

● PERSPECTIVE

Is it time to rethink the Alzheimer's disease drug development strategy by targeting its silent phase?

Alzheimer's disease (AD) is the most frequent cause of dementia in the western world. In clinical terms, AD is characterized by progressive cognitive decline that usually begins with memory impairment. As the disease progresses, AD inevitably affects all intellectual functions including executive functions, leading to complete dependence for basic activities of daily life and premature death. Around 47 million people live with AD worldwide and the number of patients is estimated to surge to 130 million in 2050 if we don't find a cure (Prince et al., 2015). By 2018 it will become a trillion-dollar disease and this economic cost inflicts a significant financial burden on individuals and families. In the US, out-of-pocket costs for families affected by Alzheimer's account for more than \$8,000 on average each year. It makes Alzheimer's disease the most expensive illness for families during the last five years of life (Kelley et al., 2013). Unfortunately, there are no effective treatments against AD, although some drugs can alleviate the symptoms associated with it.

One century ago, Dr. Alois Alzheimer described the first AD patient: Dr. Alois Alzheimer identified the cerebral lesions of the disease more than a century ago. His patient, Auguste Deter, was displaying progressive memory loss, impaired thinking, disorientation, and changes in personality. At a microscopic level, Dr. Alzheimer identified two main cerebral aggregates of the disease: the senile plaques and the neurofibrillary tangles. However, it was not until 1984 that researchers revealed that the main components of senile plaques were amyloid peptides resulting from amyloid precursor protein (APP) cleavage. Only few years after these discoveries, neurofibrillary tangles were characterized as hyperphosphorylated Tau aggregates (Jellinger, 2006). These major discoveries marked the beginning of more than 3 decades of heavy research.

Despite 30 years of intensive research, almost 100% of clinical trials have failed: As of today, two main events of AD are well established. AD is characterized by a progressive accumulation of β -amyloid peptide ($A\beta$) that leads to a gradual Tau hyperphosphorylation. As a consequence, patients display a progressive decline of their cognitive functions that is followed by senile plaques deposition and fibrillary tangles formation. At the ultimate stage, dementia appears (events sequence known as "amyloid cascade"). The neurological assessment of the patient and concurrent diagnosis may be made only after the first signs of dementia appeared. Despite billions of dollars invested in R&D to find an effective treatment, AD clinical trials still have the lowest success rate of any disease area – less than 1% compared with 19% for cancer (Cummings et al., 2017). This high failure rate could be attributed to the "too late" stage targeted during clinical trials (the dementia stage), to a lack of fundamental knowledge of the disorder and to current animal models, which do not fully replicate the human AD course. Especially, the pathophysiological link between APP processing (including soluble $A\beta$ peptides production) and Tau pathology remains challenging in AD animal models. Therefore, the lack of animal models mimicking the key events observed in human AD raises the question of the validity of the modelling technologies used.

Thus, the question is, "can we reduce the failure rate of an AD clinical trial through an improvement in its design (by treating patients long before clinically-diagnosed dementia) and using preclinical models that better mimic the AD progression?"

No early diagnosis, no possible salvation: Until recently, the diag-

nosis of AD was exclusively based on a neuropsychological assessment. Despite recent advances in biomarkers, their sensitivity and specificity remain insufficient. The first biological signs of the disease appear 20 years before the clinical diagnosis. Thus, the diagnosis is established when most of the damages have occurred to the brain and when the patient is already suffering from severe dementia (Sperling et al., 2014), making the chances of successful treatment very low. It is difficult to analyze the AD patient's brain and biological fluids before current clinical diagnosis. However, understanding this silent phase will be a decisive step for the development of effective therapeutic molecules and diagnosis. To overcome this difficulty, it is essential to have the most faithful models to human disease. However, transgenic animal models are not consistent with human pathology.

Transgenic AD models' limitations can explain why therapeutic strategies working in mice failed in patients: Most of AD models used in laboratories are transgenic mice expressing human mutated genes associated with familial forms of AD (APP, PSEN1 and PSEN2). Because each of these mutations leads to an increased $A\beta$ production, these models are pertinent to quickly mimic the amyloid plaques deposition in a very short time. In addition, they are suitable models to develop pertinent positron emission tomography (PET) or magnetic resonance imaging (MRI) tracers to identify senile plaques or neurofibrillary tangles in the brains of patients. However, these existing transgenic animal models have at least three limitations. Several studies showed that the development of AD hallmarks in transgenic mice depends on the expression of the transgene(s). Consequently, aging, which is the strongest risk factor for AD, is often ignored in AD studies because most of mice models present an AD-like phenotype just in a few months. The fact that all these mice develop an accelerated senescence not similar to the human disease may be considered as the first limitation (i). Regarding the second hallmark of AD, no genetic mutations in Tau gene have been found in AD patients. Thereby, mice models have been developed using MAPT mutations found in a subset of tauopathies to develop neurofibrillary tangles. Crossings between several lines have been performed to generate transgenic models developing both amyloid and tau pathologies, such as the 3xTg-AD mouse (Duyckaerts et al., 2008). However, both pathologies appear independently: $A\beta$, which is a causative pathogenic factor based on amyloid cascade trigger tau pathology, is not designed and represents a second limitation (ii). Moreover, the transgenes that are overexpressed in transgenic animals are not overexpressed in patients (except for the AD form developed by patients with Down syndrome), which is why the quantities of neurotoxic peptides such as $A\beta$ are much higher in these transgenic models than in AD patients' brain (Audrain et al., 2016). The last limitation is the supra-pathological concentration of pathological metabolites expressed by transgenic AD models (iii). Furthermore, other modelling strategies have been developed such as the injection-based animal models, induced by intracerebral injections of amyloid or tau peptides directly into the brain (Puzzo et al., 2017). Similar limitations to the transgenic models may also be addressed here. Despite these limitations, existing animal models of AD have provided numerous data that had led to the understanding of neurological AD lesions and the evaluation of various potential therapeutic strategies. Overall, the research community regrets the lack of adequate models. This absence of human-close AD models appears as a limiting factor for the development of diagnoses and active treatments for human beings (Lecanu and Papadopoulos, 2013). In any cases, key factors including (i) aging, (ii) influence of soluble $A\beta$ peptides toward tau pathology and (iii) faithful clinical $A\beta$ concentrations remain challenging and should be designed in adequate AD animal models.

