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Article *in* Expert Review of Neurotherapeutics · March 2015

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The role of positron emission tomography imaging in understanding Alzheimer's disease

Expert Rev. Neurother. Early online, 1–12 (2015)

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PET is a non-invasive imaging technique which allows the visualization and quantification of molecular processes, offering sensitive and early disease detection. Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to memory loss and other functional impairments. By employing different tracers targeting neurodegeneration, amyloid and tau aggregates, cholinergic neurotransmission, neuroinflammation and other processes, PET imaging enhances our understanding of the potential triggers of AD, the chronology of molecular events in AD, the detection of early AD, differentiation of AD dementia from other dementia disorders and the development of better drugs to treat AD. As such, PET imaging at different disease stages (asymptomatic, prodromal and dementia stages) is on its way to becoming a valuable routine clinical biomarker and a drug testing and research tool in AD.

Keywords: Alzheimer's disease • amyloid • mild cognitive impairment • nicotine • positron emission tomography • tau

PET is a non-invasive imaging technique which, by making use of specific radiotracers, allows the imaging and quantification of biochemical as well as physiological processes on a molecular level. As such, this functional imaging technique offers sensitive and early disease detection of molecular processes prior to the manifestation of clinical symptoms.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is, after a longer pre-symptomatic phase, clinically characterized by memory loss and impairment of other cognitive functions to a degree in which activities of daily living are affected. The histopathologic hallmarks of AD which are known to be present in the brain long before the clinical manifestation of this disease are extracellularly deposited β-amyloid plaques and intracellular neurofibrillary tangles which consist of aggregated hyperphosphorylated tau [1]. AD dementia is the most common form of dementia in the elderly, affecting more than 5 million people in the USA and more than 25 million people worldwide [2].

AD emerges to be one of the most important applications of brain PET imaging, in the context of both clinical routine and research. By employing different tracers over the last several years, PET imaging has improved our understanding of the potential triggers of AD, the chronology of events in AD, the differentiation of AD dementia from other dementing disorders and improvement in the testing of new drugs against AD. This review provides an overview of these multiple emerging roles of PET imaging in AD.

PET tracers to image AD

As AD involves multiple pathologic processes and reactive changes in brain, there are several central nervous system targets relevant to the disorder which can be investigated with PET. Table 1 provides an overview of these different PET targets, the respective tracers and the typical findings which have been reported to date in imaging AD patients.

Neuronal/synaptic activity in the brains of AD patients is visualized by employing the glucose derivative [^{18}F]fluorodeoxyglucose ([^{18}F]FDG). Its uptake is a mixed readout of glucose 1 transporter and hexokinase activities, both requiring viable tissue. As such, this tracer acts as a general brain tissue viability marker showing, in the case of AD dementia

 10.1586/14737175.2015.1023296 ISSN 1473-7175

Table 1. PET targets, respective tracers and typical findings in Alzheimer's disease.

Target	PET tracer	PET findings in AD
Neuronal/ synaptic activity	[^{18}F]FDG	Reduction in precuneus/posterior cingulate cortex at MCI stage, additional reduction in bilateral temporoparietal at dementia stage [59]
β-Amyloid plaques	[^{11}C]PIB, [^{18}F]Florbetapir, [^{18}F]Florbetaben, [^{18}F]Flutemetamol, [^{18}F]NAV4694	Increase in neocortex already at pre-symptomatic stage [60]
Tau aggregates	[^{18}F]T807, [^{18}F]THK5117, [^{11}C]PBB3	Increase in mesial temporal areas at MCI/early dementia stage, additional increase in neocortex at later disease stages [61]
TSPO	[^{11}C]-[R]-PK11195	Increase as sign of microglia activation already at MCI stage in brain regions later affected by neurodegeneration [62]
Cholinergic system	[^{11}C]PMP, 2-[^{18}F]A85380	No relevant alteration of AChE [63], reduction of α4β2-nAChRs already at MCI stage in brain regions later affected by neurodegeneration [64]
Dopaminergic system	[^{18}F]FDOPA, [^{11}C]raclopride	No relevant alterations of dopamine synthesis [65], conflicting results concerning D2/D3 receptor state [66,67]
Serotonergic system	[^{11}C]DASB, [^{18}F]MPPF	Reduction of SERT [68], conflicting findings concerning 5-HT1A receptor state [69–71]

