

# Animal models of Alzheimer's disease and drug development

Bart Laurijssens<sup>1,\*</sup>, Fabienne Aujard<sup>2</sup>, Anisur Rahman<sup>2</sup>

<sup>1</sup>BEL Pharm Consulting, Moulin d'Ozil, 07140 Chambonas, France

<sup>2</sup>CNRS UMR 7179, MNHN, 1 Av du Petit Château, 91800 Brunoy, France

**Animal disease models are considered important in the development of drugs for Alzheimer's disease. This brief review will discuss possible reasons why their success in identifying efficacious treatments has been limited, and will provide some thoughts on the role of animal experimentation in drug development. Specifically, none of the current models of Alzheimer's disease have either construct or predictive validity, and no model probably ever will. Clearly, specific animal experiments contribute to our understanding of the disease and generate hypotheses. Ultimately, however, the hypothesis can only be tested in human patients and only with the proper tools. These tools are a pharmacologically active intervention (in humans) and a clinical trial suited to evaluate the mechanism of action. Integration of knowledge in quantitative (sub) models is considered important if not essential in this process.**

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disease affecting an estimated 5.4 million people globally, mainly the elderly. There is currently no cure for AD, but some symptomatic treatments are available. The disease is characterized by its hallmark histopathological findings of extracellular  $\beta$ -amyloid ( $A\beta$ ) plaques and intracellular neurofibrillary tangles of tau and by neuronal and synaptic

## Section editor:

Oscar Della Pasqua – Leiden/Amsterdam Center for Drug Research, Leiden, The Netherlands.

loss in brain regions involved in learning and memory processes (<http://www.alz.org/>) [1].

The interest in finding a cure or prevention for AD is understandably great. Proper animal models of human AD are considered desirable if not essential in this process and much research effort has been put into that effect. As no perfect model exists, the question becomes whether 'the best models available' are good enough. What exactly can be inferred from the results and what not? Or, differently put, how do they contribute to our understanding and decision-making.

The objective, therefore, of this brief review is to discuss the potential role of the current animal disease models for AD in drug development. Specifically, the aim is to discuss why the animal models of AD should have such a limited success in predicting successful treatments in the clinic (or rather, clinical trials), and to provide some thoughts on how to use animal experiments in drug development.

## Animal models of Alzheimer's disease

The aetiology of AD is unknown, but there is still a general consensus in favour of the 'amyloid hypothesis' [1,2], even if it has been questioned [3]. A wide range of animal models have been developed to mimic the human context of the disease for the purpose of developing therapeutics or disease modifying agents. In fact, in most of the animal models the first goal is to simulate the neuropathological findings of AD

\*Corresponding author: B. Laurijssens ([bart.laurijssens@orange.fr](mailto:bart.laurijssens@orange.fr))

followed by the correlation of cognitive function without knowing whether the neuropathological agents have similar biological consequences in humans and in animal models. It is beyond the scope of this review to discuss all the different models. Here we will briefly summarize some potential animal models and their translation towards the clinical settings (Table 1). Animal models used in AD can be broadly divided into three categories: Natural models, Genetic models and Interventional models.

#### Natural models

Several animals including polar bears, dogs, cats, goats and sheep and some non-human primates spontaneously develop some AD-related neuropathological features [4]. The few

animal models reviewed in this section either show major AD-related neuropathology or are in close phylogenetic proximity to humans. In recent years dogs have been considered as a useful animal model for AD due to the close proximity of canine and human brain aging. Dogs develop extensive A $\beta$  and diffuse plaque deposition and the extent of A $\beta$  deposition correlates with the decline of some measures of cognitive function in the absence of neurofibrillary tangles (NFTs). Moreover, the amino acid sequence of A $\beta$  is fully conserved between dogs and humans [5]. Because of spontaneous age-related A $\beta$  deposition and relative ease of cognitive function assessment, aged dogs were used to evaluate interventions with the aim of reduction of A $\beta$  load and subsequent cognitive improvement (Table 1).

**Table 1. Some animal models of AD and clinical trials**

Model	Treatment active in model	Clinical trial
Natural models		
Dog	Active immunization with A $\beta$ <sub>1-42</sub> [49] Antioxidant [49]	Trial stopped due to CNS side effects [50] No effects [51]
Rhesus monkey	Acetylcholinesterase inhibitor, Donepezil [14] Nicotinic receptor agonist ABT-418 [14] Glutamate receptor blocker Memantine [14]	Approved for AD Discontinued for side effects Approved for AD
Mouse lemur, Octodon, Chimpanzees, Orang-utans, Polar bear, Cat, Sheep, Goat, Wolverine	No pharmacological trial has been reported yet.	
Genetic models <sup>a</sup>		
PDAPP mice	Active immunization with aggregated A $\beta$ <sub>1-42</sub> [52] Passive immunization with IgG [53] Vasogenic oedema and microhaemorrhage in some patients, no significant cognitive benefit, Phase-III trials underway [53]	Trial stopped due to CNS side effects [50]
APP mice	$\beta$ -Secretase inhibitor [54]	Reduce plasma A $\beta$ significantly, Under phase-III clinical trial [62]
Tg2576 mice	$\gamma$ -Secretase inhibitors [55]	Trial halted because the drug failed to stop disease progression, associated with worsening cognitive function and increased risk of skin cancer [63]
	$\gamma$ -Secretase modulators [56] Acetylcholinesterase inhibitor [57]	No benefit, trial discontinued [62] Approved for AD
Cognitive decline cannot be prevented, cognitive benefits with treatment associated with adverse effects reported [64]		
PSAPP mice	Non-steroidal anti-inflammatory drugs [58]	Negative results [65]
Tg-tau mice	Simvastatin [59]	No cognitive benefit [66]
Ttpa <sup>-/-</sup> APPsw mice	$\alpha$ -tocopherol [60]	No efficacy [67]
Fruit-fly	Doxycycline [61]	Together with Rifampin. Some positive effect on cognitive performance [68]
Interventional models		
Rat		
Intrahippocampal amyloid infusion model	Curcumin [69]	In Phase-II [4]
Intracerebroventricular A $\beta$ infusion model	GSK3 inhibitor [70]	Marginal benefits, larger trial ongoing [71]

