Pharmacological activation of LANCL2 provides immunometabolic support for the restoration of cognitive function markers in a mouse model of Alzheimer's disease

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LANCL2 is a member of a three-protein family of receptors that acts as the mammalian receptor for abscisic acid (ABA). LANCL2 expression is significantly diminished both with the brains of 3xTg mice as well as human patients with Alzheimer's disease. Immunologically, LANCL2 expression is closely associated with regulatory CD4+ T cells and their suppressive capacity as well as within microglia. Treatment of mice with ABA improved spatial memory and reduced amyloid beta load. To study the effects of LANCL2 activation in vivo, we created a novel agonist. After 12 weeks of daily oral treatment at 20 mg/kg, LANCL2 activation significantly reduced inflammatory immune cells including neutrophils, Th17 and Th1 cells in 3xTg mice relative to vehicle control. Further, treatment with LANCL2 agonists reduced the expression of key markers in the brain associated with impaired cognitive function to levels observed within age-matched, negative control WT mice. Activation of LANCL2 prevents the polarization of microglia into a reactive phenotype and supports the metabolic demands of amyloid beta phagocytosis. LANCL2 drugs can combat Alzheimer's disease progression, given that reactive microglia polarization not only diminishes microglial phagocytic capacity but also increases the production of inflammatory cytokines. The LANCL2 pathway's transformative potential merits development as a promising therapeutic mechanism for Alzheimer's disease and diverse age-related cognitive impairment.

We evaluated the behavior of CD4+ T cells isolated from 3xTg mice relative to WT cells (**Fig. 1**). When exposed to a Treg-differentiating cytokine mixture, 3xTg cells fail to develop a CD25hi FOXP3+ phenotype. These Tregs also express lower levels of CTLA4. Further, 3xTg Tregs express lower levels of STAT5 while increasing Jak3 expression, indicating a dysregulation of the standard JAK-STAT signaling induced by CD25. Higher expression of the lipid metabolic marker (Cpt1a) and an endosomal trafficking marker associated with the inhibition of the autophagosome (Vps8) were also observed. 3xTg cells were also observed to be hyporesponsive in IL17 production when stimulated with either TLR7 or TLR4 activators.

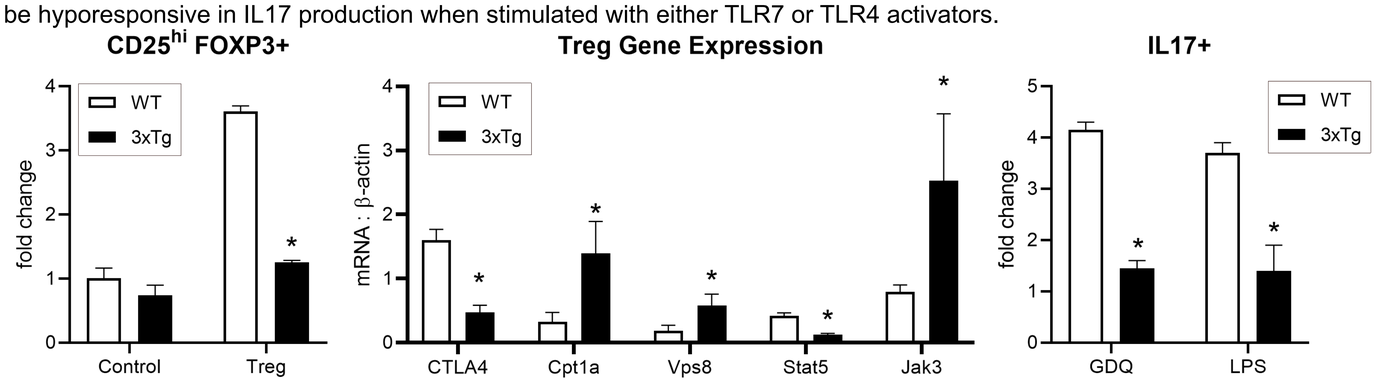

Figure 1. Alterations in Treg differentiation and expression in 3xTg mice (n = 6).

LANCL2 expression is downregulated early and throughout the life span of 3xTg mice in the cortex (**Fig. 2**). Similarly, LANCL2 is downregulated in the entorhinal cortex of ALZ patients relative to non-ALZ (GSE5281). When Tregs are transferred to 3xTg mice, the loss of LANCL2 decreases the ability of these cells to reduce IFNy+ and IL17+ CD4+ T cells (**Fig. 2**).

