Investigational phosphodiesterase inhibitors in phase I and phase II clinical trials for Alzheimer’s disease

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ABSTRACT

Introduction: Phosphodiesterase (PDE) inhibitors improve signaling pathways in brain circuits by increasing intracellular cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP). In the last decade, the first clinical studies investigating selective PDE inhibitors in Alzheimer’s disease (AD) have been initiated, based on their positive effects on cognitive processes and neuroprotection in numerous animal studies.

Areas covered: This article reviews the clinical studies investigating the pro-cognitive/neuroprotective effects of PDE inhibitors in patients with AD, as well as in age-associated memory impaired elderly and patients with mild cognitive impairment (MCI), the prodromal stage of AD. PDE inhibitors will also be discussed with respect to adverse effects including safety and tolerability.

Expert opinion: The limited available data of clinical studies with PDE inhibitors tested in different populations of AD patients do not allow the drawing of any concrete conclusion yet. Currently, studies with a PDE3 (cilostazol) or PDE9 inhibitor (BI 409,306) are still ongoing in patients with MCI or AD, respectively. Studies with PDE4 inhibitors (HT-0712, roflumilast and BPN14770) in healthy elderly and elderly with age-associated memory impairments indicate that the optimum dose and/or inhibiting the most relevant PDE isoform hold great promise when tested in the appropriate population of patients with MCI or AD eventually.

1. Introduction

In 1886, the activity of phosphodiesterases (PDEs) was actually first described as it was noted that caffeine had bronchodilator properties. In the early 1960s, this effect could be attributed to the cyclic nucleotide cAMP and that caffeine inhibited a cAMP-specific PDE [1]. Thus, caffeine was the first known PDE inhibitor. Since the early 1970s, PDEs were first identified in rat and bovine tissue and it was demonstrated that PDEs hydrolyze the phosphodiesteric bond of cAMP and cGMP [2]. Actually, caffeine turned out to be a nonselective PDE inhibitor as it also inhibits cGMP-specific PDEs including PDE type 5 (PDE5). cGMP causes vasodilatation in blood vessels by regulating their smooth muscles physiology [3]. Until now 21 classes of genes for PDEs in humans, rats and mice have been identified and characterized.

PDEs have been classified into 11 families (PDE1-PDE11) based on several criteria such as subcellular distributions, mechanisms of regulation, and enzymatic and kinetic properties [2,4]. Figure 1 provides an overview of the specificity of the PDE1 to PDE11 family for its substrate cAMP and/or cGMP. Most of these families have more than one class of gene products (e.g. PDE4A, PDE4B, PDE4C, PDE4D). In addition, each gene product may have multiple splice isoform variants (e.g. PDE4D1-PDE4D9). It has been estimated that more than 100 specific human PDEs exist [2].

Since the early 1970s, it is known that PDEs have key functions in the brain. Based on a wealth of animal research, PDE inhibitors have been suggested as a suitable tool for the treatment of a variety of cognitive deficits related to neurodegenerative disorders including Alzheimer’s disease (AD) (for a review see Ref. [6]).

The most prominent cause of dementia is AD (~70%). AD is characterized by a progressive disturbance in cognition including memory, attention, personality, intellect and speech. Its neuropathological hallmarks are amyloid plaques and neurofibrillary tangles in brain, in particular in the basal cholinergic forebrain (e.g. nucleus basalis of Meynert), as well as progressive degeneration of the cholinergic innervation of the hippocampus and cerebral cortex [7]. At present, there are two classes of drugs approved that are aimed to improve cognition in AD [8–10]. The first class comprises the acetylcholinesterase inhibitors, which elevate the endogenous levels of acetylcholine by inhibiting its degrading enzyme. Three acetylcholinesterase inhibitors are currently on the market: donepezil (Aricept®, Eisai), rivastigmine (Exelon®, Novartis), galantamine (e.g. Reminyl®, Johnson & Johnson). However, the efficacy of these drugs is very modest in mild AD and early treatment discontinuation is quite common due to adverse side effects which are gastro-intestinal or emetic in nature. The second class of drugs is NMDA antagonists. Currently, only one NMDA antagonist (mementine, e.g. Namenda®/Ebixa®; Merz, but...
The limited amount of clinical studies with rather high doses and non-isomorselective PDE inhibitors tested in AD patients do not allow to draw any concrete conclusion yet.

The future for an AD-specific PDE inhibitor lies in the development of isomorfselective inhibitors that are most effective for cognitive enhancement and neuroprotection with limited side-effect profiles.

It is of utmost importance to know the expression levels of the separate PDE splice variants in relevant brain areas of patients with MCI or AD, especially PDE splice variants with high or increased expression are interesting to inhibit.

A low dose of a PDE inhibitor might be beneficial for cognitive enhancement and neuroprotection, while not inducing any unwanted side-effects.

When testing a PDE inhibitor the appropriate population, i.e. age-associated cognitive impaired elderly, MCI patients or patients at different stages of AD, needs most careful consideration.

First results with PDE4 inhibitors suggest that indeed the optimum dose and/or inhibiting the appropriate PDE isoform hold great promise when tested in the right population of patients with MCI or AD eventually.

This box summarizes key points contained in the article.

now generic) is approved for AD treatment and it has been shown to have only modest effects on cognition in moderate-to-severe AD, and no effect in mild AD [8]. NMDA antagonists are aimed to have neuroprotective effects via blocking the NMDA channels and hence preventing excitotoxicity. Like the acetylcholinesterase inhibitors, the effects of memantine are rather small and not without side-effects.

The specific localization of the different PDEs in the brain and the body will determine which particular physiological function may be influenced by some PDE inhibitors, but not by others. Since PDE10A is highest expressed in the striatum [11] where it regulates signal transduction in the cortico-striatothalamatic circuit, it is an interesting target for schizophrenia and related disorders of basal ganglia function. This likewise accounts for PDE1, but this PDE type is also highly expressed in the hippocampus [11]. The latter is important as PDEs expressed in higher ‘cognitive’ brain structures including hippocampus and prefrontal cortex could be considered as a most useful target for treatment of cognitive deficits, in particular memory impairments, and AD. Based on mRNA expression levels of more than 20% compared to the PDE subtype with highest (100%) mRNA expression in the hippocampus and frontal cortex [11], a selection can be made of the following PDE subtypes: 1B, 1C, 2A, 4A, 4B and 8B (see also Ref. [12]). However, the therapeutic potential of other PDE subtypes cannot be excluded, as activity of the enzymes is not per se linked to the level of availability. However, this knowledge is almost completely lacking at the moment. It is assumed that an increase in PDE activity, which reduces cyclic nucleotide signaling in pathways important for memory function and brain plasticity (see Section 2), is causal, while a decrease in PDE activity might be considered as compensatory [13]. In general, PDE expression is assumed to decrease with aging, whether this is age-related decrease or a compensatory mechanism is not known [14].

2. Mechanism of action of phosphodiesterase inhibitors

The second messengers cAMP and/or cGMP, which are hydrolyzed by PDEs, are synthesized by adenylate and guanylate cyclase, respectively. However, the intracellular concentrations of both cyclic nucleotides are especially regulated by PDE activity as its hydrolysis capacity far exceeds the capacity for synthesis. Besides this absolute and temporal regulation of cyclic nucleotides, PDEs contribute to their compartmentalized signaling as different PDEs are localized at specific sites in the cell such as the plasma or nuclear membrane, or cytosol [4]. Thus, PDEs play a role in the intracellular signal transduction pathways in various biological systems as is illustrated in Figure 2.