<sup>a</sup> **PDAPP mice:** Mutation of human APP with valine at residue 717 substituted by phenylalanine under the control of human platelet derived growth factor promoter. **APP mice:** Mutation of three isoforms [695, 751 and 770] of human APP under the control of various promoters. **Tg2576 mice:** Swedish double mutation [K670N/M671L] on the 695 human APP under the control of hamster prion protein promoter. **PSAPP mice:** Swedish double mutation [K670N/M671L] plus Presenilin 1 exon 9 deleted. **Tg-tau mice:** Human normal shortest 3R tau isoform inserted into the mouse genome under the promoter. **Ttpa<sup>-/-</sup>APPsw mice:** Tg2576 mice crossed with  $\alpha$ -tocopherol transfer protein knockout mice [31,60].

Among the non-human primate models, mouse lemurs seem to be a potential animal model that exhibit amyloid plaque, NFTs and some other AD related neuropathology [6]. This small prosimian primate lives 8–10 years in captivity and shows age-related changes similar to those of aging humans [6]. About 20% of mouse lemurs aged five years or older show significant brain atrophy [7], extensive accumulation of amyloid plaques, neurodegeneration [8] and/or loss of cholinergic neurons [9]. The genes responsible for the formation of senile plaques are highly similar between mouse lemurs and humans [8]. The presence of amyloid deposition has been recently linked to cerebral atrophy in aged individuals, giving new insight into the understanding of pathological aging in this non-human primate species [10]. Moreover, a comparable decline in declarative memory and executive function has been reported in both aged human and aged mouse lemur whereas procedural memory appears to be conserved in both species [11]. In a recent study it has been shown that cognitively impaired aged mouse lemurs have cerebral atrophy especially those brain regions that are responsible for cognitive functions [12]. It should be noted that there is a difference in distribution of A $\beta$  deposits and plaques between human and mouse lemur. In humans the A $\beta$  depositions usually start in the hippocampus but in mouse lemur they appear first in cortical regions [13]. As with human AD, currently no diagnostic tools are available that can predict which adult mouse lemur will develop AD-like symptoms. However, this model provides an opportunity to search for these predictors.

Rhesus monkeys and humans share many diseases of aging and probably are the most successful models to identify diagnostic markers and the development of safe and effective treatments for human brain disorders. Monkeys of over 19 years old showed significant amyloid-plaque-like lesions in areas of brain responsible for cognitive function. However, most of the aged monkeys do not show an Alzheimer's-like syndrome and age-related rapid cognitive decline commonly found in AD patients is not usually found in them either [14]. Moreover, their long developmental period, low reproductive output, long captive life and risk of serious zoonotic disease transmission are major disadvantages for using these animals in a wider range of AD research [15].

Octodon degu, a rodent of South American origin has recently been found to have spontaneous development of AD-related neuropathology at older age. Localisation of both intracellular and extracellular amyloid deposits and NFTs-like intracellular deposition has been detected in different layers of cortex and hippocampus of old animals. Adult octodon cortex shows the presence of cholinergic neurons as in humans but with a different distribution. They also demonstrate age-associated cognitive impairment but whether these cognitive deficits are also associated with cholinergic neurodegeneration similar to AD patients is not known yet.

Another interesting finding is the presence of extensive astrogliosis in the aged octodon brain, which is a characteristic feature of human AD brain. It has been hypothesized that high homology (97.5%) of octodon A $\beta$  and human A $\beta$  might be an important factor in the appearance of AD markers in this rodent. Breeding difficulty and comparatively longer lifespan are limitations of this rodent model [16,17].

#### Genetic models

Transgenic technology provides unique opportunity to reproduce the cause of familial AD by transfecting a mutant human amyloid precursor protein (APP). Mice have extensively been used as transgenic models and facilitated our understanding of the molecular mechanisms associated with A $\beta$ -production, deposition and clearance and the effects of A $\beta$  on neuronal network and synapses that play important role in cognitive function. The APP mouse model successfully produced a wide range of parenchymal and vascular amyloid deposits similar to those of human AD [18]. Although morphological similarity does exist, there is a difference in biochemical composition of deposited A $\beta$  between mouse models and AD brain [19]. Moreover, these transgenic mouse models failed to develop neurofibrillary tangles (NFTs), an important histopathological hallmark of AD result from intraneuronal aggregation of hyperphosphorylated tau protein [20]. Oddo and colleagues [21] presented for the first time a triple transgenic mouse model where both plaques and NFTs were found in AD-relevant brain regions. These mice also developed extracellular amyloid beta deposits before the formation of NFTs and exhibited impaired synaptic plasticity including long-term potentiation, the key basis for cognitive function. Furthermore, transgenic mouse models also provided valuable information regarding the role of inflammation, oxidative stress and mitochondrial dysfunction in the pathogenesis of AD [22,23]. However, progressive neuronal loss in hippocampus and specific neocortical regions of the human AD brain [24] is not evident in most of the transgenic mouse models, and this is a major limitation of these murine models. In addition, these transgenic mice represent only those who are suffering from familial AD, which is <1% of all AD patients. There is no mouse model that can fully reproduce the features of disease progression of vast majority of AD cases that is sporadic/late-onset AD.