PET tracers listed are exemplary representatives for each target category.
[^{18}F]FDG: [^{18}F]fluorodeoxyglucose; 5-HT: 5-Hydroxytryptamine; AChE: Acetylcholine esterase; AD: Alzheimer's disease; DASB: 3-Amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile; FDOPA: 3,4-Dihydroxy-6-fluoro-l-phenylalanine; MCI: Mild cognitive impairment; MPPF: 2'-Methoxyphenyl-(*N*-2'-pyridinyl)-*p*-[(18)F]fluoro-benzamidoethylpiperazine; nAChR: Nicotinic acetylcholine receptor; PIB: Pittsburg compound B; PMP: Methyl-4-piperidinyl propionate; SERT: Serotonin transporter; TSPO: Translocator 18 kDa protein (formerly known as peripheral benzodiazepine receptor).

and, to a certain extent, already at the prodromal mild cognitive impairment (MCI) stage of AD, a rather typical uptake deficit pattern. Taking the wide experience available for this tracer in imaging AD, the broader tracer availability and the comparably positive reimbursement situation for this tracer into account, [^{18}F]FDG has established itself over the last several years as the standard scintigraphic method for AD assessment.

A more recent and valuable addition, especially for early AD diagnosis, is the β-amyloid plaque-targeting PET tracers which have been just recently approved for clinical use both by the US FDA and the EMA (Table 1). These tracers bind with high affinity to the neuritic (and potentially also to diffuse) plaques. Respective 'hot spot' PET images show brain amyloid burden already at pre-dementia stage of AD. Amyloid PET is, in clinical routine, currently recommended in MCI cases that, according to the referring dementia expert, would benefit from greater certainty of the underlying pathology and in whom clinical management would change as a result of this greater certainty, as well as in atypical or early-onset AD dementia [3]. Furthermore, it is employed in anti-amyloid AD drug testing, both as screening tool to enrich the study populations with true AD cases/to confirm the presence of the drug target as well as to monitor biological treatment effects. As amyloid PET tracers were just recently approved, there is so far limited reimbursement and, therefore, lower utilization in clinical practice.

A more recent development which is still in early phases of human testing/optimization of the tracer characteristics is tau PET imaging (Table 1). These tracers bind with varying affinity, selectivity and signal-to-background image contrast to paired helical filaments of hyperphosphorylated tau aggregates. According to the amyloid cascade theory which assumes the occurrence of amyloid plaques to be the initial event in AD triggering a cascade of other events like tau aggregation [4], the tau tracers render pathological PET finding at a later disease stage as compared to the amyloid tracers. On the other hand, with the potential advantage of the PET signal being closely correlated to the cognitive state, tau PET is attractive as a potential disease progression surrogate marker. However, as knowledge on the role of tau PET tracers in the diagnostic AD toolbox is still limited, and as the currently available tracers are not yet optimal with regard to their imaging characteristics [5], more research is clearly required in this area.

In addition to the universal neurodegeneration marker [^{18}F]FDG and the PET tracers that target amyloid and tau, the histopathologic hallmarks of AD, other PET tracers were successfully used in the past to visualize and quantify neuroinflammatory processes as well as changes in different neurochemical systems in AD (Table 1). Here, especially in the field of nicotinic acetylcholine receptor PET tracers, much work has been carried out recently to develop optimized compounds which might be suitable for routine clinical use, such as (−)-[^{18}F]Flubatine, [^{18}F]AZAN and [^{18}F]Nifene [6–8]. This is due to fact that the availability of α4β2-subtype of nicotinic acetylcholine receptor seems to be closely correlated with cognition [9], a finding which might have future implication for evaluation of disease progression or drug effects in AD dementia and other dementia patients using PET imaging.

PET imaging of potential triggers of AD

As mentioned earlier, AD is histopathologically characterized by extracellular β-amyloid aggregates (cored/neuritic and diffuse plaques) and intracellular neurofibrillary tangles which contain hyperphosphorylated tau aggregates [10]. The trigger of AD is

poorly understood till date. The leading hypothesis, the amyloid hypothesis, posits the formation of pathological amounts of β-amyloid aggregates as the decisive initial event in this disease triggering a cascade of other biochemical processes which finally lead to neurodegeneration and cognitive decline [4,11].

