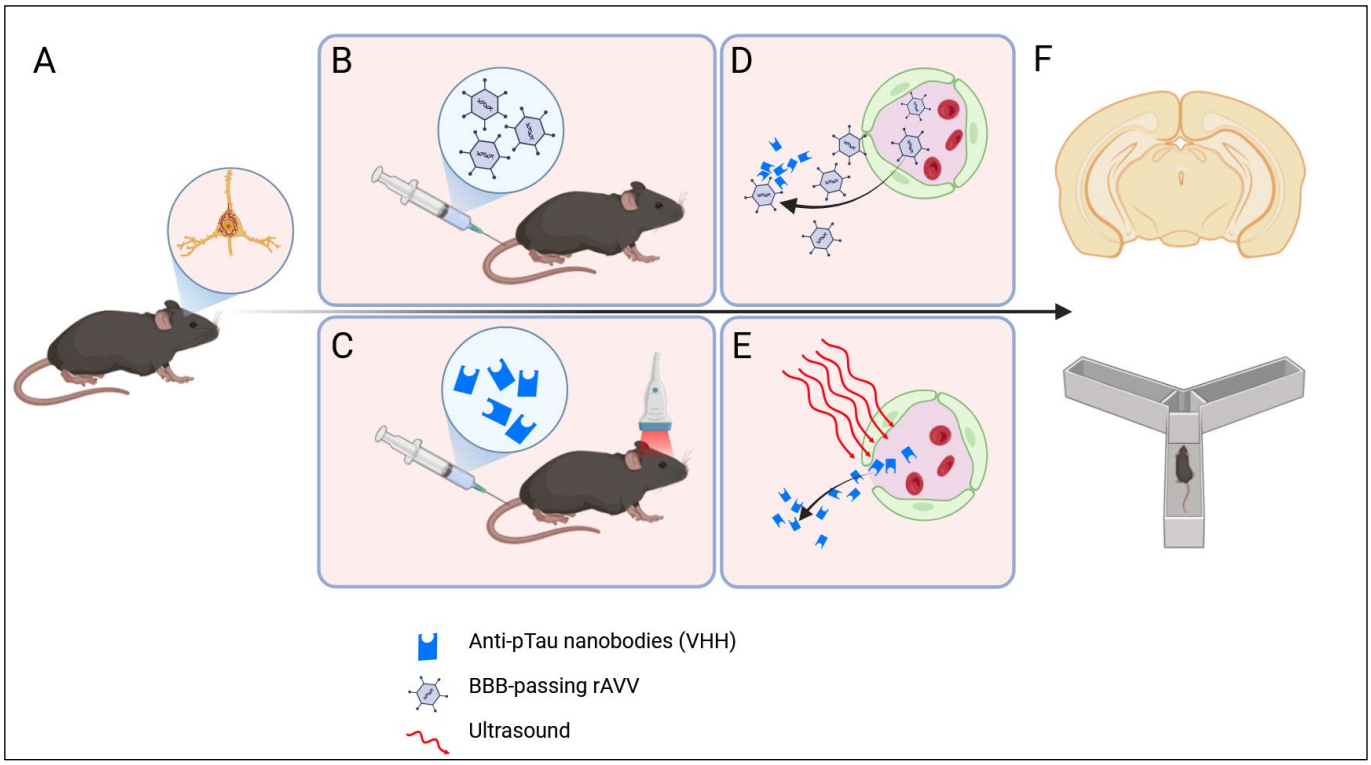
PROJET DE RECHERCHE

Graphical abstract



P301S tau transgenic mice harbor Alzheimer-like tauopathies characterized by the intracellular accumulation of neurofibrillary tangles (**A**). Augmented anti-tau immunotherapies will be performed in P301S mice using dedicated camelid nanobodies (VHHs) that will be developed and optimized. Two different strategies will be applied: (1) peripheral injection of recombinant blood-brain passing adenovirus allowing the intraparenchymal expression of anti-tau VHHs (**B** and **D**) or (2) standard chronic passive immunotherapies with anti-tau VHHs coupled to transient blood-brain barrier opening by ultrasound (**C** and **E**). The effects of anti-tau immunotherapies will be assessed by comprehensive neuropathological analysis and by functional evaluation of mice in neurological-cognitive tests (**F**).

Scientific background and general aim

Alzheimer's disease (AD) is, at the neuropathological level, characterized by the accumulation of extracellular plaques made of amyloid-ß (Aß) peptides and of intraneuronal neurofibrillary tangles (NFTs) formed by the accumulation of hyperphosphorylated tau proteins that disorganize cell skeleton.
After years of clinical failures, recent anti-Aß therapies, relying on standard passive immunotherapy protocols with intravenously (iv) injected immunoglobulins, led to some "disease-modifying" effects. However, despite reducing parenchymal amyloid plaques in the brain, these treatments still exhibit a modest clinical efficacy, providing patients a slight delay in cognitive decline [1]. Clinico-pathological evidence, while pointing to Aß as an early trigger in the disease, indicate that tau pathology in AD is more strictly correlated to symptoms than amyloid plaques [2]. However, for now, immunotherapies targeting tauopathies in AD did not lead to significant clinical improvements. It is highly likely that the blood-brain barrier (BBB) hamper the brain pharmacokinetics of used anti-tau monoclonals, hence reducing their efficiency. Also anti-tau antibodies have to cross neuronal cell membranes to reach their cytosolic targets and IgGs may lack such intrabody behavior.
Due to their short size and single-domain structure, camelid nanobodies (VHHs) have a unique capacity to diffuse through biological barriers, circulate within the brain parenchyma and access complex extra- and intracellular epitopes.
The purpose of the present PhD project is specifically to develop enhanced immunotherapy strategies using in-house anti-tau VHHs. More precisely, **the PhD student will evaluate, in a tauopathy mouse model, the efficacy of VHHs targeting abnormal tau species on neurodegeneration and behavior-cognition, using different routes of administration (repeated intravenous injections combined with transient BBB opening by ultrasound vs continuous in situ intracellular production using a viral vector).**