Consequently, the cognition enhancing and neuroprotective actions of PDE inhibitors are related to their stimulation of intracellular signaling pathways leading to a cellular response [15]. This response in neurons can be transient of nature like for instance more neurotransmitter release itself or receptor trafficking (e.g. [16–18]), as well as tonic including structural changes like the formation of receptors, dendrites, synapses or even new neurons (e.g. [19]) (see Figure 2). The exact net response is linked to the duration of PDE inhibitor treatment, i.e. a single treatment is likely to have an acute cognition enhancing effect via for instance an increase in neurotransmitter release, while structural changes including formation of receptors, dendrites or synapses might already start as well. Chronic treatment with a PDE inhibitor will add up to this and thus have a more neuroprotective mode of action due to changes even up to the level of new neurons.

3. Expression of phosphodiesterases in the brain of patients with Alzheimer’s disease

From a therapeutic perspective, inhibition of PDEs with increased expression appears most promising. In this way, deficits in cognition and plasticity resulting from impaired cyclic nucleotide signaling might be improved by inhibiting specific PDE isoforms. However, an interesting observation was that cAMP-specific PDE4 inhibition actually might have negative effects on cognition and plasticity when PDEs are already downregulated and cAMP levels and PKA activity are high. In this scenario, elevated cAMP levels might go over a physiological level and disrupt signaling. Along this line, high doses of the classic PDE4 inhibitor rolipram impaired prefrontal cognitive function in aged, but not young monkeys, which is likely due to
overstimulation of the already disinhibited cAMP/PKA signaling pathway in the aged prefrontal cortex [20]. This further supports arguments to target specifically PDEs that are overexpressed.

To our knowledge, PDE expression in the brain of AD patients has only sporadically been investigated, i.e. case studies or only at one gene product level. In addition, different techniques have been used to measure gene expression including qPCR and the less accurate in situ hybridization. Table 1 gives an overview of the distribution of the different PDE subtypes in the brain of patients with AD.

When focusing on increased PDE expression, a case study using qPCR reported that PDE4D1 and PDE4D2 were increased in the hippocampus of an AD patient [22]. PDE4A-C have not been studied in AD patients to our knowledge. Another in situ study reported an increase in hippocampal PDE8B expression, in particular in patients with the neurofibrillary tangle-associated Braak stage III–IV [24]. In these studies it was further found that PDE4D4 [22] and PDE7B showed no change in AD [24].

Decreases in expression were found for PDE4D3 and PDE4D5-9 [22], PDE7A and probably PDE8A [24]. A recent study using qPCR found no change in overall PDE4D expression in the temporal cortex of AD patients [23]. This very likely reflects a net effect and further underlines the need to measure isoform specific splice variants.

PDE5 expression, which is already very low in the brain, was found to be extremely low or even absent in the hippocampus of aged subjects and AD patients [21]. However, when using qPCR instead of in situ hybridization, PDE5 expression was more reliably detectable and appeared even increased in the temporal cortex of AD patients [23].

PDE10 did not show any changes in expression in the temporal cortex of AD patients using qPCR [23]. PDE9 in the hippocampus and temporal cortex neither showed any change in gene expression in AD patients, irrespective of the technique used [21,23]. Finally, PDE2, which is the most abundantly PDE expressed in the brain, did not show any changes in expression in the hippocampus of AD patients as assessed with in situ hybridization [21].
4. Phosphodiesterase inhibitors in phase I and phase II clinical trials for Alzheimer’s disease

Highly selective, potent inhibitors for each PDE subtype have been synthetized which have good brain penetration properties. Yet, first selective inhibitors at the level of the gene product, are currently being developed as for instance for PDE4, i.e. PDE4B and PDE4D [25,26]. This already allows more specificity between functional effects, in this particular case to distinguish between affect (PDE4B) and cognition (PDE4D) [27]. However, to our knowledge none of available PDE inhibitors is selective at the level of PDE isoform variants (e.g. PDED1-PDE4D9), and they are therefore probably still not optimally efficacious and have side-effects.

In this review we focus on the current status of specific PDE inhibitors and their effects in clinical studies on cognitive enhancement, in particular related to AD (see Table 2 for a complete overview). The specific PDE inhibitors will also be discussed with respect to efficacy and adverse effects including safety and tolerability. Information reported was obtained from ClinicalTrials.gov, PubMed Central, published conference proceedings and press releases on Internet. In case no relevant clinical studies are available we will evaluate the clinical potential of a specific PDE type on basis of available other clinical or preclinical data. For an overview of approved patents of PDE inhibitors claiming a positive effect on cognition, in particular learning and memory, we refer to Blokland et al. [12].

4.1. Phosphodiesterase 1

PDE1 is a dual substrate enzyme hydrolyzing both cAMP and cGMP. First PDE1 inhibitors developed including IBMX [51] were not very specific for PDE1. For instance IBMX also inhibits PDE5 [15]. Only the drug vinpocetine has been tested for cognitive enhancement. Recently, more selective PDE1 inhibitors have been selected of which ITI-214 appears to be furthest in development. This novel PDE1 inhibitor is developed by Intra-Cellular Therapies [52]. ITI-214 improved memory processes of acquisition, consolidation, and retrieval across a broad dose range in rats [53]. In a series of Phase I single and multiple ascending dose studies oral dosing of ITI-214 was shown to be safe and well-tolerated (ClinicalTrials.gov Identifier: NCT01900522; healthy subjects and schizophrenia patients). Further clinical development of ITI-214 is currently being considered.

4.1.1. Vinpocetine

Vinpocetine (Cavinton®, Inteectol®; Richter Gedeon) is the classical PDE1 inhibitor and has been tested for its cognition enhancing properties for almost 30 years [54]. In clinical studies vinpocetine was tested up to Phase IV trials for the treatment of cognitive impairment. Positive effects on cognition of vinpocetine were observed in a study with healthy females [28]. Likewise, positive effects were claimed in a study testing a vinpocetine/Ginko biloba compound in healthy adults [29]. In a similar population, vinpocetine is being planned to be tested on cognition as an ingredient in SPRINT (Nootrobox) capsules after a single administration (ClinicalTrials.gov Identifier: NCT02857829).

In elderly, vinpocetine was tested as the nutritional supplement Cognitex® (Life Extension) on memory impairments and was reported to have a positive effect (ClinicalTrials.gov Identifier: NCT00719953) [30]). However, this was not a placebo-controlled study but an open label study and Cognitex® was a mixture of vinpocetine and other ingredients. Participants were tested at baseline and following 2 and 12 weeks of treatment, cognitive performance of all participants was evaluated. In addition, vinpocetine was ineffective in improving cognitive impairments in AD patients [32,33]. Together, this resulted in vinpocetine never being approved by the United States Food and Drug Administration (FDA) for the treatment of memory impairment. Nevertheless, vinpocetine is still widely used as a supplement for vasodilatation and as a nootropic claiming to improve memory in for instance Mild Cognitive Impairment (MCI) [31], which is the prodromal stage of AD, and elderly with chronic cerebral dysfunction [55]. Of note, possible positive effects of vinpocetine might actually be related to vasodilatation. However, the therapeutic relevance of such possible effect of vinpocetine is still questioned.

4.2. Phosphodiesterase 2

PDE2 is a dual substrate enzyme hydrolyzing both cAMP and cGMP. Much of the early preclinical work on PDE2 and cognition in aged rodents and mouse models of AD was done using the promising BAY 60–7550 compound (e.g. [56–58]), which however never made it to the clinic. Another early compound was ND-7001 (developed by Université de Strasbourg/Neuro3d), which only made it up to Phase I clinical trials to investigate safety, tolerability and pharmacokinetics (for the indication anxiety and depression). The compound was reported to be safe and well tolerated but this research was discontinued in 2010 [59]. Due to renewed interest in PDE2 as a target for cognition enhancement in recent years, several new PDE2 inhibitors have been developed by pharmaceutical companies including Boehringer Ingelheim, Pfizer, Johnson & Johnson, Merck, Altana Pharma and Takeda (for a review see [59]). However, to our knowledge no clinical trials on cognition are currently ongoing yet.