The fruit-fly *Drosophila melanogaster* is a widely used and well-appreciated animal model of neurodegeneration including AD [25]. The fruit-fly is small in size, has a simple well-studied anatomy and possesses a well-organized brain. Although the fruit-fly brain has only a fraction of the cells of the human brain and a different neuroanatomical organization, it is similar in the fundamental aspects of cell biology, in terms of regulation of gene expression, membrane trafficking, neuronal connectivity, cell signalling, synaptogenesis and cell death [26,27]. Moreover, its short life cycle and completely sequenced genomes are added experimental

advantages of this model. Importantly, the transparent cuticle of the larvae of fruit-fly allows the study of the disease progression in living intact animals which is rather difficult in vertebrates [28]. The task for associative learning and memory such as Pavlovian olfactory conditioning can be measured in this model, which is homologous to the classical conditioning of the eyeblink response found to be impaired in patients diagnosed with AD [26]. The fact that hippocampal-dependant cognitive functions, which are impaired early in human AD, cannot be tested in invertebrates due to the lack of these brain structures is a major limitation of this animal model. In spite of the lack in homology between fruit-fly and human brain, this simple invertebrate animal provided insight in the disease mechanism ranging from genetics to some cognitive functions that could be followed in living intact animals.

#### *Interventional models*

Introduction of pharmacological or chemical substances into the brain or the induction of lesions in specific brain regions may replicate some of the characteristic features of AD. Many models involve the introduction of A $\beta$  peptide into the brain of, for example, the rat [29] or rhesus monkey [30]. Although these models induce some of the clinical signs, they do not directly resemble AD pathology [31]. Other chemical interventional models include scopolamine-induced amnesia, introduction of inflammation with endotoxins or interference with brain metabolism [4].

The lesion models involve the chemical or physical destruction of specific brain areas, which are generally either cholinergic (i.e. the nucleus basalis magnocellularis in rodents, e.g. [32]) or involved in cognition (i.e. hippocampus, striatal and cortical brain regions). Major disadvantages of the lesion models include non-specificity of the lesion, and their failure to capture the disease progression and the more global aspects of the disease (too specific) [4].

As a model of disease, interventional models would generally be better at identifying symptomatic or corrective treatments, rather than disease modifying therapies that halt or slow down progression, unless the 'intervention' represents a damage early in the disease progression.

Furthermore, a wide range of variability including species, animal husbandry, site of injection or induction, model protocol and concentration and volume of substances may influence the experimental outcome. However, these models can provide important insights such as scopolamine-induced amnesia model contributed to the role of cholinergic system in cognition [33], specific brain lesions induced memory deficit models and the neuronal mechanism underlying memory dysfunction [34] and the A $\beta$ /pharmaco-chemical substance induced model and the understanding of inflammation, neurotoxicity, neurodegeneration and synaptic function [35].

#### **Animal models of disease**

It is obvious that many of the animal models of AD have contributed, and continue to contribute, to our understanding of the processes that may or may not underlie human AD. But can they really be called animal models of AD, and can they therefore be used as such?

The perfect animal model of a disease would be a scaled down replica of the human disease, representing all important components from cause via structural damage to symptoms (Fig. 1). It would be structurally and quantitatively identical to the human. For this, both the animal model and the human disease need to be very well understood. Such a model would have face-, predictive- and construct validity [36] (see Box 1). None of the models above satisfy this criterion and no animal model probably ever will, especially for a disease such as AD, given its complexity [36,37]. It could be argued, therefore, that such a model should not even be an objective.

Even predictive validity, although very useful, is not trivial to establish. It requires effective drugs in the clinic, ideally with different mechanisms, and a good understanding of the false positives and false negatives. The latter is particularly hard to evaluate because compounds that do not work in the animal model are often not tested in the clinic (or reported). In a way, one could say that the availability of an animal model with established good predictive validity is unfortunately inversely correlated with the unmet medical need.

All the disease models for AD listed have at best face validity, where the animal model merely shares phenomenological similarities with the disorder under study. Hence the problem of 'translation' and recent interest in 'translational sciences'.

The schematic in Fig. 1 illustrates the complexity and the many possible pitfalls in trying to create an animal analogue for human disease. A single relevant difference, whether structural or quantitative, can 'invalidate' the model. This is, when it is not understood and considered in the translation to the human condition.

