A high throughput drug screening revealed novel pharmacological agents to treat Cerebral Cavernous Malformation

Maximiliano Arce[1], Hua Huang[1], Charlotte Rorsman[1], Joppe Oldenburg[1], Anna Klemm[2], Veronica Sundell[1], Anna Eriksson[3], Emeli Hermansson[1], Carolina Wählby[2], Peetra Magnusson[1], Elisabetta Dejana[1,4]

[1] Immunology, Genetics and Pathology department, Uppsala University, Sweden.
[2] Department of Information Technology, Uppsala University, Sweden.
[3] Departament of Chemistry, Umeå University, Sweden.
[4] FIRC Institute of Molecular Oncology, Milan, Italy.

Cerebral Cavernous Malformations (CCM) is an intricate disease that significantly affects the brain vasculature. The inherited mutation in one of the three CCM genes (CCM1, CCM2, and CCM3) specifically affects the endothelial cells of the brain, causing mulberry-like vascular malformations. Lesion leakage, rupture, and bleeding of the malformations are the leading cause of focal neurological deficit, migraines, seizures, and hemorrhagic stroke in CCM patients. Nowadays, there are no pharmacological treatments available, and therefore, there is an urgent need to find druggable targets to improve the situation for the patients by avoiding bleeding and reducing lesion growth. We have performed a high throughput screening of 5200 compounds (including FDA/EMA approved drugs) on CCM3 knockout cells, finding a long list of drugs that were able to rescue the CCM-related phenotype *in vitro*. Moreover, one of the compounds was particularly effective upon KLF4 and RhoA-ROCK pathways, two detrimental pathways involved in lesion progression and endothelial barrier disruption. Concomitantly, in vitro treatment with the compound inhibits endothelial hyperproliferation, excessive F-actin stress fibers, vascular leakage and promotes junctional stability in CCM3 knockdown primary human brain endothelial cells. Interestingly, we have demonstrated that the dual mechanism of this compound is probably through the inhibition of Erk5 phosphorylation and Cofilin activation. Further *in vivo* validation using an endothelial-specific CCM3 knockout mouse model, demonstrated that this compound was able to significantly reduce the lesioned area in the brain vasculature. Permeability studies are now ongoing to understand the effect of this compound on vascular leakage during CCM.
Overall, among 5200 compounds, we found a promising candidate with a novel and unexpected dual role against KLF4 and RhoA-ROCK pathways, two independent and central pathways in CCM. Therefore, this compound could benefit CCM patients by targeting vascular leakage and reducing lesions progression, but could also serve as a novel pharmacological tool to enhance vascular integrity.