Maybe interesting to mention are PDE inhibitors in development for other indications than cognitive enhancement and AD per se. Examples in this respect are two PDE2 inhibitors which recently entered clinical evaluation for the treatment of central nervous system (CNS) disorders. The Pfizer compound PF-05180999 was tested in two Phase I trials for the treatment of migraine. However, one trial was terminated prematurely due to safety concerns and the other trial was withdrawn prior to participant enrollment (ClinicalTrials.gov Identifier: NCT01981486 and NCT01981499). Recently, Takeda started a Phase I trial to examine the degree and duration of brain PDE2 enzyme occupancy and target engagement as a function of their PDE2 inhibitor TAK-915 to guide dosing and schedule for future clinical studies in schizophrenia (ClinicalTrials.gov Identifier: NCT02584569).
| Agent (Sponsor) | Dosing, treatment (always oral) | Subjects | Status | Results | ClinicalTrials.gov identifier/reference |
|-----------------|---------------------------------|----------|--------|---------|----------------------------------------|
| **PDE1**        |                                 |          |        |         |                                        |
| Vinpocetine (University of Leeds, UK) | 10, 20, 40 mg daily for 3 days | Healthy females | Completed (Phase II) | Sternberg Memory Scanning Test + (only the 40 mg dose) | [28] |
| Vinpocetine as ingredient in SPRINT (Nootrobox) | 40 mg single administration | Healthy young adults | Not yet open for participant recruitment (Phase IV) | Not yet available | NCT02857829 |
| Vinpocetine/Ginko biloba compound (The Scripps Research Institute, USA) | 10 mg three times daily for 2 weeks | Healthy adults | Completed (Phase II) | Cognometer Test Battery +? | [29] |
| Vinpocetine as ingredient in Cognitex (Tel-Aviv Sourasky Medical Center/Enzymotec) | 20 mg daily for 12 weeks | Elderly with memory complaints | Completed (Phase IV) | Cognition +? | NCT00719953, [30] |
| Vinpocetine (VA Medical Center San Diego, USA) | Undisclosed dosing for 18 months | MCI patients | Completed (Phase II) | MMSE, ADAS-Cog + | [31] |
| **PDE3**        |                                 |          |        |         |                                        |
| Cilostazol (Juntendo University School of Medicine, Tokyo, Japan) | 100 mg daily for 1–13 months as add-on to 5 mg donepezil | Mild to moderate Alzheimer's disease patients | Completed (Phase IV) | MMSE + | [34] |
| Cilostazol (Tokyo Medical University, Japan) | 100 mg daily for 6 months | Patients with Alzheimer's disease and cerebrovascular disease | Completed (Phase IV) | ADAS-cog, Revised Wechsler Memory Scale + | [35] |
| Cilostazol (Seoul National University Hospital/Korea OIAA) | 200 mg daily for 24 weeks as add-on to 10 mg donepezil | Mild to moderate Alzheimer's disease patients with subcortical white matter hyperintensities | Completed (Phase IV) | MMSE, ADAS-cog = NCT01409564 |
| Cilostazol (Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan) | 50 mg twice daily for 12 months as add-on to AChEIs (types and doses undisclosed) | Patients with Alzheimer’s disease | Completed (Phase II) | MMSE, CDR-SB + | [36] |
| Cilostazol (Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan) | 50–100 mg daily for at least 3 months (retrospectively) | Elderly | Completed (Phase II) | Reduced risk incidence of dementia including Alzheimer's disease, mainly in cilostazol users with ischemic heart disease and cerebral vascular disease | [37] |
| Cilostazol (National Cerebral and Cardiovascular Center Hospital, Osaka, Japan) | Average 130 mg daily for at least 6 months (retrospectively) | Elderly, MCI patients and demented patients | Completed (Phase IV) | MMSE + (only in MCI patients) | [38] |
| Cilostazol (National Cerebral and Cardiovascular Center Hospital, Osaka, Japan) | Average 121 mg daily for at least 6 months with donepezil (dose undisclosed) (retrospectively) | Mild Alzheimer’s disease patients and moderate/severe Alzheimer’s patients on donepezil | Completed (Phase IV) | MMSE + (only in mild Alzheimer’s patients) | [39] |
| Cilostazol (National Cerebral and Cardiovascular Center Hospital, Osaka, Japan) | 50 mg twice daily for 96 days | MCI patients | Currently recruiting participants (Phase II) | Not yet available | NCT02491268 |
| **PDE4**        |                                 |          |        |         |                                        |
| MEM 1414 (Memory Pharmaceuticals/Roche) | Undisclosed | Healthy adults and healthy elderly | Completed (Phase I) | Safety + | [40] |
| MK-0992 (Merck) | Undisclosed | Patients with mild to moderate Alzheimer’s disease | Terminated (Phase II) | Undisclosed | NCT00362024 |
| HT-0712 (DART Neuroscience) | 45 mg daily for 28 days | Elderly with age-associated memory impairment | Completed (Phase II) | Verbal learning (delayed recall) + | [41] |
| HT-0712 (DART Neuroscience) | 50 mg daily for 6 weeks | Elderly with age-associated memory impairment | Completed (Phase II) | Undisclosed | NCT02013310 |

(Continued)
| Agent (Sponsor) | Dosing, treatment (always oral) | Subjects | Status | Results | ClinicalTrials.gov identifier/reference |
|----------------|--------------------------------|----------|--------|---------|-----------------------------------------|
| Roflumilast (Takeda) | Undisclosed doses, single administration with or without donepezil (10 mg) | Healthy adults with scopolamine-induced cognitive deficits | Completed (Phase I) | Verbal learning (immediate recall) + (only the combination treatment) | NCT02051335 |
| Roflumilast (Maastricht University, The Netherlands) | 0.1, 0.3, 1 mg single administration | Healthy young adults | Completed (Phase II) | Verbal learning (immediate recall) + (only the 0.1 mg dose) Sensory gating + (only the 0.1 mg dose) | NCT01433666, [42] |
| Roflumilast (Maastricht University, The Netherlands /Takeda) | 0.1, 0.25, 1 mg single administration | Healthy elderly and elderly with age-associated memory impairment | Completed (Phase II) | Undisclosed ISRCTN registry ID ISRCTN96013814; EudraCT Number 2013–001223-39 | |
| BPN14770 (Tetra Discovery Partners) | 10, 20 mg and one undisclosed high dose twice daily for 2 weeks | Healthy adults and Healthy elderly | Completed (Phase I) | Safety + | NCT02648672, NCT02840279, [43] |
| BPN14770 (Tetra Discovery Partners) | 10, 20 mg and one undisclosed high dose twice daily for 2 weeks | Healthy elderly | Complete (Phase I) | Working memory + (10 and 20 mg dose) | NCT02840279, [43] |
| Denbufylline (Tel Aviv University, Israel) | 25, 50, 100 mg twice daily for 16 weeks | Patients with vascular, mixed or Alzheimer’s disease Patients with multi-infarct dementia and Alzheimer’s disease | Completed (Phase II) | MMSE + (only when combining all dosages and treatment groups) | [44] |
| Denbufylline (University of Vienna, Austria) | 100 mg twice daily for 12 weeks | Healthy males | Completed (Phase IV) | Cognition = | [45] |
| Sildenafil (University of Cologne, Germany) | 100 mg single administration | Healthy males | Completed (Phase IV) | Cognition = | [46] |
| Sildenafil (Hannover Medical School, Germany) | 100 mg single administration | Healthy males | Completed (Phase IV) | Cognition = | [47] |
| Vardenafil (Maastricht University, The Netherlands) | 10, 20 mg single administration | Healthy young adults | Completed (Phase II) | Sensory gating = | [48] |
| Vardenafil (Maastricht University, The Netherlands) | 10, 20 mg single administration | Healthy young adults | Completed (Phase II) | Cognition = | [49] |
| Tadalafil (St George’s, University of London, UK) | 20 mg single administration | Elderly with small vessel disease not diagnosed with dementia Recruiting participants (Phase II) | Not yet available | | NCT02450253 |
| PF-0447943 (Pfizer) | 25 mg daily for 12 weeks | Patients with mild to moderate Alzheimer’s disease | Completed (Phase II) | Cognition = | [50] |
| BI 409,306 (Boehringer Ingelheim) | Undisclosed doses for 12 weeks | Alzheimer’s disease patients | Completed (Phase II) | Not yet available | NCT02337907 |