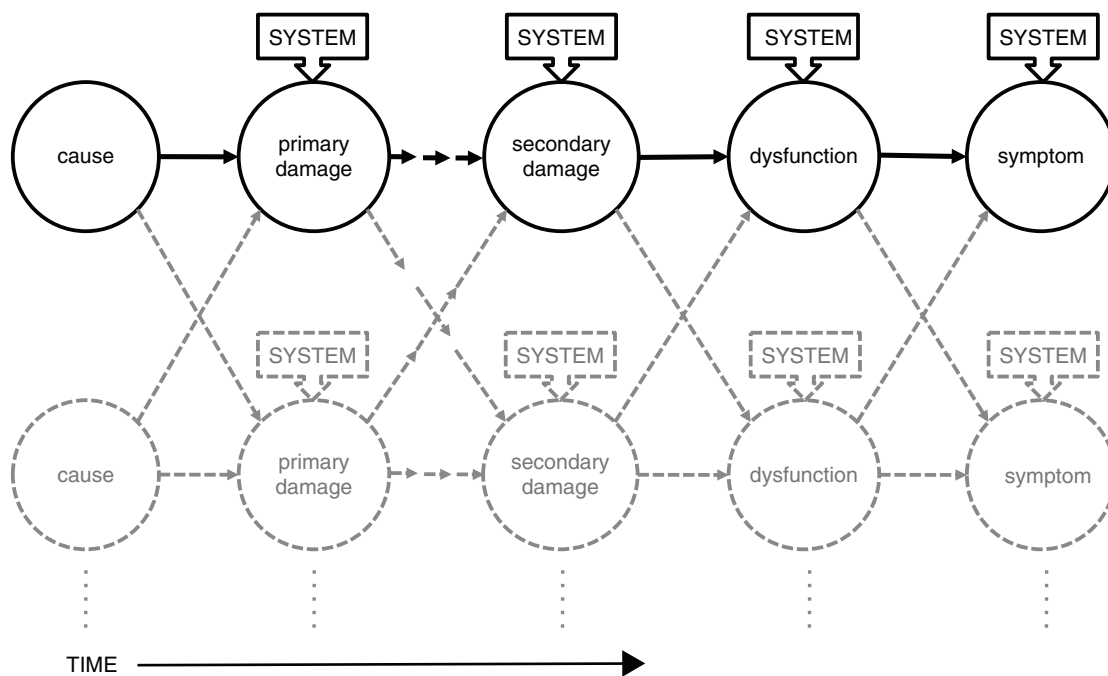
By contrast, many of the single components of the schematic in Fig. 1 will be preserved across species (normal physiology). Thus, whereas it is probably impossible to replicate the full sequence from cause to symptoms in animals, the study of components of the hypothesized sequence could

#### **Box 1. Validation criteria for animal models [36]**

**Face validity:** The animal model resembles the human disease condition on a superficial level, for example, biochemistry or symptomatology.

**Predictive validity:** The animal model can successfully discriminate between successful and unsuccessful treatments for the human disease condition.

**Construct validity:** The animal model is based on a sound theoretical rationale, requiring good understanding of the human disease condition. Notably, construct validity does not require superficial similarity.



Drug Discovery Today: Technologies

**Figure 1.** A theoretical framework for a disease model. The disease cause, which can be an event or a continuous process, would result in some primary damage, followed possibly by a downstream damage cascade, the result of which may be the disruption of function, observed as a symptom. For AD this could be a genetic defect generating an excess of A $\beta$ , downstream resulting in death of cholinergic neurons causing memory impairment, observed as a poor ADAScog score. The diagnosis of the disease may involve some or all of these components. There may be parallel paths, interacting at different stages. A different cause may either generate the same secondary damage, or independently show the same symptoms. Depending on how the disease is defined this may represent heterogeneity. Also, the same cause and primary damage can have multiple secondary damages (in multiple anatomical locations) and subsequently, multiple dysfunctions and symptoms. Importantly, these cascades are part of a system, which defines such elements as the turnover rates of components, repair (i.e. reversibility) and response (i.e. immune system), and robustness (i.e. feedback loops, collateral pathways, compensatory mechanisms). All the elements of such a model have anatomical locations, and can be described quantitatively in terms of capacity, sensitivity and rate constants. In addition, the cause has an intensity and time course associated with it. Furthermore, most if not all relationships between elements are non-linear.

identify potential rate-limiting steps, suggest possible interventions and provide a rationale for the translation to humans.

### Reasons animal models of disease can fail

So far the animal models for AD have not been very successful in identifying effective treatments (see Table 1 and [38]). Why? It is possible to highlight specific weaknesses (and strengths) for each animal model (e.g. [4,38]). Some of the issues have been raised in the previous sections. More generally, one can identify three categories of reasons why clinical trial results do not match the animal model results, and not only because of the animal model *per se*. In our discussion it is assumed that the animal experimentation is technically sound, potential issues with that being discussed elsewhere [39].

1. The animal disease model does not truly reflect the disease or represents an incorrect hypothesis. Hence the drug target is not relevant to human disease. This can ultimately only be addressed in patients, and failures like this

are an unavoidable part of drug development. A properly conducted negative clinical trial would suggest that a revision of the hypothesis is necessary. Appropriate biomarkers can help to understand the nature of the required revision. Some possible reasons are:

- a. Incorrect basic structure of the model. Key elements of the animal model are not major players on the causal path in humans. There is some similarity with reasons why biomarkers fail [40]. In addition, a key element could be regulated differently in the model species.
  - b. The model is quantitatively wrong. Critical species differences in the capacity, sensitivity or rate constants governing the relationship between elements are not captured.
2. Incorrect (quantitative) translation of the intervention to the patient. Thus the target is relevant but not appropriately engaged. Without the appropriate pharmacological biomarkers this is difficult to identify, and very little useful inferences can be made after a negative clinical trial. One cannot distinguish between wrong target and wrong dose. Possible explanations are:



- a. Incorrect dosing regimen because of species differences in pharmacokinetics, distribution to target site and/or target affinity, duration of treatment required.
  - b. Physiology of the primary target is different between species, or altered in human disease (not captured in the animal model), for example, interventions that significantly lower brain A $\beta$  in mice can fail to do so in AD patients because of species differences in A $\beta$  homeostasis.
3. Clinical trial design not appropriate to test the intervention under investigation. A negative clinical trial risks abandoning a correct hypothesis and a beneficial intervention. Although not the objective of this review, some reasons may be:
- a. Wrong dosing regimen. For example, the translation may be adequate, but target engagement is limited by safety and tolerability.
  - b. Study too short to be able to notice effects
  - c. Wrong patient population. The patients are too advanced to allow significant benefit of the intervention [41], or only a subpopulation would benefit.
  - d. Insensitive or not relevant endpoints. The modality affected by the treatment may not, or poorly so, be captured by the study endpoints.
  - e. Other factors such as placebo response and data analysis.