There are a number of amyloid PET studies in cognitively healthy elderly subjects which provided evidence for the concept of amyloid being the trigger of AD. It was reported that children of parents with late-onset AD have a higher percentage of amyloid positivity on PET imaging [12]. Furthermore, healthy controls with a positive amyloid PET scan had a faster rate of hippocampal brain atrophy [13], and showed sub-clinical decline in their verbal and visual memory over the next 3 years [14]. Also, cross-sectional PET, cerebrospinal fluid (CSF) and MRI biomarker dynamics studies support the concept of occurrence of amyloid pathology in the brain being the initial event in AD [15].

On the other hand, PET imaging within a broader array of other AD biomarker techniques also reported in recent years interesting data potentially challenging the concept of amyloid accumulation being the pathogenic trigger in AD. Jack *et al.*, for instance, evaluated serial amyloid PET, FDG PET and MRI in cognitively normal subjects and reported that 42% of elderly subjects with incident amyloid PET positivity in follow-up showed evidence of neurodegeneration in prior imaging. From these results, the concept of 'neurodegeneration-first' AD (or suspected non-AD pathology) was developed [16]. In another recent longitudinal cohort study including 311 cognitively normal subjects older than 65 years, a suspected non-AD pathology frequency of 23% was reported [17]. It is currently unclear whether such suspected non-AD pathology cases represent tau-dominant or other pathologies. However, with the recent emergence of potential tau PET tracers [5], it now seems to be possible to further investigate this phenomenon.

Another interesting finding related to potential triggers of AD for which PET imaging might – at least in the future – play a relevant role is cholinergic neurotransmission. This is based on the cholinergic hypothesis of AD which states that in this disorder, cholinergic neurons in the basal forebrain degenerate, which is associated with a loss of cholinergic neurotransmission and cognitive decline [18]. The relevance of this hypothesis is supported by the fact that acetylcholinesterase inhibitors, drugs increasing the transmitter level in the synaptic cleft, are effective in AD to a certain degree [19]. Interestingly, preclinical data in AD models showed that the cholinergic deficit might be present as early as β-amyloid oligomers occur, for example, before β-amyloid aggregates into plaques [20]. PET imaging, by employing a combination of amyloid and cholinergic tracers, has a great potential to, in a translational manner, further investigate the question of cholinergic deficits potentially playing a co-trigger role in AD pathogenesis.

A similar potential of future PET imaging is in the evaluation of early neuroinflammatory changes in AD for their potential trigger function. This is because preclinical PET imaging research suggests that microglia activation, as detected via tracers targeting the over-expressed translocator 18 kDa protein, might precede β-amyloid plaque accumulation [Nordberg A, Pers. Comm.], a feature which is, likewise (like the above cholinergic deficit), poorly understood for its pathogenetic relevance. Providing human evidence for neuroinflammatory changes playing a trigger role in AD, the respective PET imaging techniques would bear the potential to improve early disease diagnosis and anti-inflammatory drug testing/monitoring.

PET imaging of chronology of events in AD from a drug testing perspective

From the perspective of histopathologic diagnosis, the two primary histopathologic features of AD are the presence of abnormal β-amyloid protein in the form of diffuse and neuritic plaques found in the brain parenchyma and intraneuronal tau protein aggregates composed of paired helical filaments forming neurofibrillary tangles. The presence of plaques and tangles is required for the diagnosis of AD. The timing with regard to the formation and spread of these protein aggregates has been subject to much study and debate. Both protein species have been associated with neuronal degeneration. Recently, there has been intense interest in AD therapeutics designed to ameliorate or reduce brain amyloid burden [21]. This is partly based on the evidence of very early involvement of β-amyloid accumulation in the brain [22], the deleterious impact on neuronal viability of β-amyloid accumulation in animal models of AD [23] and the direct evidence from *in vitro* models of neuronal toxicity by soluble forms of β-amyloid [24]. This concept that amyloid is an early and necessary component of AD pathology has led to the routine use of PET amyloid imaging for determining the eligibility of potential subjects recruited into therapeutic trials. These clinical trials have used PET amyloid imaging as a biomarker for eligibility into the study and for disease monitoring. Germane to the present discussion, extensive clinical investigation of PET amyloid imaging has been particularly helpful in sorting out the timing of brain amyloid appearance and proliferation with regard to the onset of cognitive impairment in patients who subsequently have a diagnosis of probable AD dementia.