When stimulated in vitro, 3xTg antigen presenting cells have higher expression of co-stimulatory molecules like CD86 (**Fig. 3**). We examined whether the interaction between such cells and Tregs were responsible for the impaired phenotype. Neither WT or 3xTg astrocytes were able to induce IL10 expression in 3xTg Tregs to the level of WT Tregs, suggesting a Treg-intrinsic mechanism is altered. Notably, 3xTg astrocytes induced lower IL10+ expression in WT Tregs when stimulated with amyloid-beta.

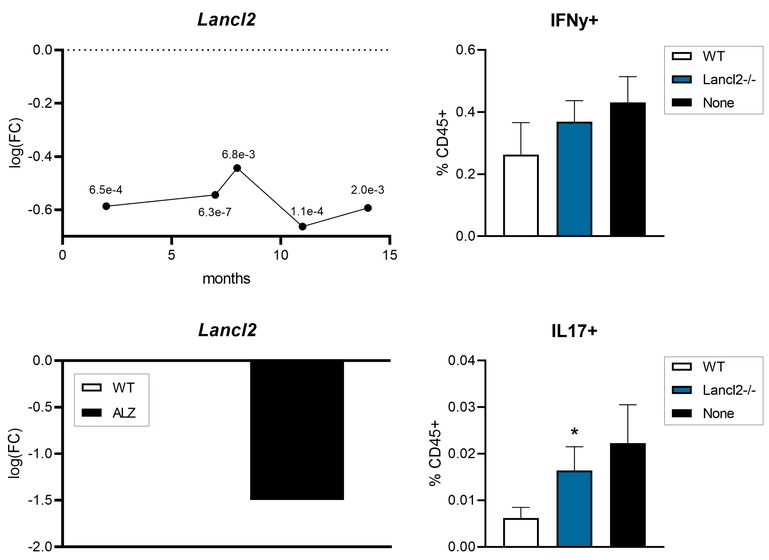

Figure 2. Downregulation of Lancl2 in mouse and human ALZ

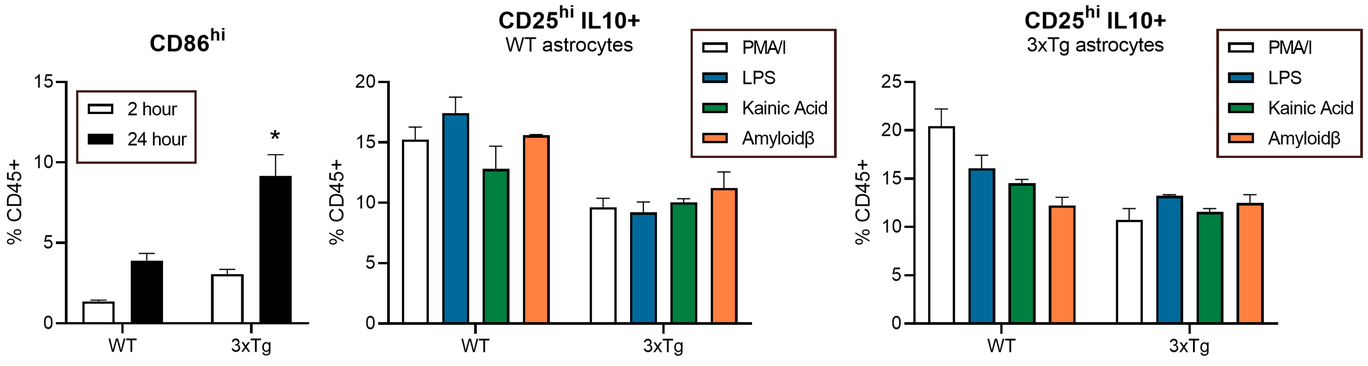

Figure 3. Impaired IL10 induction in 3xTg Tregs by astrocytes (n = 6).

Upon observing the potential role of Tregs and suppressed LANCL2 in ALZ, we conducted studies to examine the efficacy of an LANCL2 agonist in the 3xTg model of ALZ (**Fig. 4**). In a light-dark test, LANCL2 activation reduced anxiety responses as evidenced through an increased time spent in the light and number of full body transitions. LANCL2 activation was also beneficial to activity and exploration as measured in an open-field scoring of arousal and time spent in the inner zone. In a Morris Water Maze, treatment reduced the time needed to find the platform. At necropsy, treatment significantly reduced spleen weight.