The first two categories are failures to translate animal results to human patients. One cannot translate if the crucial components leading up to a particular endpoint are not identified. This is where a conceptual framework such as shown in Fig. 1 would help by capturing the current state of knowledge and assumptions explicitly. One could argue that this understanding could also contribute to avoid some failures of the third category, for example, timing of the intervention, identification of surrogate or biomarkers or the modality monitored for treatment effect.

### Animal experimentation and Alzheimer's disease

How can animal experimentation contribute to some of the challenges facing drug development in general and our search for a cure for AD in particular? Three major challenges mirror the reasons for failure listed above and they are: first, target selection, second, the (pharmacological) intervention and third, the clinical patient trials.

Target selection is probably the most difficult challenge in AD and is directly related to our, limited, understanding of the disease. An obvious role of animal experiments is to increase this understanding, as they already greatly have, and to generate hypotheses. However, the correctness of the hypothesis can only be addressed in human patients with the right tool. Some have suggested to focus more on research in human patients [37] and the need to simultaneously

engage multiple targets to treat complex disorders [38]. Multiple targets may be required to significantly affect one aspect of the disease, or to address the multidimensionality of the disorder. In addition, animal models can be used to screen series of compounds, and identify potential targets by their ability to modulate an aspect of the hypothesized disease model.

The main objective for the pharmacological intervention is to ensure that the dose regimen that will be tested in the human patient does what it pharmacologically is meant to do: sufficiently, long enough and at the right place. Animal experimentation can contribute to our understanding of the pharmacokinetics of the compound and the target pharmacology, as long as properly scaled between species, and provide guidance on what may be the required target engagement. One objective is to verify this in humans early on in clinical development, as is done for pharmacokinetics and, if possible, with biomarkers of pharmacology.

Animal experimentation does not seem to contribute directly to the clinical trial design. However, as mentioned above, the right experiments can provide guidance regarding the timing of treatment and regarding which modalities would be consistent with the target pharmacology.

Thus, animal experimentation remains part of the scientific method that iterates between hypothesis generation, predictions and experimentation. The hypothesis is in fact conceptual and should be a human disease model, attempting to address the components mentioned in Fig. 1. Given the complexity, describing and connecting parts of the model quantitatively is an important part of this process [41–45]. Among other benefits, modelling and simulation provide a way of integrating currently available facts and beliefs, identifying gaps and raising questions [45] and, for drug development, ultimately some sense of the uncertainties of a particular development program.

For drug development some specific quantitative aspects of a theoretical human model to consider are suggested below, all of which are a potential source of critical species differences.

#### Target selection:

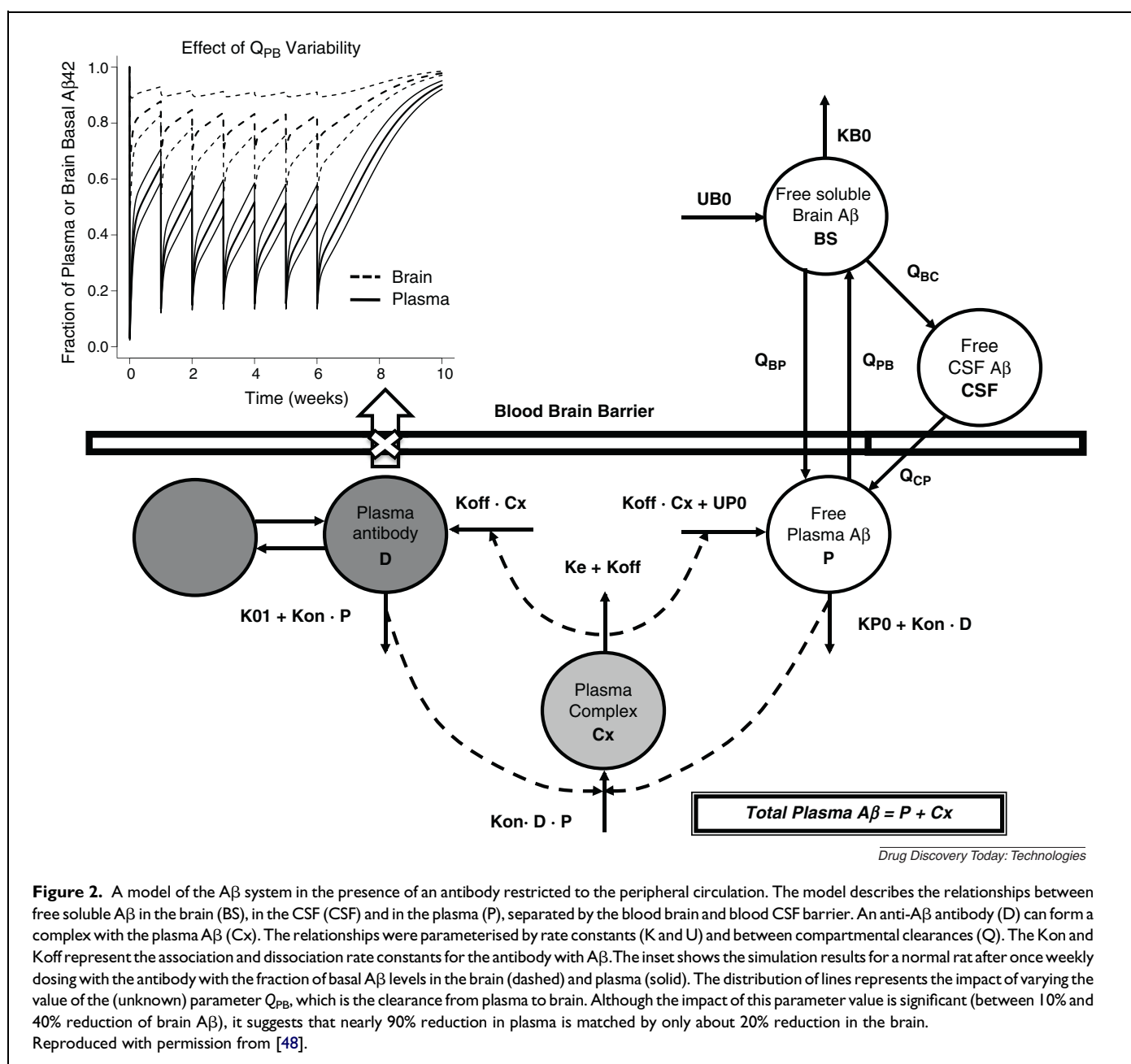
- Relationship between target modulation and subsequent events. How much 'change' of the target is required to cause changes downstream (sensitivity) and when can these changes be observed? What is the relative contribution of the target pathway to the endpoint of interest? Is there a rationale for multiple targets?
- The homeostasis of the target and/or critical downstream elements, for example, A $\beta$ , or memory function. How is it maintained and can it be modulated?
- Related to the above, robustness and redundancies in the system.