One of the early findings in the amyloid PET imaging literature was [^{11}C]-Pittsburg compound B PET showing unexpectedly high rates of amyloid positivity in the scans of otherwise healthy older individuals who were serving as controls for the AD dementia subjects [25,26]. This phenomenon could represent several possibilities including that amyloid accumulation is a function of normal aging with some people unaffected by brain amyloid accumulation or, alternatively, imaging is sensitive to early prodromal changes in the brains of individuals who will subsequently go on to develop cognitive impairment and dementia with adequate longitudinal follow-up.

The amyloid PET studies in cognitively healthy elderly subjects have already been mentioned above. Several other lines of evidence supporting the amyloid cascade hypothesis suggest the latter possibility may be correct. These include: the consistent

doi: 10.1586/14737175.2015.1023296

Table 2. Failed clinical amyloid-targeting drug trials in Alzheimer's disease.

Drug	Company	Development phase	Status
Alzhemed	Bellus Health	3	Failed
Avagacestat	Bristol-Myers Squibb	2	Failed
AN1792	Elan/Johnson & Johnson	2	Failed
Bapineuzumab	Elan/Johnson & Johnson	3	Stopped
ACC-001	Elan/Johnson & Johnson	2	Stopped
Avandia	GlaxoSmithKline	3	Failed
ELND005	Johnson & Johnson	3	Stopped
Semagacestat	Eli Lilly	3	Failed
m266	Eli Lilly	2	Stopped
Solanezumab	Eli Lilly	3	Failed
Dimebon	Medivation	3	Failed
Tarenflurbil	Myriad Genetics	3	Failed
Tramiprosate	Neurochem	3	Failed
Huperzine	Neuro-Hitech	3	Failed
Lipitor	Pfizer	3	Failed
PBT2	Prana Biotechnology	2	Failed

association between the APO E4 allele as a risk factor for both AD and brain amyloid accumulation detected by PET [27,28], the demonstration of amyloid PET positivity in Down's individuals occurring in midlife is associated with dementia [29] and the finding that MCI subjects who have positive scans are much more likely to go on to develop probable AD dementia than those MCI subjects with negative PET amyloid scans [30].

Both APO E4 and the replication of chromosome 21 in Down's syndrome (DS) are genotypes which increase the brain amyloid burden and are both associated with the development of cognitive impairment. For the former, APO E4 dose, going from heterozygous to homozygous for the allele, represents increasing susceptibility risk for dementia, and interestingly, in healthy volunteers, increasing amyloid positivity based on composite standard uptake value ratios (SUVr) [31]. DS serves as a natural model of brain amyloid deposition in that all affected individuals would develop amyloid accumulation and clinical dementia. Chromosome 21 contains several genes implicated in neurodegenerative mechanisms involving amyloid precursor protein and β-site amyloid precursor protein cleaving enzyme, among others. The extra copy of chromosome 21 causes a fourfold to fivefold overexpression of the *APP* gene. Histopathologic studies demonstrate β-amyloid plaques in all individuals with DS, who are 40 years and older, with neurofibrillary tangles occurring generally after the age of 40. Several studies evaluating DS with amyloid PET suggest that changes in brain amyloid occur in the age range 40–55, which parallels the onset of dementia [29,32,33]. This time course of amyloid accumulation may be more rapid in DS than in AD, but nonetheless serves as a model for the amyloid hypothesis and suggests that amyloid targeting therapeutics might be beneficially evaluated in DS.

Despite the support from the evidence cited above, that amyloid is a key initial component of the pathophysiology of AD, several factors have led, as noted above, to some skepticism about the amyloid cascade hypothesis. One major criticism has been the failure of numerous clinical trials designed to ameliorate the impact of amyloid in brain. The fact that treatments targeting the reduction or removal of brain amyloid have not led to the clinical improvements in mild to moderate AD dementia research volunteers has led to questioning the veracity of the amyloid hypothesis. There have been multiple clinical trials of putative amyloid targeted treatments with antibodies (e.g., bapineuzumab, summarized below) or small molecules (e.g., semagacestat), which demonstrated lack of clinical efficacy in well-characterized AD dementia subjects (Table 2).