In case of data reported: +: positive effects; =: no effect reported; +?: questionable positive effect due to drug constraints (PDE5); MMSE: mini-mental state examination; ADAS-cog: Alzheimer’s disease assessment scale (cognitive part); CGI: Clinical Global Impression; CDR-SB: clinical dementia rating sum of boxes; AChEIs: Acetylcholinesterase inhibitors.
4.3. Phosphodiesterase 3

4.3.1. Cilostazol

PDE3 is a dual substrate enzyme hydrolyzing both cAMP and cGMP. The PDE3 inhibitor cilostazol (Pletal®; Otsuka Pharmaceutical) is approved by the FDA for the treatment of intermittent claudication and has additionally been investigated as a prevention for stroke recurrence in two Phase IV studies (ClinicalTrials.gov Identifier: NCT00216749, NCT00234065; no results disclosed). Cilostazol has been tested preclinically in mice which received central injections with Aβ. Repeated administration of cilostazol attenuated the accumulation of Aβ and tau phosphorylation [60] and improved cognitive performance in several behavioral paradigms [61].

Several clinical studies with cilostazol have been conducted regarding AD. In a first pilot trial, 10 mild to moderate AD patients received 100 mg cilostazol per day as add-on to donepezil (5 mg per day) for a variable period ranging between 1 and 13 months [34]. This study was an open-label, uncontrolled trial. In this small group, an improvement on the Mini Mental State Examination (MMSE) was reported during the first 6 months of follow up, suggesting that cilostazol may reduce the cognitive decline in these AD patients already on donepezil.

In a second study, the effects of 100 mg cilostazol daily for 6 months on cognition and regional cerebral blood flow (rCBF) in elderly patients with AD and cerebrovascular disease were investigated [35]. The cilostazol group did not show any changes in cognitive function as reflected in their test scores, whereas the control group showed a cognitive decline on the AD Assessment Scale-Cognitive Subscale (Japanese version), Revised Wechsler Memory Scale (logical memory-I) and Trail Making Test-A. Analysis of treatment effect revealed that the cilostazol group showed increased rCBF in the right anterior cingulate lobe compared with baseline, whereas the control group showed decreased rCBF in the left middle temporal gyrus compared with baseline. The latter may, at least in part, be responsible for the positive effects of cilostazol. Remarkably, an increase in cerebral blood flow was not found after acute treatment in healthy subjects [62], which suggests that long-term treatment is necessary to exert effects on cerebral blood flow.

A third and similar study was initiated in 2011 by the Seoul National University Hospital (Korea) (ClinicalTrials.gov identifier: NCT01409564). In total, 36 mild to moderate AD patients with subcortical white matter hyperintensities treated with donepezil (10 mg per day) were included. Subjects were equally divided over a cilostazol (100 mg twice a day) group and placebo group and treated for a period of 24 weeks. However, no difference between groups was reported on ClinicalTrials.gov for cognitive measures which included the MMSE and the cognitive scale of the cognitive part of the AD Assessment Scale.

In a very recent fourth case-control study, 60 patients with stable AD and treated with acetylcholinesterase inhibitors (types and doses not disclosed) were equally divided into a placebo group and a group receiving cilostazol (50 mg twice per day) as an add-on therapy for at least 12 months [36]. Cilostazol was found to improve the MMSE scores and clinical dementia rating sum of boxes scores in the AD patients already being treated with acetylcholinesterase inhibitors.

Two retrospective studies with patients already on cilostazol (e.g. for the prevention of stroke or peripheral artery disease) reported that average daily doses above 100 mg for at least 6 months improved MMSE scores in MCI patients [38] and mild AD patients as add-on to 10 mg donepezil per day [39]. The same research group at the National Cerebral and Cardiovascular Center Hospital (Osaka, Japan) has started a new study investigating the effects on MMSE scores of cilostazol 50 mg twice a day for 96 weeks in patients with MCI (ClinicalTrials.gov identifier: NCT02491268). Estimated primary completion date is 2018. Interestingly, a recent retrospective study reported that patients already using cilostazol 50–100 mg daily for at least 3 months had a decreased risk of incident dementia compared with patients not using the drug [37]. Of note, this effect was mainly in cilostazol users with ischemic heart disease and cerebral vascular disease.

Interestingly, cilostazol has also been tested in an open-label pilot study for cognition enhancing effects in schizophrenic patients [63]. One memory task and six cognitive tasks assessing prefrontal functioning were performed. Results on the Trial Making Test showed a significant decrease after eight weeks of cilostazol treatment as compared to baseline. This suggests improved visu-motor search skills, simple attention and processing speed. However, it cannot be ruled out that the resulting improvement is an interaction effect with the drugs the patients were already receiving.

Taken together, due to the FDA approval of cilostazol, PDE3 has traditionally been one of the earliest PDE subtype investigated in clinical trials in the field of AD. Five studies have been conducted in different populations of AD patients and resulted in general in a positive indication for cognition enhancement. Also in MCI patients it appears effective as reported in one retrospective study, which is currently being followed-up in a new treatment study. Linked to this, another retrospective study reported that cilostazol appears to reduce the risk incidence of dementia including AD. Of note, possible side-effects of the PDE3 inhibitor cilostazol include most commonly headache, diarrhea, abnormal stools, and since it is a quinolinone derivative also irregular heart rate and palpitations [64]. Therefore, it is dangerous for people with severe heart failure and can only be given to people without this indication. It is also contraindicated in patients with severe hepatic or renal impairment.

4.4. Phosphodiesterase 4

PDE4 is cAMP specific. PDE4 was initially considered as a target for the treatment of depressive disorders [65]. In this respect, the classic PDE4 inhibitor rolipram has been widely investigated. First clinical studies showed good antidepressant response to rolipram treatment [66,67]. However, rolipram produces severe dose-limiting side-effects including headache, gastric hyper secretion and emesis (nausea and even vomiting). The emetic effects are likely due to inhibition of PDE4D in the area postrema and nucleus of the solitary tract [68]. Especially these emetic effects have put a serious hold on the further development of rolipram and other related inhibitors of the PDE4 subtype. It even prevented rolipram from
reaching the market. Nevertheless, a clinical Phase II trial had begun in 2006 by the US National Institutes of Health to reevaluate the antidepressant properties of rolipram ([ClinicalTrials.gov identifier: NCT00369798]). This study has been completed in 2016, yet no data has been disclosed.