Evaluation of the intervention:

- Pharmacokinetics
- Can the intervention modulate the component(s) of interest (target engagement), and if so, what is the required timing of the intervention? And, again, how long does it take before these changes can be observed, which also depends on the endpoint? In other words, pharmacokinetic–pharmacodynamic relationships for the target pharmacology.
- Are there bio- or surrogate markers that can inform on the target pharmacology, and, if so, what is the relationship between target function and biomarker level? Again, considering relative contributions and time courses. A recent

example is some modelling and simulation of brain atrophy as biomarker [46]. Multiple competing factors contributing to brain volume loss measurements were identified and incorporated in a model. These included, for example, normal aging, disease, volume of amyloid deposits and inflammation. The model could explain why treatment with an anti-amyloid vaccine would result in a temporary acceleration of brain volume loss, as was observed in clinical trials.

Another example is the complexity underlying interventions attempting to lower soluble A $\beta$  in the CNS of patients, suggested as potentially beneficial by the A $\beta$  hypothesis (e.g. [1]). The relationships between the different forms of A $\beta$



Drug Discovery Today: Technologies

across the different compartments of interest, for example, brain, CSF and plasma, are not immediately obvious. This issue was highlighted for the rational selection of biomarkers by Thompson and Lockhart [47], for example A $\beta$  concentrations in the CSF as marker for CNS concentrations, but is equally important for the rationalization of interventions. Preliminary work attempting to address this was presented by Simeoni *et al.* [48], and an example of the model, with an intervention included, is shown in Fig. 2. Interestingly, the preliminary simulations with the model suggest that an antibody against A $\beta$ , restricted to the systemic circulation, would only modestly lower CNS levels of A $\beta$ , even if plasma levels were greatly reduced (Fig. 2). This is clearly a red flag when in the process of developing such an antibody, and merits further investigation of the system and assumptions. The model made specific assumptions about the structure of the model and some of the parameters and ignored some non-linearities that must be present in the system. Nevertheless, this work could guide specific future experiments and the impact of parameter assumptions can be explored by sensitivity analysis (as was done for this presentation).

Thus, even if animal models of AD cannot be considered models of disease with construct (or even predictive) validity, it is not so that animal experiments have not been or will not be useful in understanding of the disease or that the hypotheses they aim to reflect are incorrect. The challenge is to identify which part of the overall human disease model a particular animal experiment addresses.

Lastly, although not specifically addressed here, anticipating safety issues of the intervention under study is obviously important. In particular, adverse pharmacology associated with the primary and/or secondary targets of the compound can, should, be addressed applying similar principles as for efficacy.

## Conclusions

The value of many of the so-called animal models for AD lies not in the fact that they attempt to replicate the full spectrum of the human disease, but in that they could help us, together with clinical data, to generate hypotheses and understand the complex physiology relevant to the human disease. In that sense, they are not really animal models of disease but 'just' animal experiments. These experiments, then, need to address specific questions that allow the construction of the hypotheses for the whole or parts of a quantitative human disease model. The conceptual human model is where interventions should be evaluated: for example, target selection, dosing regimen and timing, biomarker and clinical endpoint selection and safety. Integration of information and ideas into a quantitative conceptual (sub) model of human disease and pharmacology is therefore considered essential.

For drug development, whereas there is likely to remain great uncertainty around target selection and the associated

hypotheses, it is crucial to identify a pharmacologically active dosing regimen to test in patients. Then a negative clinical patient study, although disappointing, will at least increase our knowledge regarding the disease.

## Acknowledgements

FA and AR are funded as part of the Pharma-Cog consortium by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under Grant Agreement no. 115009. For further information please refer to <http://www.pharmacog.org/>.