Advent of non-transgenic models which are closer to the human pathology: In order to mimic in an *in vivo* model the progression of the disease in a manner that reproduce more faithfully the clinical observation, an innovative AD rat model, the AAV-AD rat, was recently developed through the injection of adeno-associated viruses

(AAV) coding for human mutant APP and presenilin 1 (PS1) genes into the hippocampi of adult rodents.

This approach has allowed the localized production of APP and PS1 proteins in a small number of neurons. These neurons produce A β 42 peptide which progressively diffuses throughout the hippocampal tissue. The majority of the hippocampal cells thus have no genetic modification, making it a relevant model for non-genetic forms of the disease that represent more than 92% of cases (Prince et al., 2015). Its pathophysiological relevance has been validated by comparing it to post-mortem samples of AD patients. The concentration of A β 42 peptide gradually increases to reach at the late stage concentrations comparable to those measured in the hippocampus of AD patients. As hyper-phosphorylation of the endogenous Tau protein gradually take place, the memory capacity simultaneously declines, reproducing the chronology of events progression seen in clinics. Amyloid plaques and cerebral amyloid angiopathy develop only in aged AAV-AD rats. Intraneuronal aggregates of hyperphosphorylated Tau protein confirm a full commitment of the Tau pathology (Audrain et al., 2017).

This model could thus be described as follows:

1) A disruptive technology: The technology used is not based on a transgenic approach. Because AD induction is conducted only on adult animals, AAV-AD rats do not suffer from developmental compensation or genetic drift. Moreover, the pattern of APP expression in AAV-AD model may mimic the genomic mosaicism recently described in the sporadic form of human AD, in which an increase in copy number was observed for the APP gene in a limited subset of neurons (Bushman et al., 2015). The AAV-AD rat model could thus be considered as a closer model of the sporadic form of AD than transgenic animals.

2) A time course closer to the human progression of AD: Induced APP pathology appears similar to the human one in terms of the amount of amyloid peptide and A β 42/40 ratio. The induced amyloid pathology leads to pathophysiological mechanisms including progressive Tau hyperphosphorylation. Slow progression of the APP pathology allows the progressive development of an endogenous Tau pathology to take place without the occurrence of a would-be interfering early inflammation and plaque formation. These steps could be considered as the silent phase of AD, beginning in patients at least 18 years before the current clinical diagnosis (Rajan et al., 2015). The next phase of AD disease progression consists of the appearance of AD-related cerebral lesions such as senile plaques, cerebral amyloid angiopathy and tangle-like aggregates, which only appear in aged AAV-AD rats.

3) A study model for early diagnosis development: Proteomic techniques on plasma pools of AAV-AD rats and age-matched control samples identified dysregulated plasmatic proteins between AAV-AD animals and controls (41 proteins at 8 months, 21 proteins at 30 months including 3 identical, AgenT personal communication). These results confirm that despite the AD-induced pathology is restricted to the hippocampus of AAV-AD rats, plasma protein dysregulation could constitute the first step to define an early diagnosis.

All these features make the AAV-AD rat model a powerful tool to better predict the drug efficacy during clinical trials. It could thus accelerate the development of therapies specifically acting during silent AD phases. This model also constitutes a study system to characterize new biomarkers or panel of biomarkers of early diagnosis, disease progression, target engagement and drug efficacy.

Is it time to rethink the AD drug development strategy? Drug development in the field of Alzheimer's disease is certainly among the most challenging and has been suffering dramatic setback regardless of the strategy developed. A reason may lie in the lack of reliable animal models that would constitute a relevant tool to the development of new drugs. Although transgenic mice helped in characterizing some of the pathological pathways occurring in AD, they failed for

the past 30 years at helping to release new treatments to the market. Re-thinking drug development strategy in AD cannot be done without new animal models that address unmet needs, namely the infra-clinical phase of the disorder. With the advent of powerful modeling technologies and our non-transgenic AAV-AD rat model, we are ready to rethink the Alzheimer's disease drug development strategy.

Benoit Souchet, Mickael Audrain, Baptiste Billoir, Laurent Lecanu, Satoru Tada, Jérôme Braudeau

AgentT, 4 rue Pierre-Fontaine, 91058 EVRY Cedex, France

*Correspondence to: Jérôme Braudeau, Ph.D.,

jerome.braudeau@agent-biotech.com.

orcid: 0000-0002-9920-4112 (Jérôme Braudeau)

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Open peer review reports:

Reviewer 1: Vasily Vorobyov, Russian Academy of Sciences, Russian.

Comments to authors: This is a timely and clearly written manuscript renewing our interest to early stages of AD development. The main idea about the AAV-AD rats as an adequate model for AD studies is sufficiently supported.

Reviewer 2: Alessandro Tonacci, Istituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche, Italy.

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