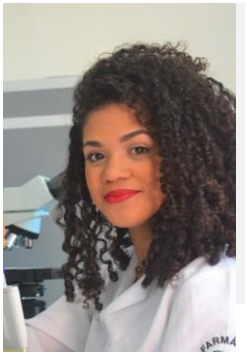
ImmunoTools *special* Award 2024



Juliana Helena Castro e Silva, Post-doctoral fellow

P.I.: Professors Davide Lecca and Maria Pia Abbracchio

University of Milan, Department of Pharmaceutical Sciences.
Via Giuseppe Balzaretti, 9 – 20133 – Milan, ITALY

Targeting GPR17 receptor to counteract oligodendrocyte functional failure in multiple sclerosis inflammation

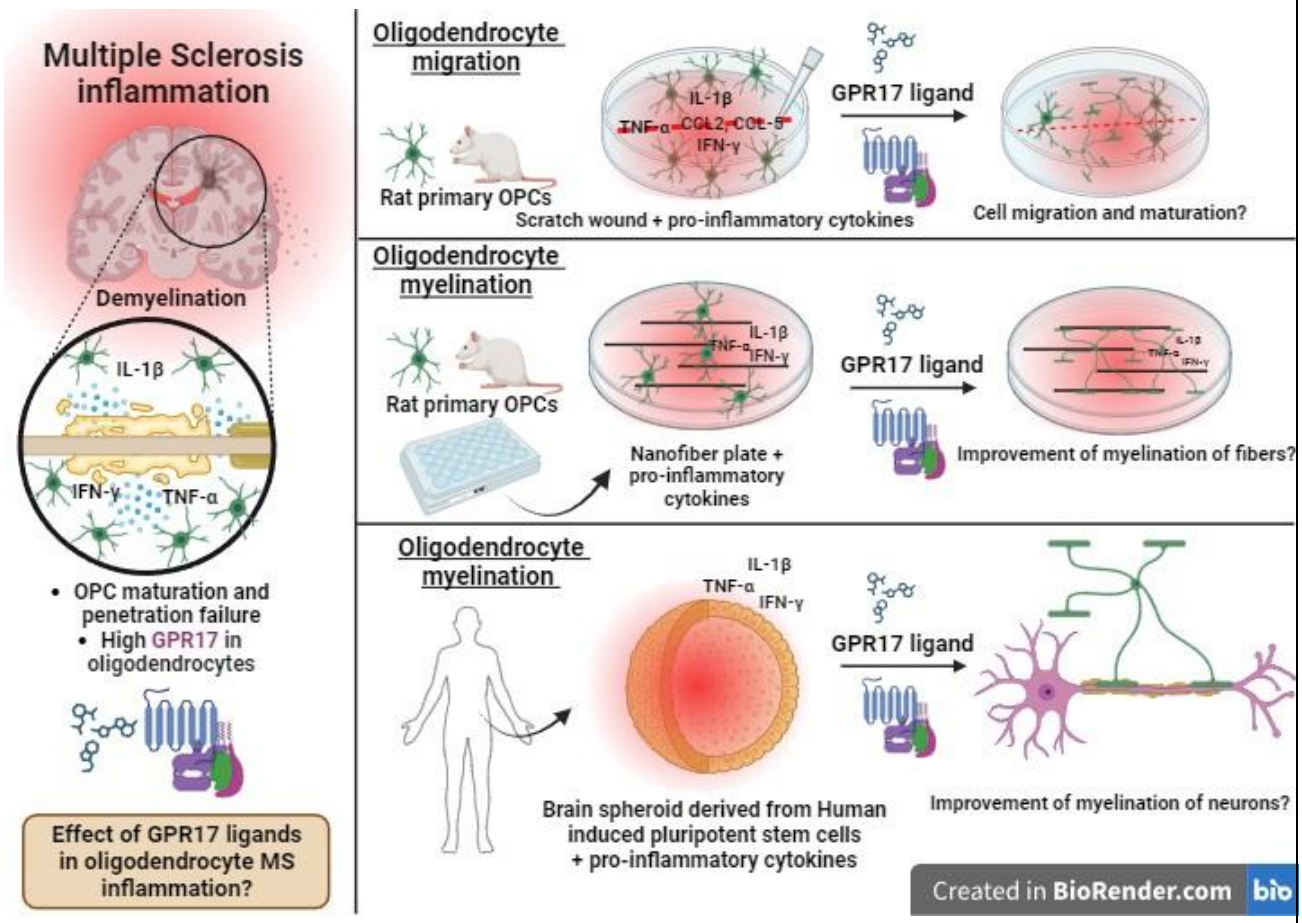
Multiple Sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system (CNS). MS is characterized by the immune-mediated attack to myelin, the fatty substance the covers the axons of neurons and allow rapid conduction of the electric impulse. This generates various incapacitating symptoms, depending on the location of demyelinating lesions. Current therapy for MS comprises mostly immunomodulatory drugs, which offer relief for the most prevalent MS forms but cannot support remyelination of lesions in MS most aggressive forms. This happens because we still lack in therapies that target the CNS myelin-producing cells: oligodendrocytes (OLs) (*Klotz, Antel & Kulhman, 2023 Nat. Rev. Neurol.*).