## References

- Mattson, M.P. (2004) Pathways towards and away from Alzheimer's disease. *Nature* 430, 631–639
- Hardy, J.A. and Higgins, G.A. (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185
- Schnabel, J. (2011) Little proteins, big clues. *Nature* 475, S12–S14
- Van Dam, D. and De Deyn, P.P. (2011) Animal models in the drug discovery pipeline for Alzheimer's disease. *Br. J. Pharmacol.* 164, 1285–1300
- Johnstone, E. *et al.* (1991) Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. *Mol. Brain Res.* 10, 299–305
- Languille, S. *et al.* (2012) The grey mouse lemur: a non human primate model for ageing studies. *Ageing Res. Rev.* 11, 150–162
- Dhenain, M. *et al.* (2003) Regional atrophy in the brain of lissencephalic mouse lemur primates: measurement by automatic histogram-based segmentation of MR images. *Magn. Reson. Med.* 50, 984–992
- Bons, N. *et al.* (2006) *Microcebus murinus*: a useful primate model for human cerebral aging and Alzheimer's disease? *Genes Brain Behav.* 5, 120–130
- Mestre, N. and Bons, N. (1993) Age-related cytological changes and neuronal loss in basal forebrain cholinergic neurons in *Microcebus murinus* (Lemurian, Primate). *Neurodegeneration* 2, 25–32
- Kraska, A. *et al.* (2011) Age-associated cerebral atrophy in mouse lemur primates. *Neurobiol. Aging* 32, 894–906
- Picq, J.L. (2007) Aging affects executive functions and memory in mouse lemur primates. *Exp. Gerontol.* 42, 223–232
- Picq, J.L. *et al.* (2012) Age-related cerebral atrophy in nonhuman primates predicts cognitive impairments. *Neurobiol. Aging* 33, 1096–1109
- Giannakopoulos, P. *et al.* (1997) Quantitative analysis of tau protein-immunoreactive accumulations and  $\beta$  amyloid protein deposits in the cerebral cortex of the mouse lemur, *Microcebus murinus*. *Acta Neuropathol.* 94, 131–139
- Buccafusco, J.J. (2008) Estimation of working memory in macaques for studying drugs for the treatment of cognitive disorders. *J. Alzheimer's Dis.* 15, 709–720
- De Magalhaes, J. and Costa, J. (2009) A database of vertebrate longevity records and their relation to other life-history traits. *J. Evol. Biol.* 22, 1770–1774
- Braidy, N. *et al.* (2012) Recent rodent models for Alzheimer's disease: clinical implications and basic research. *J. Neural Transm.* 119, 173–195
- Inestrosa, N.C. *et al.* (2005) Human-like rodent amyloid- $\beta$ -peptide determines Alzheimer pathology in aged wild-type *Octodon degu*. *Neurobiol. Aging* 26, 1023–1028
- Games, D. *et al.* (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein. *Nature* 373, 523–527
- Kalback, W. *et al.* (2002) APP transgenic mice Tg2576 accumulate A $\beta$  peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. *Biochemistry* 41, 922–928