While these negative studies call into question the amyloid hypothesis, several important considerations are merited. First, it is not known which is the most beneficial time for intervention with an amyloid targeting treatment. Intuitively, early intervention would seem ideal insofar as there would be relatively more preservation of at-risk neurons. Furthermore, the concept of efficacy is complex; the treatment would need to show clinical slowing or improvement on objective clinical instruments as the primary outcome measure of these trials. Biomarkers such as CSF measures of amyloid, tau, or p-tau or PET imaging changes in amyloid deposition, changes in MRI brain volumetrics, or metabolism serve as adjunct efficacy markers or support demonstration of mechanistic engagement. Changes in biomarkers in the absence of relevant clinical effects are less compelling to the regulatory authorities and clinical community, while concordance between clinical measures and imaging biomarkers provides powerful endorsement of the treatment mechanism and efficacy. As noted earlier, the timing of treatment with regard to the severity of disease has been proposed as an important potential explanation for the failure of amyloid treatments to date. According to this hypothesis, anti-amyloid treatment is only efficacious in earlier AD dementia, MCI or prodromal patients where there is presumably more function to preserve. This concept is perhaps demonstrated in the Phase III solanezumab trials (Expedition 2 trial) where there was a significant trend toward slowing the rate of reduction on the ADAS-Cog in the mild AD dementia cohort versus placebo controls and compared to more severe patients. PET imaging results were consistent with the clinical data and were compelling enough to launch an additional Phase III trial in mild AD dementia subjects, even though the original Phase III trial did not achieve its specified clinical outcomes.

PET imaging for differential diagnosis in dementia

As noted above, AD dementia is the most common form of dementia in the elderly. It accounts for about 60% of all

doi: 10.1586/14737175.2015.1023296

Table 3. Typical brain patterns of neuronal/synaptic activity as imaged by [^{18}F]fluorodeoxyglucose PET and stages of amyloid load as imaged by different PET tracers in dementia subtypes.

	AD dementia	FTLD			DLB	PDD
		FTD	*SD*	*PNFA*		
FDG uptake	Reduction: Bilateral temporoparietal and precuneus/PCC	Reduction: Bilateral frontal and ACC	Reduction: Left anterior temporal	Reduction: Left frontal	Reduction (less pronounced in PDD as compared to DLB): Bilateral temporoparietal, precuneus/PCC and occipital	
Amyloid tracer uptake	Increase in 100% of cases[†]	Increase in about 30% of cases	Rarely increased	Rarely increased	Increase in about 70% of cases	Increase in about 30% of cases

Typical FDG uptake reductions are given as evident at the respective earlier dementia stage. These reductions tend to expand to other brain areas at advanced dementia stages.
[†]The presence of β-amyloid plaques is one criterion required to establish the gold standard histopathologic AD diagnosis.
ACC: Anterior cingulate cortex; AD: Alzheimer's disease; DLB: Dementia with Levy bodies; FDG: Fluorodeoxyglucose; FTD: Frontotemporal dementia; FTLD: Frontotemporal lobar degeneration; PCC: Posterior cingulate cortex; PDD: Parkinson's disease dementia; PNFA: Progressive non-fluent aphasia; SD: Semantic dementia.
Table adapted from [72].

dementia cases in the Western world. This is followed by vascular and Lewy body dementia (DLB), each of which is believed to represent (either as a sole entity or mixed with AD dementia features) 15% of dementias. Frontotemporal lobar degeneration and other dementia forms (like normal pressure hydrocephalus and others) constitute about 5% of all dementia cases.

Differential diagnosis of these dementia forms is of importance both in clinical routine as well as in drug research because the different entities respond, at least in part, to different medical interventions and certain drugs are contraindicated in some dementia types (examples are shunt surgery, which is effective only in normal pressure hydrocephalus [34], and neuroleptics, which are occasionally prescribed in dementia patients, especially in case of behavioral symptoms [35], but are relatively contraindicated in DLB [36]).

Nevertheless, the first-line diagnostic tool to diagnose AD dementia and other dementia diseases, that is, clinical testing, is known for its limited accuracy in dementia-type discrimination: only 70–90% of dementia patients characterized by clinical testing as 'probable AD dementia' were confirmed as AD according to the National Institute of Neurological and Communicative Disorder and Stroke-Alzheimer's Disease and Related Disorder Association criteria by post mortem gold standard histopathology [37,38], meaning that clinical testing misdiagnosed other dementing diseases in 10–30% of cases. In prodromal stages of AD, that is, MCI, where there is increased appreciation of the advantages of early diagnosis for clinical drug testing, this number of wrongly classified dementia types is probably even higher.

