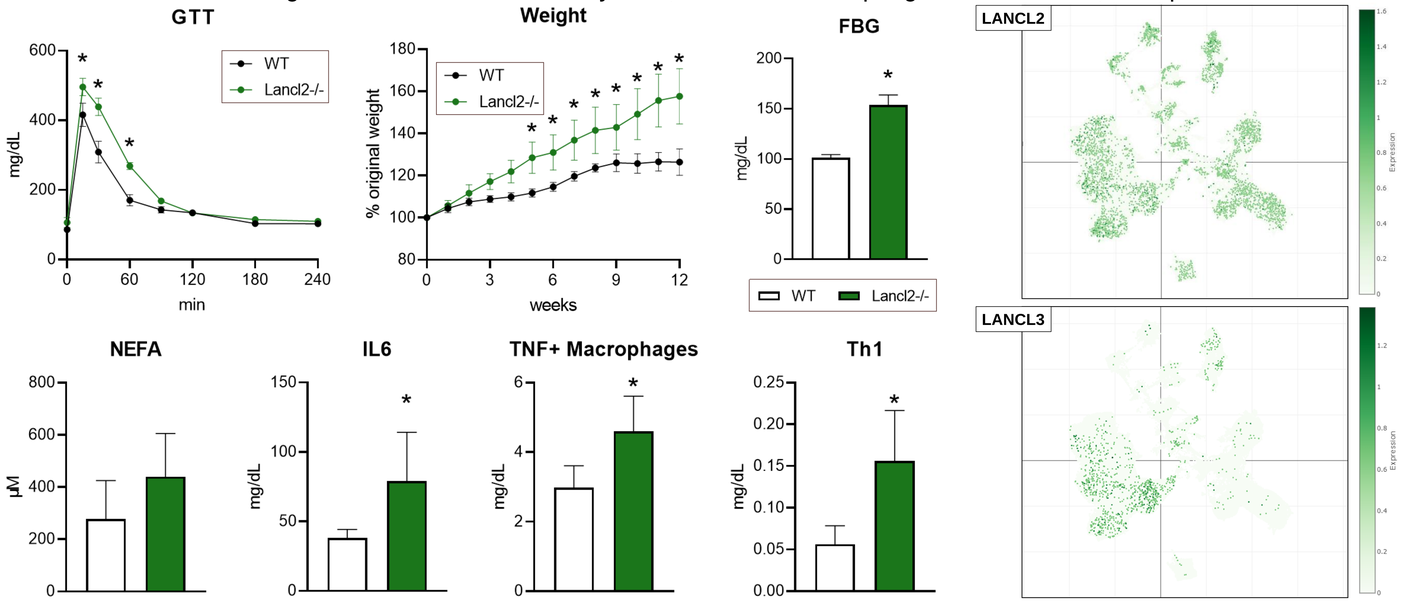

Figure 1. Loss of Lancl2 negatively impact metabolism and inflammation in a HFD model (n = 8).

To evaluate if LANCL2 is also implicated in the fibrotic aspect of MASH, hepatic stellate cells were isolated from WT and Lancl2-/- mice. When stimulated with either TNF and palmitic acid or TGFβ, Lancl2-/- cells produce greater levels of collagen. In a choline-deficient, amino acid defined diet (*Research Diets* A06071302) model, Lancl2-/- mice had higher liver weights, liver triglycerides and CD4+ IL17+ cells in the spleen after 10 weeks of CDAA diet (**Fig. 2**). Conversely, treatment with an LANCL agonist for 6 weeks resulted in reduced liver weights, liver triglycerides, and Masson's trichrome positive area after 10 weeks of CDAA diet (**Fig. 3**). LANCL activation also decreased inflammatory cells like CD4+ IL17+ cells and CD11b+ TNF+.