- 20 Goedert, M. *et al.* (2006) Tau protein, the paired helical filament and Alzheimer's disease. *J. Alzheimer's Dis.* 9, 195–207
- 21 Oddo, S. *et al.* (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A [beta] and synaptic dysfunction. *Neuron* 39, 409–421
- 22 Du, H. *et al.* (2008) Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat. Med.* 14, 1097–1105
- 23 Lee, Y.J. *et al.* (2010) Inflammation and Alzheimers disease. *Arch. Pharm. Res.* 33, 1539–1556
- 24 Gomez-Isla, T. *et al.* (1996) Profound loss of layer II entorhinal cortex neurons distinguishes very mild Alzheimers disease from nondemented aging. *J. Neurosci.* 16, 4450–4491
- 25 Driscoll, M. and Gerstbrein, B. (2003) Dying for a cause: invertebrate genetics takes on human neurodegeneration. *Nat. Rev. Genet.* 4, 181–194
- 26 Iijima, K. and Iijima-Ando, K. (2008) Drosophila models of Alzheimer's amyloidosis: the challenge of dissecting the complex mechanisms of toxicity of amyloid- $\beta$  42. *J. Alzheimers Dis.* 15, 523–540
- 27 Sang, T.K. and Jackson, G.R. (2005) Drosophila models of neurodegenerative disease. *NeuroRx* 2, 438–446
- 28 Sinadinos, C. *et al.* (2009) Live axonal transport disruption by mutant huntingtin fragments in Drosophila motor neuron axons. *Neurobiol. Dis.* 34, 389–395
- 29 Nakamura, S. *et al.* (2001) Progressive brain dysfunction following intracerebroventricular infusion of beta1-42-amyloid peptide. *Brain Res.* 912, 128–136
- 30 Li, W. *et al.* (2010) A nonhuman primate model of Alzheimer's disease generated by intracranial injection of amyloid-beta42 and thiorphan. *Metab. Brain Dis.* 25, 277–284
- 31 Duyckaerts, C. *et al.* (2008) Alzheimer disease models and human neuropathology: similarities and differences. *Acta Neuropathol. (Berl.)* 115, 5–38
- 32 Fine, A. *et al.* (1985) Cholinergic ventral forebrain grafts into the neocortex improve passive avoidance memory in a rat model of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* 82, 5227–5230
- 33 Ebert, U. and Kirch, W. (1998) Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur. J. Clin. Invest.* 28, 944–949
- 34 Castane, A. *et al.* (2010) Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats. *Behav. Brain Res.* 210, 74–83
- 35 Frautschy, S. *et al.* (2001) Phenolic anti-inflammatory antioxidant reversal of A [beta]-induced cognitive deficits and neuropathology. *Neurobiol. Aging* 22, 993–1005
- 36 Willner, P. (1986) Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 10, 677–690
- 37 Horrobin, D.F. (2003) Modern biomedical research: an internally self-consistent universe with little contact with medical reality? *Nat. Rev. Drug Discov.* 2, 151–154
- 38 Geerts, H. (2009) Of mice and men: bridging the translational disconnect in CNS drug discovery. *CNS Drugs* 23, 915–926
- 39 van der Worp, H.B. *et al.* (2010) Can animal models of disease reliably inform human studies? *PLoS Med.* 7, e1000245
- 40 Frank, R. and Hargreaves, R. (2003) Clinical biomarkers in drug discovery and development. *Nat. Rev. Drug Discov.* 2, 566–580
- 41 Dollery, C.T. (2010) The challenge of complexity. *Clin. Pharmacol. Ther.* 88, 13–15
- 42 Aderem, A. (2005) Systems biology: its practice and challenges. *Cell* 121, 511–513
- 43 Butcher, E.C. *et al.* (2004) Systems biology in drug discovery. *Nat. Biotechnol.* 22, 1253–1259
- 44 Boxenbaum, H. (1992) Pharmacokinetics: philosophy of modeling. *Drug Metab. Rev.* 24, 89–120
- 45 Massoud, T.F. *et al.* (1998) Principles and philosophy of modeling in biomedical research. *FASEB J.* 12, 275–285
- 46 Cedarbaum, J.M. and Wagg, J.K. (2011) Predicting initial changes in brain volume with effective anti-amyloid agents: just ask alice. *Alzheimer's Demen.* S46
- 47 Thompson, P.W. and Lockhart, A. (2009) Monitoring the amyloid beta-peptide in vivo-caveat emptor. *Drug Discov. Today* 14, 241–251
- 48 Simeoni M, *et al.* (2008) Modelling beta amyloid system: sensitivity analysis at steady state and in dynamic conditions. *Population Analysis Group Europe (PAGE) Meeting, Marseille, France* (Abstract 1396)
- 49 Cotman, C.W. and Head, E. (2008) The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches. *J. Alzheimers Dis.* 15, 685–707
- 50 Check, E. (2002) Nerve inflammation halts trial for Alzheimer's drug. *Nature* 415, 462
- 51 Fillenbaum, G.G. *et al.* (2005) Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. *Ann. Pharmacother.* 39, 2009–2014
- 52 Schenk, D. (2002) Amyloid- $\beta$  immunotherapy for Alzheimer's disease: the end of the beginning. *Nat. Rev. Neurosci.* 3, 824–828
- 53 Morgan, D. (2011) Immunotherapy for Alzheimers disease. *J. Intern. Med.* 269, 54–63
- 54 Hussain, I. *et al.* (2007) Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases  $\beta$ -cleavage of amyloid precursor protein and amyloid- $\beta$  production in vivo. *J. Neurochem.* 100, 802–809
- 55 Townsend, M. *et al.* (2010) Oral treatment with a  $\gamma$ -secretase inhibitor improves long-term potentiation in a mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* 333, 110–119
- 56 Kounnas, M.Z. *et al.* (2010) Modulation of [gamma]-secretase reduces [beta]-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *Neuron* 67, 769–780
- 57 Dong, H. *et al.* (2009) Effects of donepezil on amyloid-[beta] and synapse density in the Tg2576 mouse model of Alzheimer's disease. *Brain Res.* 1303, 169–178
- 58 Choi, J.K. *et al.* (2010) Anti-inflammatory treatment in AD mice protects against neuronal pathology. *Exp. Neurol.* 223, 377–384
- 59 Boimel, M. *et al.* (2009) Statins reduce the neurofibrillary tangle burden in a mouse model of tauopathy. *J. Neuropathol. Exp. Neurol.* 68, 314–325
- 60 Nishida, Y. *et al.* (2009) Depletion of vitamin E increases Amyloid  $\beta$  accumulation by decreasing its clearances from brain and blood in a mouse model of Alzheimer disease. *J. Biol. Chem.* 284, 33400–33408
- 61 Costa, R. *et al.* (2011) Testing the therapeutic potential of doxycycline in a *Drosophila melanogaster* model of Alzheimer's disease. *J. Biol. Chem.* 286, 41647–41655
- 62 Golde, T.E. *et al.* (2010) Targeting A [beta] and tau in Alzheimer's disease, an early interim report. *Exp. Neurol.* 223, 252–266
- 63 Exrance, A. (2010) Alzheimer's failure raises questions about disease-modifying strategies. *Nat. Rev. Drug Discov.* 9, 749–751
- 64 Sabbagh, M. and Cummings, J. (2011) Progressive cholinergic decline in Alzheimer's disease: consideration for treatment with donepezil 23 mg in patients with moderate to severe symptomatology. *BMC Neurol.* 11, 21
- 65 Imbimbo, B.P. (2009) An update on the efficacy of non-steroidal anti-inflammatory drugs in Alzheimer's disease. *Expert Opin. Invest. Drugs* 18, 1147–1168
- 66 Feldman, H. *et al.* (2010) Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease. *Neurology* 74, 956–964
- 67 Isaac, M. *et al.* (2008) Vitamin E for Alzheimer's disease and mild cognitive impairment. *Cochrane Database Syst Rev (Online)* CD002854
- 68 Loeb, M.B. *et al.* (2004) A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. *J. Am. Geriatr. Soc.* 52, 381–387
- 69 Ahmed, T. *et al.* (2010) Curcuminoids enhance memory in an amyloid-infused rat model of Alzheimer's disease. *Neuroscience* 169, 1296–1306
- 70 Hu, S. *et al.* (2009) GSK3 inhibitors show benefits in an Alzheimer's disease (AD) model of neurodegeneration but adverse effects in control animals. *Neurobiol. Dis.* 33, 193–206
- 71 Martinez, A. *et al.* (2011) Glycogen synthase kinase 3 inhibitors in the next horizon for Alzheimer's disease treatment. *Int. J. Alzheimers Dis.* 2011, 280502