At the moment, PDE4 inhibitors are being developed which are supposed to have less emetic side-effects, and are being studied for other disorders besides that of depression, like AD. One approach is the development of negative allosteric inhibitors of PDE4D, which do not fully inhibit the enzyme [69]. Another approach was to develop a full PDE4 inhibitor (Maastricht University/Columbia University/University of Genoa) with probably less affinity for the PDE4D isoforms involved in emesis [70]. This first compound Gebr-7b was effective in improving cognition in a mouse APP/PS1 model of AD, as was also found for the follow-up compound Gebr-32a in Tg2576 mice [71]. Gebr-7b had no effect on hippocampal Aβ load [72]. The latter was also reported in previous studies with the classic PDE4 inhibitor rolipram, yet it rescued decreased CREB activation [73,74]. Thus, activation of the pCREB pathway is likely to make to the synapses and neurons more resistant to the damaging effects of Aβ, i.e. protection by PDE4 works independently of Aβ. Linked to this it is important to mention that it has very recently been shown that rolipram promoted clearance of aggregated tau in the frontal cortex and hippocampus, and improved cognition in a mouse rTg4510 model of tauopathy [75]. This might possibly be mediated via increased ATP-dependent proteasome phosphorylation/activation and a linked drop in ubiquitin conjugate levels. These are processes described to be dysfunctional in AD. Thus, it would be interesting to also investigate whether PDE4 inhibition could prevent neurodegeneration in AD by reducing levels of aggregated tau. Till now PDE4 inhibitors have been tested in the field of AD for memory enhancement up to clinical Phase II studies.

### 4.4.1. MEM 1414

In February 2005, Roche completed a Phase I clinical trial program for their PDE4 inhibitor MEM 1414. The Phase I program consisted of four trials: a double blind, placebo-controlled study to evaluate the safety, tolerability and pharmacokinetics of single ascending doses of MEM 1414; a bioequivalence study; a scopolamine challenge study; a multiple ascending dose study for 14 days. At all doses tested in this Phase I program, MEM 1414 was generally safe and well tolerated as reported in an Annual Report of Memory Pharmaceuticals [40], though it has emetic potential [76]. However, in April 2005, Roche stopped further clinical development of MEM 1414 and its back-up candidate, MEM 1917, and Memory Pharmaceuticals regained rights to these agents, intending to move them into Phase II trials. However, Roche acquired the biotech company in a 2008 merger, and it not very likely that the development of MEM 1414, or any of the other PDE4 inhibitors are currently being pursued.

### 4.4.2. MK-0952

MK-0952 (Merck) [77], was investigated in a Phase II study in patients with mild to moderate AD and completed in 2008, but no results were ever disclosed ([ClinicalTrials.gov Identifier: NCT00362024]). Whether MK-0952 is emetic is not known.

### 4.4.3. HT-0712

HT-0712 (Dart Neuroscience) [78] given for 28 days at a daily dose of 45 mg was reported in a press release in 2008 to improve in a phase II study the long-term (word list) memory of subjects with an age-associated memory impairment [41]. Recently, a follow-up phase II study with the same class of subjects being treated daily with 50 mg HT-0712 for 6 weeks has been completed in 2015 ([ClinicalTrials.gov Identifier: NCT02013310]). However, results have not been disclosed. Whether HT-0712 has emetic effects is not yet known.

### 4.4.4. Roflumilast

Roflumilast (Daxas®, Daliresp®; AstraZeneca, initially Nycomed/Takeda) had been approved in 2011 as an anti-inflammatory drug to reduce the risk of chronic obstructive pulmonary disease (COPD) exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations [79]. Compared to rolipram it has reduced emetic effects, i.e. it causes mild to moderate nausea in approximately 5% of the COPD patients at the dose of 0.5 mg, which helped being accepted for treatment [80]. Besides emesis, the most commonly reported side-effects of roflumilast include diarrhea, weight loss, abdominal pain and headache. Three suicides have been observed in a COPD patient pool versus none in the placebo pool [80], although none of the suicides was identified as being related to the study medication. There are no indications for cardiovascular effects associated with roflumilast use.

The availability of roflumilast and its reduced emetic effect paved the way for testing it for other indications, including cognition enhancement. Takeda tested in a Phase I study whether a scopolamine-induced cognitive impairment is attenuated by a single administration of roflumilast (dose unknown) with or without donepezil (10 mg) in healthy adults ([ClinicalTrials.gov Identifier: NCT02051335]). Study results have been disclosed on ClinicalTrials.gov and show that separate treatments appeared not effective, while the combination of roflumilast and donepezil was able to attenuate an impaired verbal learning performance. Recently, a Phase II study was finished by Maastricht University (The Netherlands) investigating whether roflumilast improves cognition per se in healthy adults ([ClinicalTrials.gov Identifier: NCT01433666]). At the conference proceedings of the Society for Neuroscience it was reported that a single dose of 0.1 mg improved immediate recall performance on the 30-word Verbal Learning Task (VLT) and was accompanied by an enhanced P600 peak during the presentation of the words in the third immediate recall trial [42]. Additionally, the same 0.1 mg dose of roflumilast improved sensory gating of the P50 peak at the Fz electrode in that study. Doses of 0.3 and 1 mg were ineffective. Interestingly, the effective low dose is 5 times lower than the approved dose for COPD treatment and, therefore, it is devoid of the side-effects typical for PDE4 inhibitors (in particular emesis). Another recent study ([ISRCTN registry ID ISRCTN96013814; EudraCT Number 2013–001223-39] of Maastricht University in collaboration with Takeda investigated the effects of roflumilast (single administration 0.1, 0.25, and 1 mg) on cognition in elderly...
subjects and subjects with age-associated memory impairment (all aged 60–80 years old). Results have not been disclosed yet.

Interestingly, roflumilast has also been tested by Takeda in a Phase I study to determine whether cognitive impairment associated with schizophrenia is attenuated by add-on roflumilast administration for 8 days (0.1 or 0.25 mg, once daily) to second generation antipsychotics (ClinicalTrials.gov Identifier: NCT02079844). Results disclosed on ClinicalTrials.gov showed that a dose of 0.25 mg resulted in a clinical meaningful effect size of 0.55 in the Hopkins Verbal Learning test. However, this observation was not supported by any significant differences with the placebo treatment. No serious adverse effects were observed.

4.4.5. **BPN14770**

Currently, for PDE4 the gene product PDE4D appears to be the most promising target for cognition improvement [14,81]. However, PDE4D is also the target with the most prominent emetic effect [68]. One approach to have less emetic effects is the development of negative allosteric inhibitors of PDE4D, which do not fully inhibit the enzyme (Imax ~80–90%) [69]. BPN14770 (Tetra Discovery Partners) [69] is a negative allosteric inhibitor of PDE4D and has been evaluated in two Phase I (single and multiple) ascending dose studies in healthy young and elderly subjects (ClinicalTrials.gov Identifier: NCT02648672 and NCT02840279). In addition, cognitive benefit was explored in the elderly subjects (age 60 and older) enrolled in the multiple ascending dose study. In a press release it has been reported that BPN14770 was safe and well-tolerated in both studies. Headaches were the most frequently reported adverse event in the high dose group of the elderly. In addition, a post hoc analysis of the elderly subjects demonstrated significant improvements with an effect size >0.70 in two measures of working memory at the low and mid doses of 10 and 20 mg, respectively, given twice on a day over a period of two weeks [43].

4.4.6. **Denbufylline**

Denbufylline, a xanthine derivate with PDE4 inhibitory activity, has been given for 16 days to patients with different types of dementia including AD [44]. Patients were assigned to one of four treatment groups (placebo, 25, 50 or 100 mg twice a day for 16 weeks). Every four weeks patients were tested on a cognitive battery consisting of the MMSE, digit symbol substitution subtests of the Wechsler Adult Intelligence Scale (WAIS), and the vocabulary subtest of the WAIS. MMSE scores were found to be higher among patients who received denbufylline when the different types of dementia were pooled into a single group, regardless of the diagnosis or dosage regimen. Additionally, mildly to moderately demented patients including patients with AD, were assigned to a 12 week treatment period of either denbufylline (100 mg twice a day) or placebo [45]. Patients were assessed on the Clinical Global Impression, the MMSE, the Sandoz Clinical Assessment Geriatrics (SCAG) and the Digit-Symbol Substitution Test. Secondary target variables were the Trail-Making Test and the Digit Span Test. In addition, electrophysiological correlates were included. In both groups, patients showed treatment-induced improvements on all tasks, with significantly stronger increases in the denbufylline group as compared to the placebo group. Clinical Global Impression (CGI) was reduced with one point in the denbufylline group, based on which the authors concluded that the denbufylline-induced changes were clinically relevant.