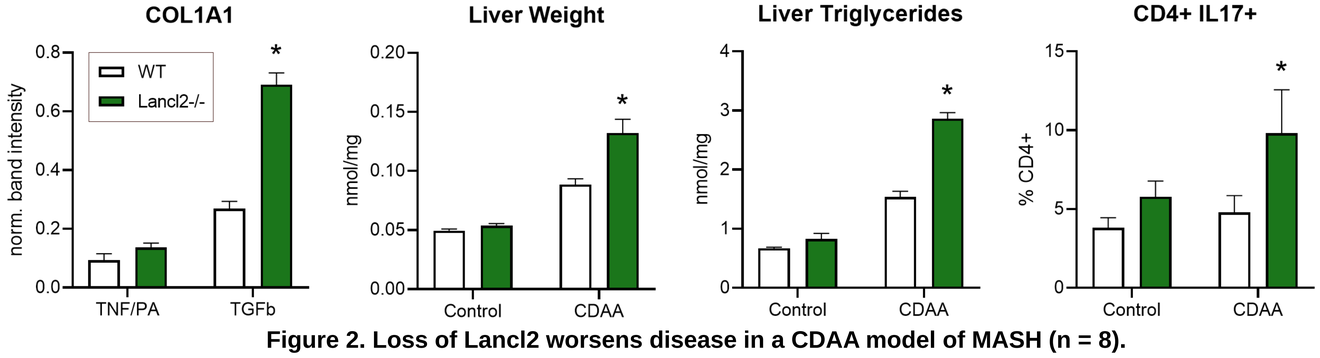

Figure 2. Loss of Lancl2 worsens disease in a CDAA model of MASH (n = 8).

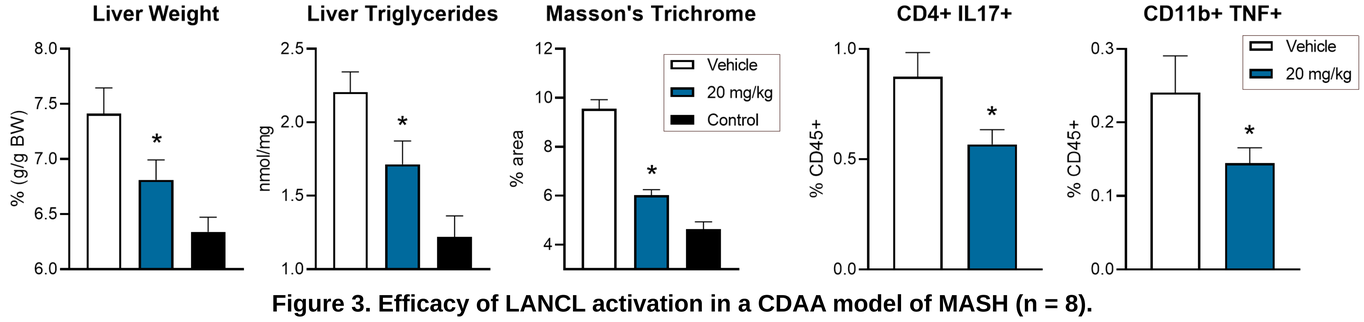

Figure 3. Efficacy of LANCL activation in a CDAA model of MASH (n = 8).

To further test the ability of LANCL activation in ameliorating liver damage, WT mice were injected twice weekly with 0.5 μL/g CCl4 for 4 weeks. Treatment with an LANCL agonist or vehicle was given once daily beginning at 2 weeks. Treatment with 10 and 20 mg/kg significantly reduce picrosirius red area and increased splenic Tregs (**Fig. 4**). Further, 20 mg/kg normalized total collagen, perivascular collagen and bridging to control levels.

