EphA4 inhibition as a therapeutic strategy for ALS

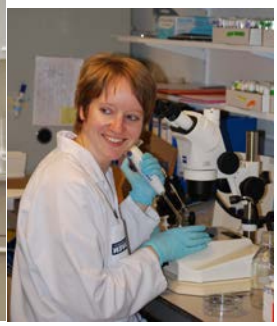
Acronym: EphrALS

Principal Investigator: Pr Philip Van Damme

Co-Investigator: Pr Wim Robberecht

Grant: 210,000€

Project duration: three years



The team working on the project: Pr Philip Van Damme on the left, with Lindsay Poppe (PhD student), Lies Schoonaert (PhD student) and Laura Rué (post doc). In the picture: Mieke Timmers (lab technician) at the zebrafish injection set-up.

Summary of the research project

In previous (Thierry Latran Foundation funded) research, we have performed a genetic screen of a zebrafish model for ALS to identify disease modifiers of ALS. We found that EphA4 is such a modifier in patients and animal models. EphA4 is a receptor in the ephrin system, an axon repellent pathway. Genetic deletion and antagonists of the EphA4 receptor prolong survival and preserve motor neurons in animal models for ALS. In ALS patients, low expression of EphA4 is associated with later age of onset and longer survival. These findings suggest that an EphA4-mediated pathway is a modifier of motor axon degeneration and that the use of EphA4 antagonists is a potential therapeutic strategy in ALS.

In the current project, we intend to translate these biological findings in preclinical data needed for further development of EphA4 antagonists in patients with ALS.

We will investigate the cell type through which the protective effect of the deletion of EphA4 on motor neuron degeneration is exerted, and study whether the effect exerted by EphA4 inhibition is mediated by forward signaling or through retrograde signaling in the cell which expresses the ligand for EphA4. This is important as this will establish whether compounds that inhibit EphA4 tyrosine kinase may be useful in ALS.

The EphA4 receptor is promiscuous and binds type A and type B ligands. We intend to study the presence of EphA4 ligands in the spinal cord and identify the cell type in which these are expressed. In preliminary experiments, we found one such ligand, ephrin-B2, to be abundantly

upregulated in reactive astrocytes in ALS. Therefore, we will study the effect of specific deletion of ephrin-B2 from astrocytes in the $SOD1^{G93A}$ model.
We intend to further explore the therapeutic potential of inhibiting the EphA4 receptor. We will investigate the therapeutic effect of the KYL peptide, an EphA4 antagonist. Furthermore, we have developed a panel of nanobodies against the extracellular region of EphA4 and will study whether EphA4 nanobodies may be a therapeutic strategy in ALS. Finally, we will investigate whether interfering with the intracellular EphA4 cascade using ROCK (Rho-associated protein kinase**)** inhibitors may affect motor neuron degeneration in ALS.
If successful, these experiments will establish a robust basis for the use of ephA4 antagonists in ALS, and will lead to further development of these compounds in this disease

Philip Van Damme (MD, licensed neurologist, PhD) is since 2008 permanent member of staff in the Neurology Department of Pr Wim Robberecht at University Hospital Leuven with main interest in clinical neurophysiology and neuromuscular disorders. He is also since 2009 associate professor at K.U. Leuven, clinical investigator of the fund for scientific research Vlaanderen (FWO-Vlaanderen) and staff scientist in Vesalius Research Center, Flanders Institute of Biotechnology, Leuven.

Pr Van Dammes' five recent publications relevant to this research:

- Van Hoecke, A., et al. EphA4 is a modifier of amyotrophic lateral sclerosis in rodent models and humans. Nature Medicine, 2012; 18 (9): 1418-1422
- Van Damme, P., et al. Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2. Neurology 76, 2066-2072 (2011).
- Schrooten, M., et al. Benefit of the Awaji diagnostic algorithm for amyotrophic lateral sclerosis: a prospective study. Ann Neurol 70, 79-83 (2011).
- Van Damme, P., et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J Cell Biol 181, 37-41 (2008).
- Van Damme, P., et al. Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity. Proc Natl Acad Sci U S A 104, 14825-14830 (2007).