4.5. **Phosphodiesterase 5**

PDE5 is specific for cGMP hydrolysis. In humans, PDE5 inhibition causes relaxation of smooth muscles in blood vessels, hence its importance for the treatment of erectile dysfunction (ED) [3]. Three PDE5 inhibitors are approved by the FDA for treatment of ED: sildenafil (Viagra®, Pfizer), vardenafil (Levitra®, BAYER/GSK) and tadalafil (Cialis®, Eli Lilly/ICOS). Because of its vasodilatory properties, sildenafil and tadalafil are also FDA approved under the names of Revatio® and Adcirca®, respectively, for the treatment of hypertension of the pulmonary artery. For the same application, vardenafil has been evaluated in a phase III trial (ClinicalTrials.gov Identifier: NCT00718952; No results have been disclosed). Recently, sildenafil has been evaluated in a Phase I study for its neuroprotective properties in the treatment for stroke (ClinicalTrials.gov Identifier: NCT00452582). However, in 2011 this study was terminated because of a failure to recruit in the expected time period.

Sildenafil clearly improves cognition and reduces hippocampal Aβ load in different AD mouse models [82,83], but see [84] in which sildenafil was reported to restore cognitive function without affecting β-amyloid burden. Tadalafil is considered to have a poor brain penetration and therefore less efficacious for cognition enhancement as compared to sildenafil or vardenafil [82]. Yet it has been shown to cross the blood brain barrier after chronic treatment is a J20 mouse model of AD [85]. Tadalafil also improved cognition, yet it was not able to reduce A load in the hippocampus, though it interestingly reduced tau phosphorylation. A possible explanation for these positive effects could be that the blood brain barrier was altered in these AD mice thus facilitating access of tadalafil to the CNS.

Sildenafil, vardenafil and tadalafil (actually PDE5 inhibitors in general) have side effects such as headaches, facial flushing, nasal congestion and dyspepsia (indigestion). However, these effects are transient. All three PDE inhibitors can act on PDE6, which is present in the retina, and high doses have been reported to cause adverse visual events, including nonarteritic anterior ischemic optic neuropathy, and thus can cause vision problems (e.g. blurred vision). Moreover, tadalafil also potently inhibits PDE11. Because of the possible cardiovascular effects including increasing heart rate and decreasing peripheral blood pressure [86], these compounds are not suited for patients with cardiovascular indications or hypotensives. Also, one might argue that possible cerebrovascular changes could influence cognitive function after PDE5 inhibitor treatment. But a regular dose of sildenafil has no effect in humans on blood flow in the middle cerebral artery, just as there are no changes in radial and temporal artery diameters [86,87]. This suggests that possible effects on cognition after a PDE5 inhibitor administration are
not likely to be related to cerebrovascular mechanisms. Early FDA approval of PDE5 inhibitors for ED and the positive preclinical findings paved the way for clinical studies in the field of cognition and AD, even though PDE5 is expressed at low levels in the human hippocampus and frontal cortex [11].

4.5.1. Sildenafil
100 mg sildenafil was tested on a range of cognitive functions in healthy subjects [46]. Sildenafil given before testing enhanced performance in a simple reaction time test, but showed no effects on short-term memory, divided attention and other psychomotor tasks. Another study confirmed the lack of effect of sildenafil on memory performance in healthy subjects [47]. The same dose of sildenafil induced no direct cognition enhancing effect on auditory attention and word recognition. Yet, sildenafil did change certain components of event-related potentials (ERPs), indirectly indicating improved focused attention. Improved attention could also explain the effects on the simple reaction time task in the previous study. Also, a reduced negativity in the electroencephalogram (EEG) was found in the word recognition experiment after sildenafil treatment. The latter may indirectly indicate an effect on information processing [47].

Interestingly, sildenafil has also been tested in relation to cognitive symptoms in schizophrenia [88]. Sildenafil, as add-on to antipsychotic treatment, did not affect cognitive performance in schizophrenia patients.

4.5.2. Vardenafil
In two recent studies the potent PDE5 inhibitor vardenafil was tested for its cognition enhancing effects in healthy subjects on information processing (sensory gating), reaction time responding, executive function and memory performance (e.g. verbal learning test) [48,49]. Memory and executive functioning were tested while EEG activity was recorded. None of the ED recommended doses of 10 and 20 mg vardenafil given before testing induced any prominent effect on information processing, reaction time responding, cognition or EEG measures.

4.5.3. Udenafil
A study investigating the effects of repeated dosing of the PDE5 inhibitor udenafil (Zydena®; Dong-A Pharmaceutical) in patients suffering from ED, demonstrated that a daily dose of 50 mg udenafil for 2 months improved performance on the Korean version of the MMSE, and on an assessment battery measuring frontal executive function [89].

4.5.4. Tadalafil
Despite a supposedly bad brain penetration [82], tadalafil crossed the blood brain barrier and improved cognition in a mouse model of AD [85]. This might be related to a possible alteration in the blood brain barrier of the AD mice. Along similar lines, tadalafil could be effective in AD patients when its brain penetration is sufficient. Interestingly, a recent clinical trial has been initiated at St George’s (University of London) to test tadalafil in patients with symptomatic small vessel disease in the brain but who have not been diagnosed with vascular cognitive impairment. The rationale is that the chronic lack of blood flow to deep brain areas common in AD and other dementias is leading to vascular cognitive impairment [90]. The vasodilatory properties of tadalafil should improve blood flow to these brain areas as measured by MRI-Arterial Spin Labeling and thus prevent vascular cognitive impairment. A single oral dose of 20 mg will be given which is higher than the recommended dose of 10 mg to treat erectile dysfunction. Cognitive performance will be measured as well. This study is currently recruiting subjects (ClinicalTrials.gov Identifier: NCT02450253).

4.6. Phosphodiesterase 6
In the nervous system the cGMP specific PDE6 is exclusively expressed as a photoreceptor PDE in the pineal gland. PDE6 is not under clinical investigation for any CNS indication.

4.7. Phosphodiesterase 7
Like PDE4 and PDE8, PDE7 is highly specific for cAMP. Preclinical research into PDE7 inhibitors and AD is currently starting to emerge [91]. Consequently, PDE7 inhibitors are not under clinical investigation for CNS indications. Chronic treatment with the PDE7 inhibitor S14 (Instituto de Química Médica (CSIC), Madrid, Spain) significantly decreased memory impairments in an APP/PS1 mouse model of AD [24]. S14 also lowered frontal cortex and hippocampal Aβ load likely via an enhanced phagocytosis of Aβ by astrocytes. Also a decrease in frontal cortex and hippocampal tau hyperphosphorylation was observed. This makes PDE7 an interesting target for future studies.

4.8. Phosphodiesterase 8
PDE8 is cAMP-specific. Like PDE7 inhibitors, the first PDE8 inhibitors have only recently been disclosed [92]. The gene product PDE8B appears an interesting target based on its increased expression in the hippocampus of patients with AD [24]. A preclinical study with PDE8B knock out (KO) mice demonstrated an enhancement in cognitive performance in a wide array of behavioral tests [93]. These findings indicate that selective antagonism of PDE8B may indeed be an attractive target for improvement of cognitive functions. Clearly more preclinical studies with selective PDE8 inhibitors are needed. PDE8 inhibitors are not under clinical investigation yet.

