NEXT-GENERATION NEURODEGENERATIVE DISEASES THERAPEUTICS WITH STRONG BINDING FOR AMYLOID-BETA/TAU/ALPHA-SYNUCLEIN AGGREGATES, REDUCED RISK OF ARIA, AND IMPROVED BRAIN DELIVERY.

Damien Toulorge, Julie Goemaere, Aditya Iyer, Dafni Birmpili, Elodie Hendrick, Chiara Vollaro, Sandra Parys, and Pierre Vandepapeliere
Amyl Therapeutics, Boulevard Patience et Beaujonc 3, Boîte 12, 4000 Liège, Belgium

Objectives: Neurodegenerative diseases (NDDs) such as Alzheimer's or Parkinson's diseases (AD/PD) are mixed pathologies characterized by the concomitant presence of several protein aggregates among Amyloid-β, Tau, and α-synuclein (Aβ/Tau/α-syn). Current monoclonal antibody (mAb) approaches aim at removing these amyloid aggregates from the brain, but their efficacy is limited for several reasons: they only act on a single type of amyloid aggregate; they are associated with safety issues (ARIA); and they poorly cross the blood-brain barrier. Building on Amyl Tx's pan-amyloid binding Fc-fusion protein platform, our objective is to develop next-generation products overcoming current mAb limitations, i.e. with strong binding affinities for all Aβ/Tau/α-syn aggregates, reduced ARIA and an improved brain delivery.

Methods: Twenty Fc domain variants engineered to modulate effector functions were screened in iPSC-derived microglia for simultaneous Aβ fibril phagocytosis and TNF-α release. Affinity maturation of our amyloid-binding domain (Amyl-2) was performed on >2 billion variants using AI-guided design and wet-lab screening. Final selection was based on SPR affinities to the targets and off-target screening. Eight Fc-fusion proteins fused to different antibody fragments (VHH) targeting the Transferrin Receptor (hTfR1) were screened for *in vivo* brain delivery. Benchmark comparisons were made with Lecanemab, Trontinemab and other mAbs.

Results: Fusion to a new Fc domain activated phagocytosis of Aβ fibrils to levels comparable to Lecanemab while reducing TNF-α release by 50%. Affinity maturation of our binding domain dramatically increased the affinities for Aβ/Tau/α-syn amyloid aggregates to levels comparable to that of mAbs. Brain exposure was strongly improved following conjugation to hTfR1-binding VHH.

Conclusions: These results pave the way for the development of a next-generation therapeutic solution for mixed NDD pathologies like AD/PD.