Previous results

We (BD, PL) have a long-standing collaboration and experience in VHHs engineering and characterization. We previously identified a camelid-derived VHH against pTau proteins [3]. Our anti-pTau VHH (named A2) has a good affinity for its target and can be used by IHC to identify tauopathies in different AD transgenic mouse models, in human brain sections and also, in vivo, when iv-injected at high concentrations.
Ultrasound-based methods to open transiently the BBB in tau transgenic mice has been the focus of the thesis of A. Géraudie in BD's laboratory [4,5] and we have been associated to the first clinical application of this technique in AD patients (phase 1/2 NCT03119961 [6]). In a twin project relying on anti-Aß VHHs, Jean-David Randrianaly (3rd year Phd student, co-direction PL&BD) and A. Trotier (BD's postdoc) demonstrated that VHHs, when given access to brain parenchyma (stereotaxic injections), are able to diffuse more rapidly and extensively than standard IgGs. Randrianaly and Trotier also showed that ultrasound BBB opening allows iv-injected VHHs to penetrate and diffuse in brain parenchyma and to reach their targets (amyloid plaques when using an anti-Aß VHH). Finally, using a recombinant adeno-associated virus (rAAV) with a BBB-passing serotype (PHP.eB), they were able to induce a strong VHHs production in the brain ("nanobody biofactory"), after a single iv injection of the viral vector. **All these stimulating results provide strong scientific and methodological background for the present project that will rely on similar approaches but applied to anti-tau VHHs**. In addition, we recently obtained important results showing, using in vitro biosensors systems, that the 1st anti-pTau VHH we engineered (A2) is able to mitigate the seeding activity of tau, underlining a clear therapeutic potential (collaboration E. Cecon, Institut Cochin).

Workplan and methodologies of the thesis project

Note: All in vivo experiments will be carried out, after ethics approval, using P301S transgenic mice (PS19) available on site at ICM. Experimental groups (n=10) will be composed of 50% males and females.

Task 1 (Year 1): optimization and refinement of anti-tau VHHs

Our A2 VHH targets a phospho-tau epitope at S422 that has a clear clinical interest [7,8]. To be used as a therapeutic agent (especially for chronic treatments) the stability of A2 should however be improved. Its low

stability may be explained by low thermostability. We will therefore generated A2 variants and select the most thermostable variants by panning based on increasing selection temperatures.
A number of different new epitopes on the tau protein have been identified in the recent years and may also be considered as important targets for tau immunotherapies (eg pS214 implicated in tau seeding and propagation[9], pT217 an early biomarker of AD pathology with a therapeutic potential[10]). Within the timeframe of the PhD project it will be possible to engineer 1-2 extra anti-tau VHHs and to evaluate them, in parallel to our original A2 VHH, in Tasks 2 & 3.

Task 2 (Year 2): Enhanced immunotherapy with transient BBB opening

We will made use of combined iv VHH injection with transient BBB opening by ultrasounds using a SONOCLOUD device (©CarThera) in order to facilitate VHH brain penetration and minimize antibody concentrations/bolus volume of injections.
For each experiment, we will perform 6 consecutive weekly treatments (our pilot studies showed no adverse effects of sonication using this regimen). At the end of treatment, we will perform 1) a standard neurological evaluation to evaluate improvement of motor impairments displayed by aged P301S Tg, 2) Morris water maze and object recognition tests, using protocols recurrently used at ICM, to evaluate both spatial and non-spatial memories. Mice will then be sacrificed and we will quantify by ELISA and IHC the impact of treatment on tau lesions (soluble / insoluble pTau, tangles pathology using various reference anti-tau antibodies) and associated neuropathologies (synaptic loss, neuroinflammation).

Task 3 (Year 2 M6-12, Year 3 M1-6): Enhanced immunotherapy using a viral vector

Under the supervision of F. Piguet (Head of the innovation unit GENOV, ICM), expert in gene therapy, we will pursue our strategy to produce VHHs in situ in the brain parenchyma. We will use the AAV.PHP.eB serotype, that was found to be efficient in our pilot studies, to express anti-pTau VHHs in the brain. Six weeks after iv injection of the AAV construct mice will behaviorally phenotyped and then sacrificed and neuropathologically assessed as described in Task 2.

Task 4 (Year 3 M6-12): redactions

The last months of the thesis will be dedicated to the finalization of papers redaction and sufficient time will also be allocated to the writing of the thesis manuscript.

Conclusions

This PhD project will provide new insights and innovative approaches for the treatment of AD. **It will rely on strong preliminary data and on an active collaborative network** between the two thesis directors who have different and complementary expertise. PL will have a key role in obtaining, optimization and engineering of high quality-controlled VHHs and BD will provide all necessary expertise and environment for preclinical in vivo and neuropathological studies.

Bibliography

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