4.9. Phosphodiesterase 9
PDE9 has the highest affinity for cGMP next to cAMP, and its inhibitors have shown promising cognition enhancing results in preclinical studies with healthy and pharmacologically impaired rodents [94–96]. Two PDE9 inhibitors are approved for clinical testing.

4.9.1. PF-04447943
In 2009, Pfizer entered a Phase II study with PF-04447943 to evaluate its effects on cognitive symptoms in AD. It was found that 25 mg dosing twice a day during 12 weeks had no effects
on cognition in patients with mild to moderate AD [50]. Even though this was the only study conducted with a large sample size (n = 191), the relatively short duration (12 weeks) and especially inclusion of only one dose of the tested drug, renders the negative results still of limited interpretability.

4.9.2. BI 409,306
Recently, Boehringer Ingelheim has finished several Phase I studies to investigate the safety, tolerability and relative bioavailability of their PDE9 inhibitor BI 409,306 (e.g. [97]). Efficacy studies are currently being conducted in Phase II clinical trials for both AD and schizophrenia (ClinicalTrials.gov Identifier: NCT02337907 and NCT02281773).

4.10. Phosphodiesterase 10
PDE10 is a dual-substrate enzyme hydrolyzing both cAMP and cGMP. In the CNS, PDE10 is almost exclusively expressed in the striatum [11]. As a result, initially the rather non-selective PDE10 inhibitor papaverine and later-on selective inhibitors as MP-10, PQ-10, TP-10 or TAK-063 have been extensively investigated preclinically as antipsychotics drugs to treat positive symptoms [98,99]. Already several clinical trials up to Phase II have been conducted by Pfizer (PF-02545920/MP-10), Takeda (TAK-063), Hoffmann-La Roche (RO5545965) and Amgen (AMG 579) testing the efficacy of PDE10 inhibitors in patients with schizophrenia (ClinicalTrials.gov Identifier: NCT00570063, NCT02477020, NCT02019329 and NCT01568203). The mechanism of action of PDE10 inhibition was attributed to be modulation/normalization of dopaminergic fronto-striatal function [100]. However, no positive effects have been reported thus far and currently pharmaceutical companies are re-evaluating their compounds for PD or HD (ClinicalTrials.gov Identifier: NCT02197130, NCT02074410 and NCT02061722).

Interestingly, preclinical studies with healthy rodent as well as rodent models for fronto-striatal disorders including have investigated and confirmed the memory enhancing potential of PDE10 inhibitors (e.g. [101–103]). Until now no PDE10 inhibitors have been tested in clinical trials of aging and AD.

4.11. Phosphodiesterase 11
PDE11 is a dual-substrate enzyme hydrolyzing both cAMP and cGMP. PDE11 is the most recently identified member of the PDE superfamily and therefore understanding its exact function in the brain has just started. Interestingly, subsequent preclinical studies have confirmed that although PDE11 KO mice exhibit normal short-term memory for social odor recognition and social transmission of food preference, they show impaired long-term memory. At the same time, PDE11A KO mice showed normal long-term memory for nonsocial odor recognition [104,105]. Therefore, PDE11 seems to fulfill a special role in the consolidation of social memories. Currently, the first PDE11 inhibitors have been developed [106], yet no clinical studies have been conducted yet.

5. Conclusion
The effects on cognition after chronic treatment with selective inhibitors for PDE3, 4 and 9 have been investigated in different populations of AD patients. The PDE3 inhibitor cilostazol was tested in several studies and indications were found that it could improve cognition, in particular in MCI/mild AD patients (e.g. [38,39]). The PDE4 inhibitor MK-0952 was given to patients with mild to moderate AD [77]. However, the results were never disclosed. The PDE9 inhibitor PF-04447943 was tested in patients with mild to moderate AD, yet 25 mg dosing twice daily during 12 weeks had no effects on cognition in these patients [50]. It was suggested that the treatment duration may not have been long enough and/or a less detrimental population as for instance age-associated cognitive impaired subjects could be a better choice for treatment [95]. However, it could also be speculated that the single dose tested was not the appropriate one, i.e. too low or too high. Of note, PDE inhibitors have narrow dose-response ranges in preclinical animal research. They mostly have only one to two optimum doses when using a log scale [107,108]. This makes it difficult to titrate the effective dose for human studies. To optimize the chance of success for finding the optimum dose of a PDE inhibitor, testing at least three doses in a human study is recommendable.

PDE4 inhibitors have also been tested on cognition in healthy subjects. A single administration of a low dose of the PDE4 inhibitor rolflumilast improved cognition in healthy adults [42]. The effective dose was substantially lower than the approved dose for COPD treatment, which had no effect on cognition. Thus, there is a therapeutic window between positive cognitive effects at low dosing and possible emetic effects at high dosing. Although rolflumilast is considered as a non-selective inhibitor for all PDE4 isoforms, in particular the PDE4B and PDE4D isoforms in the hippocampus [109], it might be suggested that it has differences in intrinsic properties still. This could also be concluded from our previous preclinical work comparing pro-cognitive and emetic-like effects in mice between rolflumilast and the similarly non-selective PDE4 inhibitor rolipram [107]. Rolipram induced emetic-like effects at pro-cognitive doses, while rolflumilast had a significant difference between the high doses inducing emesis-like behavior and low doses improving cognition.

Recently, a single administration of rolflumilast has been tested in healthy elderly (ISRCTN registry ID ISRCTN96013814; EudraCT Number 2013-001223-39). It would be interesting to see if the same therapeutic window can be observed as in healthy adults, yet no data has been disclosed yet. The PDE4 inhibitor BPN14770 has been tested in healthy elderly using a repeated treatment. Also this compound showed a positive effect on cognition [43]. Of note, though BPN14770 is a selective inhibitor for the gene product PDE4D, it does not completely inhibit the enzyme. Actually, it was especially designed for this purpose with the aim to reduce emetic side effects [69]. Indeed, the compound was reported to be safe and well tolerated.

Besides having being evaluated in healthy elderly, a single administration of rolflumilast has been tested in parallel in elderly with age-associated memory impairments (EudraCT Number: 2013-001223-39). Also the data of this study has
not been disclosed yet. The PDE4 inhibitor HT-0712 has been tested using a repeated administration in two studies with age-associated memory impaired elderly. The first study with HT-0712 reported a positive effect on cognition [41]. The results of the second study with HT-0712 have not been disclosed yet (ClinicalTrials.gov Identifier: NCT02013310).

Acute administration of a PDE5 inhibitor had no effects on cognition in healthy subjects [46,47,49]. Yet despite these negative findings we still cannot rule out inhibition of PDE5 as a treatment for cognitive impairment and/or AD. There is a wealth of preclinical data showing cognitive enhancement, neuroprotective effects and even decreased Aβ load in the hippocampus of a mouse model of AD after treatment with a PDE5 inhibitor (e.g. [82]). Although PDE5 expression is low in the human brain, an increase in the brain of AD patients has been observed [23]. Moreover, chronic treatment with the PDE5 inhibitor udenafil improved cognitive performance in patients suffering from ED [89]. This suggests that it would be better to proceed to (sub)chronic PDE5 inhibitor treatment using a patient population including age-related memory impaired subjects or MCI patients. Alternatively, a deficit model with healthy subjects to assess cognition enhancing effects of PDE5 inhibitors might be suited as well. A final point to consider is the dosing. The doses of PDE5 inhibitors tested thus far are all used for the treatment of ED. Based on the PDE4 studies as mentioned above it is important to realize that actually a low dose might already be beneficial for cognition. Yet this does probably not apply to a recent study investigating the effects of tadalafil on blood flow which might prevent vascular cognitive impairment in the brain of patients with symptomatic small vessel disease [90]. Here the aim is to actually make use of the high dose-related vasodilatory properties of tadalafil.