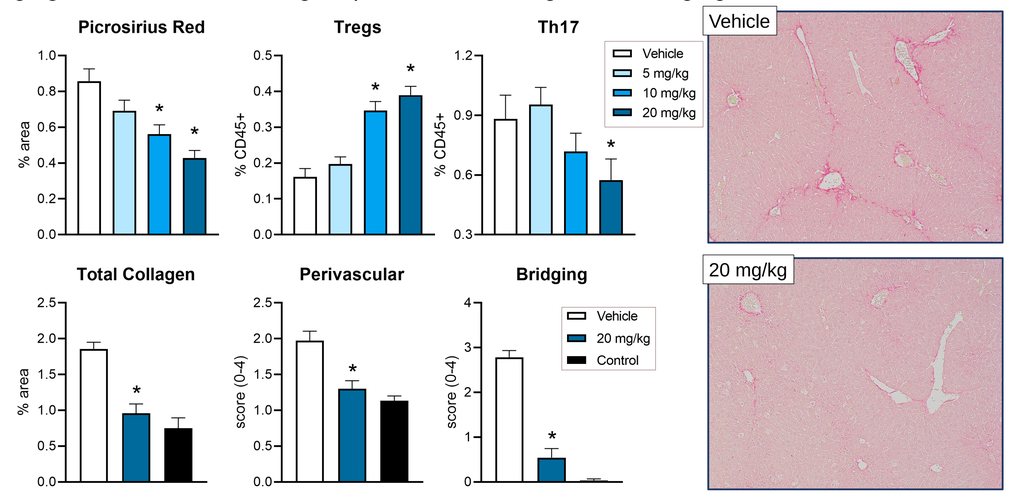

Figure 4. Efficacy of LANCL activation in a CCl4 model of MASH (n = 10).

To test LANCL activation in a chronic model of metabolic stress, WT mice were fed a 42% fat Western diet (TD.120528) supplemented with 23.1 g/L d-fructose and 18.9 g/L d-glucose in drinking water for 24 weeks. Treatment was initiated after 16 weeks. In a dose dependent manner, LANCL activation reduced liver weight and histological severity of disease in the level including picrosirius red area and lipid accumulation and fibrosis scoring (**Fig. 5**).

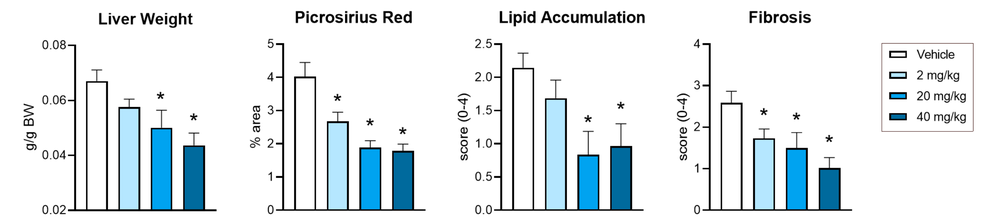

Figure 5. Efficacy of LANCL activation in a WD model of MASH (n = 8).

In human primary cells, the localization of LANCL2 is dependent on cholesterol levels, with high cholesterol inducing nuclear translocation and low cholesterol inducing cytoplasmic localization (**Fig. 6**). The ability of LANCL activation to influence cytokine production is retained in human cells with 100 nM treatment significantly decreasing IL-6, MCP1, and TNF. In primary human hepatic stellate cells, LANCL activation reduces proliferation following stimulation with TGFβ. Meanwhile, production of COL1A1, α-smooth muscle actin and MMP2 are reduced with treatment.

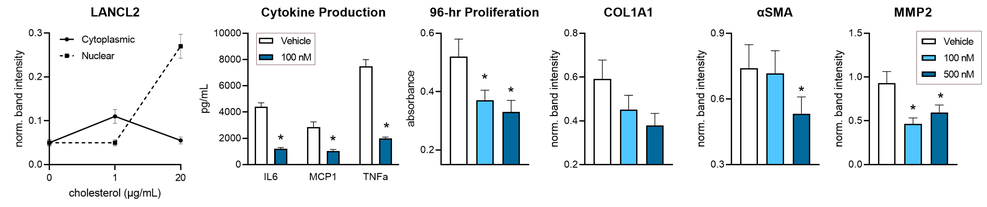

Figure 6. Efficacy of LANCL activation in primary human cells (n = 5).

Conclusions & Acknowledgements

LANCL receptors are a promising therapeutic targets for the treatment of immunometabolic diseases including MASH. The activation of these receptors has the ability to impact the underlying inflammation, metabolic stress and fibrosis to have three-fold benefit in MASH. In three models, LANCL activation consistently improves liver health, while decreasing systemic inflammation. These studies serve as the first pre-clinical proof of concept of LANCL activation in MASH.

We are currently developing orally active, systemically distributed LANCL compounds for autoimmune, metabolic and inflammatory disease in collaboration with NImmune Biopharma. NIM-5338 is a lead product candidate targeting the LANCL family of receptors currently at the pre-IND stage of development.