Bearing in mind these limitations of clinical testing for accurate differential classification of dementia, biomarkers, especially PET imaging, are gaining increasing importance. Up to this point, differential dementia diagnosis by means of PET imaging is mainly carried out employing the neuronal/synaptic activity marker [^{18}F]FDG. This is because different dementia forms bear (not in all cases and with a certain overlap) specific patterns of [^{18}F]FDG uptake reduction throughout the brain. These patterns are described in Table 3.

However, with the recent emergence of β-amyloid plaque-targeting PET tracers, for which typical findings in the different dementia types are also given in Table 3, [^{18}F]FDG might in future be challenged as a first-line PET tracer for differential diagnosis and rather be used in second line in a step-by-step PET imaging protocol potentially followed by dopaminergic imaging to discriminate AD from DLB [39].

There are two other challenges to the current role of [^{18}F]FDG in differential dementia diagnosis. First, as recently reported, dynamic or dual time-point (early after tracer administration in addition to the standard later after tracer administration image acquisition for amyloid load evaluation) amyloid PET offers the possibility of obtaining blood flow images which, as this parameter is, in most situations, closely coupled to the neuronal/synaptic activity in the brain, are a reliable surrogate of [^{18}F]FDG images [40] potentially superseding the use of the latter. Secondly, with the recent introduction of integrated PET/MRI systems, it might even be possible in the future to measure cerebral blood flow, for instance, by arterial spin labeling MRI together with amyloid load by PET in one session [41]. Providing the proof of principle for such a one-stop shop imaging approach by combined amyloid PET/arterial spin labeling MRI, this technique would certainly hold the potential to simplify and improve differential dementia diagnosis. This is supported by a comprehensive meta-analysis of separately acquired imaging-based biomarkers indicating that combined image data evaluation is valuable for differential dementia diagnosis [42].

Concerning the potential role of the emerging tau and the other PET tracers presented in Table 1 for differential diagnosis in dementia, no systematic data are so far available in the literature. This is apart from the potential role of PET or single-photon emission computed tomography imaging of presynaptic dopaminergic neurotransmission for differentiating DBL from AD, a task which has therapeutic consequences (see above) and which is difficult to achieve by clinical testing alone. Here, a dopaminergic deficit is evident in DLB [43], but

doi: 10.1586/14737175.2015.1023296

not in AD patients. Such imaging algorithms have been adopted as routine by many clinical dementia clinics.

General aspects of PET imaging in AD drug testing

As already noted, clinical drug testing in AD, regardless of whether anti-amyloid, neuroprotective or neurorestorative approaches are followed [44], is currently in a stage of flux. This is because a curative treatment is still not available and many drugs tested over the last years with huge efforts and expense failed in clinical validation, despite giving promising preclinical results. There are at least four scenarios in which PET imaging could improve this situation in the future.