To summarize, the results – if reported at all – of the first clinical studies in AD patients with a PDE3, 4 or 9 inhibitor do not allow drawing any concrete conclusion yet. This might be due to inappropriate dosing and/or having chosen the inappropriate patient population. Optimum dosing is extremely important as it will help to reduce unwanted side effects (e.g. emesis, cerebro- and/or cardiovascular effects). Currently, studies with a PDE3 (cilostazol) or PDE9 inhibitor (BI 409,306) are still ongoing in patients with MCI or AD, respectively. PDE4 inhibitors (HT-0712, roflumilast and BPN14770) are also being tested in healthy elderly and elderly with age-associated memory impairments. First results have been presented and appear promising and suggest that indeed the optimum dose and/or inhibiting the appropriate PDE isoform hold great promise when tested in the right population of patients with MCI or AD eventually.

6. Expert opinion

From a drug perspective, the current biggest challenge is to develop isoform selective PDE inhibitors to have the most efficacious effect of interest while limiting unwanted side effects. To find out which PDE subtype is the best target for therapeutic inhibition, it is of utmost importance to know the expression levels of the separate splice variants in relevant brain areas. From a therapeutic point of view, PDE splice variants with a high or increased expression are interesting to inhibit. Based on known expression levels in the brain of AD patients, PDE4D1, PDE4D2 and PDE8B appear very suited then (see Table 1). Interestingly, the PDE4D inhibitor BPN14770 is being clinically tested now, yet it is not known whether it is selective for specific PDE4D isoforms.

PDE1 expression has not been studied in AD brain yet, but appears interesting based on its relatively high expression in the frontal cortex and hippocampus [11]. Though overall expression of PDE2 and PDE9 was not changed in the AD brain, this does not rule out the possibility that these enzymes could be promising drug targets. However, if PDE levels are too low, then enzyme availability could be an issue at some point to obtain a sufficient treatment effect. This might be at hand for PDE3 or PDE5, which have a low basal expression in the human brain [11].

An important point to consider is the assumption that a decrease in expression/activity of a specific PDE subtype is compensatory [13,20], further inhibition of such PDE subtype would cause the cyclic nucleotide(s) to go over a maximum physiological level and cause a disruption of intracellular signaling. This would actually impair cognitive function. Therefore, targeting a PDE of which the expression is decreased in the AD brain (e.g. PDE4D3,5,6,7,8 or PDE7A) has to be considered with some caution. Yet again, this urges the need to develop splice variant/isoform specific PDE inhibitors (for instance three splice variants are known for PDE7A: PDE7A1-3) [41].

A promising approach to avoid side effects is combining drugs as has already been proposed based on rodent studies, like for instance a low dose of a PDE4 inhibitor together with a low dose of a PDE5 [110], PDE7, or PDE8 inhibitor [111]. The purpose of combining drugs is not merely to be additive, but actually to be synergistic. Thus, the cognitive enhancing effect of the combination therapy is greater than simply adding up the effects of the separate treatments. At the same time, dose limitations of the separate treatments due to their side effects are circumvented while increasing efficacy for cognitive enhancement and neuroprotection.

Due to the short half-life of PDE inhibitors in rodents it was possible to find out that PDE inhibitors have distinct effects on specific memory processes like for instance early consolidation versus late consolidation (e.g. [110]). Early consolidation is dependent on cGMP/PKG signaling, while late consolidation processes are dependent on cAMP/PKA signaling. However, in humans PDE inhibitors have a long half-life which is in general extended up to approximately 24 h or even longer (e.g. [47,80]). In addition, humans are given the drugs mostly chronically to achieve steady state plasma levels. Therefore, PDE inhibitors are not very well suited to exert an effect in humans on a specific memory process per se as the drug is on board continuously. But we think it is important to realize that the drugs could offer the opportunity to do so. This is something that could be of interest in the future, e.g. developing a drug with a short half-life. Consequently, a specific cognitive process could be temporarily targeted only when needed. The recent Boehringer compound BI 409,306 has a short half-life of 0.99–2.71 h in humans [97]. This would open opportunities for timed treatment of specific cognitive processes.
A main challenge in drug development in general is the translational gap between preclinical animal testing and human clinical trials which could also explain why for instance PDE5 inhibitors are effective in animals but not in humans thus far. It is very questionable if the animal tests tap into the same cognitive processes as in humans [112]. Also contributing to the high attrition of cognitive enhancers is the fact that animal tests are non-verbal, whereas human test are in particular verbal. This urges the need for more translational tests in animal as well as human testing. An interesting cognitive process to study in this respect is pattern separation, which is the cognitive ability to distinguish between rather similar stimuli. This process is dysfunctional in numerous different psychiatric disorders including AD [113]. The pattern separation task is highly translational as it allows to score episodic memory in both humans and rodents [114]. Adding to the translational value is that the performance in the verbal learning task (VLT), which is the golden standard for episodic memory testing, correlates strongly with that in the non-verbal translational pattern separation task [115]. Interesting to mention is that post-study caffeine administration improved specifically long-term memory consolidation using a pattern separation task in healthy subjects [116]. Of note, this effect of caffeine is likely related to a non-PDE mechanism (e.g. adenosine), yet the exact mechanism remains unclear.

Taken together, it is clear now that the future for a disease-specific PDE inhibitor lies in the development of isoform selective inhibitors that are more specific for the clinical target of interest with limited side-effect profiles. When focusing on AD, improving memory function eventually is the main cognitive endpoint. Thus, it is best to therapeutically target a specific overexpressed PDE in a specific brain area that is responsible for a specific cognitive function. If possible, cell-type specific PDE inhibition should be achieved, that is in different types of neurons, but possibly also astrocytes or microglia as they play a major role in AD pathology, e.g. inflammation, plaque removal. Gene transfer techniques (e.g. CRISP/Cas9) could offer a therapeutic tool to achieve this goal as the expression of a specific PDE splice variant level can be silenced in a cell type of choice. Obviously, this further underlines the need to characterize the expression of the PDE splice variants and isoforms in relevant brain areas in health and disease. But gene transfer techniques are considered not safe enough for human use and ethical constraints regarding gene therapy need to be resolved. Therefore, selective PDE inhibitors – if available- are needed as treatment. Furthermore, since AD is characterized by progressive stages which can either be described by amyloid plaques (e.g. MCI) or neurofibrillary tangles (e.g. Braak stage), it is essential to determine the time point of over-expression of a specific PDE. This will allow for exact optimal timing of treatment with an isoform selective PDE inhibitor. The earlier such treatment could start, the higher the chance of preventing or delaying the progression of AD.

Declarations of interest

P. Heckman is financially supported by the Human Enhancement and Learning (HEaL) initiative of Maastricht University, which stimulates research into cognitive enhancement to improve the quality of life. A Blakland and J Prickaerts have a proprietary interest in the PDE4 inhibitor roliflumast. J Prickaerts also has a proprietary interest in selective PDE4D inhibitors, including GEBR-32a. For the last three years A Blakland has been performing contract research into cognitive enhancing properties of specific drugs in humans for Takeda and Nootrobox. For the last three years the following activities of J Prickaerts can be disclosed: A grant was received from the Dutch Alzheimer Foundation (Alzheimer Nederland) to investigate cAMP-specific PDE inhibitors as a potential therapeutic target for cognitive decline in AD (project number WE.03-2016-07). Consultancy services have been performed for Takeda and Lundbeck. Finally, contract research has been conducted into cognitive enhancing properties of specific drugs in humans and/or animals for Takeda, Dart Neuroscience, Natural Stacks, Tetra Discoveries and Bayer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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