OLs originate from oligodendrocyte precursor cells (OPCs). To produce myelin, OPCs must undergo a series of physiological events to reach their fully mature stage. In chronic MS, OPCs are migrate to the borders but fail to enter lesions, likely due to the unfavourable inflammatory environment. Additionally, they do not reach final maturation stages, thus not contributing to remyelination.

The G protein-coupled receptor 17 (GPR17) is expressed in OPCs at intermediate stages of differentiation. We demonstrated that GPR17 expression is essential for early OLs, however, it must be downregulated to allow proper OL maturation (*Marangon et al. 2022, Cells*). Moreover, OLs accumulate at a GPR17-positive stage in various *in vivo* neurodegenerative disease models. GPR17 has a complex pharmacology and both agonists (e.g. the chemokine CXCL12/SDF-1) and antagonists (e.g. Montelukast), promote remyelination and recovery of CNS function in these models (for review, please see *Lecca et al., 2020, Glia*). Furthermore, $GPR17^{+}$ cells are accumulated in the inflamed normal-appearing white matter of

human MS post-mortem brains (*Angelini et al. 2021, Int. J. Mol. Sci.*)., suggesting that GPR17 can be a target to overcome demyelination in MS inflammation. Thus, the aim of our investigation is to target GPR17 in this context, searching for novel drugs that can promote remyelination focusing on OLs.

We have recently established an *in vitro* model that mimics oligodendrocyte differentiation impairment, typical of MS lesions. We obtained rat primary OPCs and treated them with a cocktail of MS relevant pro-inflammatory cytokines: IFN-γ, TNF-α, and IL-1β. Our preliminary results indicate that a subtoxic concentration of this cocktail significantly hinders OPC differentiation, increasing gene expression of immature markers (e.g. NG2 proteoglycan, and GPR17) and decreasing mature proteins expression (e.g. myelin basic protein – MBP). Furthermore, the cocktail induced an increase in gene expression of MS chemokines associated with the migration of leukocytes into the MS brain, CCL-2, CCL-5 and CXCL10.



We are currently selecting the most promising compounds that can restore OLs maturation after cytokines treatment. However, it is important to note that myelination is a complex event that depends not only on OL maturation stage but also on their

ability to migrate and access lesion sites; their ability to enwrap and compact myelin sheaths around axons; and the contribution of other cells of the CNS. Thus, we plan to test whether promising GPR17 ligands can modulate:
a) OL migration in a wound healing assay that simulates a lesion combined with MS-relevant cytokines (IFN-γ, TNF-α, IL-1β, and IL-17) and chemokines (CCL-2, CCL-5) – using CX3CL1 as positive control of migration;
b) OL myelination after treatment with MS-relevant cytokines using nanofiber plates that simulates axons of neurons;
c) OL remyelination in human brain spheroids after treating with pro-inflammatory MS cytokines;

We expect that our strategy allows us to broadly characterize the effects of GPR17 ligands in the context of MS inflammation and provide information on possible mechanisms of action of GPR17 ligands on the search for novel drugs that support OLs in MS inflammation.

References

Angelini, J., Marangon, D., Raffaele, S., Lecca, D., & Abbracchio, M. P. (2021). The Distribution of GPR17-Expressing Cells Correlates with White Matter Inflammation Status in Brain Tissues of Multiple Sclerosis Patients. International Journal of Molecular Sciences, 22(9). https://doi.org/10.3390/IJMS22094574

Klotz, L., Antel, J., & Kuhlmann, T. (2023). Inflammation in multiple sclerosis: consequences for remyelination and disease progression. Nature Reviews Neurology 2023 19:5, 19(5), 305–320. https://doi.org/10.1038/s41582-023-00801-6

Lecca, D., Raffaele, S., Abbracchio, M. P., & Fumagalli, M. (2020). Regulation and signaling of the GPR17 receptor in oligodendroglial cells. In GLIA (Vol. 68, Issue 10, pp. 1957–1967). John Wiley and Sons Inc. https://doi.org/10.1002/glia.23807

Marangon, D., Audano, M., Pedretti, S., Fumagalli, M., Mitro, N., Lecca, D., Caruso, D., & Abbracchio, M. P. (2022). Rewiring of Glucose and Lipid Metabolism Induced by G Protein-Coupled Receptor 17 Silencing Enables the Transition of Oligodendrocyte Progenitors to Myelinating Cells. Cells, 11(15). https://doi.org/10.3390/CELLS11152369