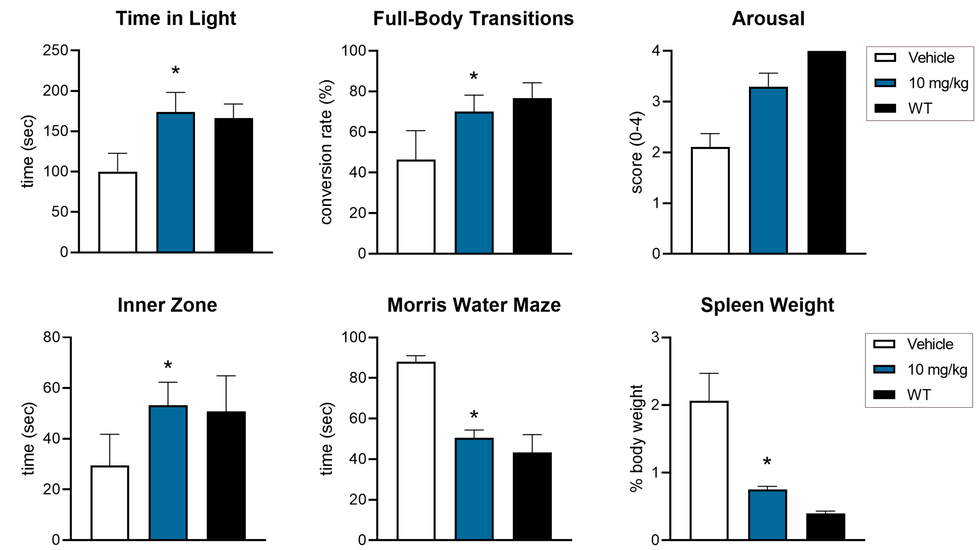

Figure 4. Efficacy of LANCL2 activation in behavioral tests of 3xTg mice (n = 10).

After conduct of behavioral tests, brains were collected and processed to obtain immune cells which were characterized by flow cytometry (**Fig. 5**). Treatment with an LANCL2 agonist reduced neutrophils, TNF+ microglia and IL17+ T cells in the brain. LANCL2 activation induced a near 3-fold increase in CD25hi IL10+ Tregs.

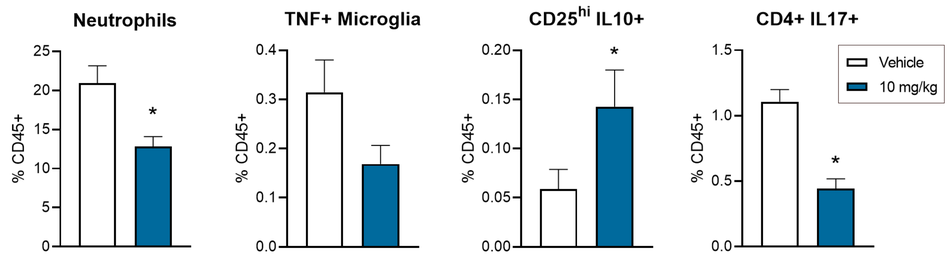

Figure 5. Flow cytometry of brain immune cells after treatment (n = 10).

RNA was isolated from the cortex of 3xTg mice after 6 weeks of treatment beginning at 28 weeks of age for gene expression by qRT-PCR (**Fig. 6**). LANCL2 activation was observed to decrease markers of ALZ (*Tuba1c*, *Pcna*) while increasing markers of Treg activation (*Il2ra*), insulin signaling (*Irs1*) and phagocytic processing (*Rictor*).

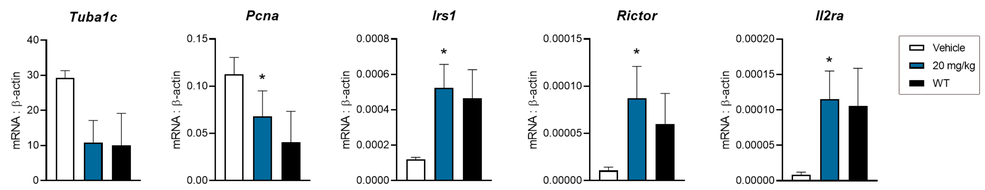

Figure 6. Cortex gene expression after LANCL2 activation (n = 6).

Conclusions & Acknowledgements

LANCL2 is a promising therapeutic target for the treatment of ALZ. LANCL2 has a Treg-centric mechanism of action but impacts numerous elements of ALZ pathogenesis including the immunometabolic control of astrocytes, microglia and neurons to reduce oxidative and inflammatory stress while maintaining phagocytic capacity to efficiently process necessary material like β-amyloid plaques. These studies serve as the first pre-clinical proof of concept of LANCL2 activation in ALZ.

We are currently developing orally active, systemically distributed LANCL2-specific compounds for ALZ with plans to initiate an IND-enabling program.