1. One new direction for future therapeutic trial strategies is based on the notion that certain anti-amyloid drugs were successful in removing amyloid out of the brain of AD dementia patients, but failed to show clinical improvement. From this, it is suggested that these drugs were given too late at the dementia stage of AD, that is, in a situation in which irreversible neurodegeneration already took place. As a consequence, these drugs are increasingly tested in earlier disease stages, that is, in MCI or even asymptomatic subjects with the risk of developing AD dementia. Here, one important role of brain PET imaging lies in the identification of subjects at risk, mainly by using amyloid PET. An example of such ongoing trials in which amyloid PET imaging is used in prodromal or preclinical AD as an enrollment tool is the A4 study in which older amyloid PET-positive subjects without cognitive impairment are enrolled [45]. For the other PET tracers available to image AD, in particular, for the promising novel tau aggregate-targeting tracers, their potential as enrollment tools in drug testing at the prodromal or preclinical AD stage awaits investigation.
2. Another important application of PET imaging is in clinical drug testing trials with improved designs and better diagnostic classification compared to the current situation in studies of subjects with MCI or in those who manifest dementia stage of AD. So far, the diagnosis of MCI/AD dementia was mainly established on clinical grounds, that is, by cognitive testing. It is, however, increasingly recognized that clinical testing has only limited accuracy for this purpose: There are considerable numbers of subjects included as AD dementia patients based on clinical testing who do not fulfill the biomarker profile of AD, for instance, being negative on amyloid PET. To support this notion, we reviewed data on a number of anti-amyloid drug testing studies in which amyloid PET was carried out at baseline to later allow therapy monitoring by follow-up imaging (see below). Here, the range of AD dementia patients enrolled into the trials while being scored as negative in the amyloid PET images was 4–34% depending on the subject pool. This would not only mean that patients with other (in this case, amyloid-negative) dementias were included, but more importantly that the drug target was not present in these subjects. This suboptimal enrollment situation causes a major problem for the study in achieving a statistically relevant positive outcome in otherwise well-designed, placebo-controlled trials. As a critical improvement to this problem, amyloid PET imaging is increasingly used as a screening tool in such trials to (by following the widely accepted rule of 'no AD dementia without amyloid') enrich the study populations with true AD cases/ensure the presence of the drug target. One example of ongoing drug testing trials in which amyloid PET imaging is used for that purpose is the Expedition 3 study [46]. As recently published, the use of amyloid PET imaging and other AD biomarkers to enrich the study populations in drug testing with true AD cases is also supported by the regulatory authorities [47,48]. For the other AD PET tracers, determination of their usefulness for this application is still pending. In principle, especially for the novel tau and cholinergic PET tracers, they are regarded as promising candidates for future study population enrichment in respective anti-tau or cholinergic drug testing trials. As a drawback to this concept, it was recently noted by Lorenzi *et al.* that enrichment of study populations by AD biomarkers is obviously associated with a relevant increase of subject numbers required at study screening due to increased number of biomarker-negative drop-outs [49], a feature which certainly needs to be considered in respective trial planning.
3. Furthermore, as already noted, PET imaging in the context of AD drug testing plays a relevant role in monitoring biological drug effects. The question on whether a particular AD patient responds toward the anti-amyloid drug on the biological level is relevant insofar as this is the requisite for symptomatic improvement.

Earliest examples in this regard are the application of the nicotinic acetylcholine receptor ligand [^{11}C]nicotine, which was employed to monitor the effect of the cholinesterase inhibitors tacrine, rivastigmine and galantamine [50–52], in the first case in combination with the muscarinergic acetylcholine receptor PET ligand [^{11}C]benztropine and in the last case in combination with the acetylcholine esterase substrate [^{11}C]methyl-4-piperidinyl propionate. Monitoring the effects of cholinergic drugs directly on the different components of cholinergic neurotransmission is an attractive approach insofar as it, in principle, would allow titrating treatment amount to the individual need toward personalized medicine.

One example of using the neuronal/synaptic activity marker [^{18}F]FDG to monitor the effect of nerve growth factor in AD is shown in Figure 1. In this trial, brain glucose consumption was absolutely quantified before and 6–8 months after treatment. On a group level, an improvement of brain metabolism after therapy was observed [53]. [^{18}F]FDG has the advantage of being closely related in its uptake to general brain tissue viability, as such making it a good candidate surrogate biological endpoint in neuroprotective and/or neurorestorative drug testing. The aforementioned study also points to another strength of brain PET imaging as compared to other AD biomarkers for therapy monitoring – it provides biological efficacy readouts directly measured within the brain in a quantitative manner.

doi: 10.1586/14737175.2015.1023296

Drugs which are tested for their potential to lower/remove β-amyloid plaque burden in the AD brain, however, profit in their biological efficacy testing by more specific PET imaging with amyloid tracers. Figure 2 provides an example of a trial in which an anti–β-amyloid antibody was successfully evaluated for its biological effect. Here, [^{11}C]-Pittsburg compound B amyloid PET was carried out in a controlled study design in AD dementia patients either receiving the humanized anti-amyloid β monoclonal antibody bapineuzumab or placebo at baseline as well as 20, 45 and 78 weeks after treatment. Change from baseline tracer retention ratio at week 78 was significantly lower in the treatment group as compared to the placebo group. This was despite no detectable differences observed in cognitive or other clinical indices of AD severity between the treatment groups [54].

The question of the capability of amyloid PET imaging and other AD biomarkers in serving as co-primary endpoint (besides clinical outcome) for testing drug candidates to treat AD was likewise recently discussed by the regulatory authorities. Other than for enrichment of study populations with true AD cases, it was noted by the FDA that (despite that fact that innovative approaches to trial design and endpoint selection are urgently needed) there is currently no consensus as to what particular biomarkers would be appropriate to support the clinical findings [47,48]. More work is certainly required here to define the potential impact of brain PET imaging as AD therapy monitoring tool. This also refers to the novel emerging tau tracers, which so far have not been systematically investigated for their potential to monitor the drug effects in AD.

4. Finally, although human data do not exist so far for this potential application, PET imaging could be applied in future to improve the detection and understanding of treatment-related side effects in the brain. One example of this is a recently published study in a non-human primate AD model in which the animals were actively immunized by aggregated Aβ_{42}. To investigate whether this causes microglia activation as a sign of brain inflammation, the PET tracer [^{11}C]PK11195 which traces the 18 kDa translocator protein, formerly known as the peripheral benzodiazepine receptor, was employed. No vaccination effect on microglia activity was observed in follow-up PET imaging, potentially pointing to a favorable safety profile of this particular vaccination approach [55]. More work on this application of PET in AD drug testing, also considering other neuroinflammatory and/or blood brain barrier integrity-related tracers, is certainly warranted to fully explore this promising approach.

The following two sections discuss two of the above applications of PET imaging in Alzheimer drug testing, namely the use of PET imaging as eligibility criterion for patient inclusion and that for therapy monitoring, in more detail.

PET imaging for early AD diagnosis in drug testing

As indicated above, interest in the amyloid hypothesis of AD has resulted in a number of clinical treatment trials aimed at reducing or slowing the accumulation of brain amyloid. These large, multicenter Phase II–III studies have used both small molecules interrupting normal β-amyloid production and trafficking, as well as antibodies targeted to amyloid. Originally, these trials recruited mild to moderate probable AD dementia patients, as the initial results showed no clinical effects in the severely impaired patients. As a consequence, the concept of targeting the earlier AD spectrum patients including those with MCI or even prodromal AD was proposed and recently implemented in several trials. This has resulted in a significant recruitment challenge among even expert clinicians seeking appropriate patients for these trials. The reason for this is the subtlety of the initial clinical presentation with significant phenomenological overlap in prodromal AD and early AD dementia compared with other causes of cognitive decline. As a result, the theoretical likelihood is high that there would be patients recruited into trials who meet the clinical eligibility criteria including the degree of cognitive impairment, but who do not have brain amyloid. Including such patients would result in significant bias toward type two errors in the clinical trial and,

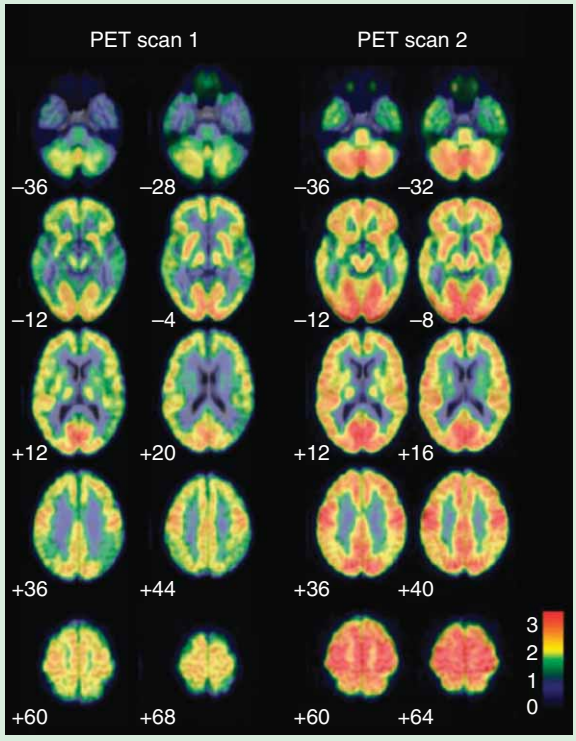

Figure 1. Example of using [^{18}F]fluorodeoxyglucose neuronal/synaptic activity PET imaging for therapy monitoring in Alzheimer's disease. Baseline (PET scan 1) and follow-up (PET scan 2; after treatment with nerve growth factor) images of the averaged transverse slices at different brain levels of four Alzheimer's disease patients. In this trial, cerebral glucose consumption was absolutely quantified (the color scale represents μmol/100 g tissue/min). A widespread average increase of brain glucose consumption was observed after treatment.
This figure was originally published in Nature Medicine by [53]

doi: 10.1586/14737175.2015.1023296

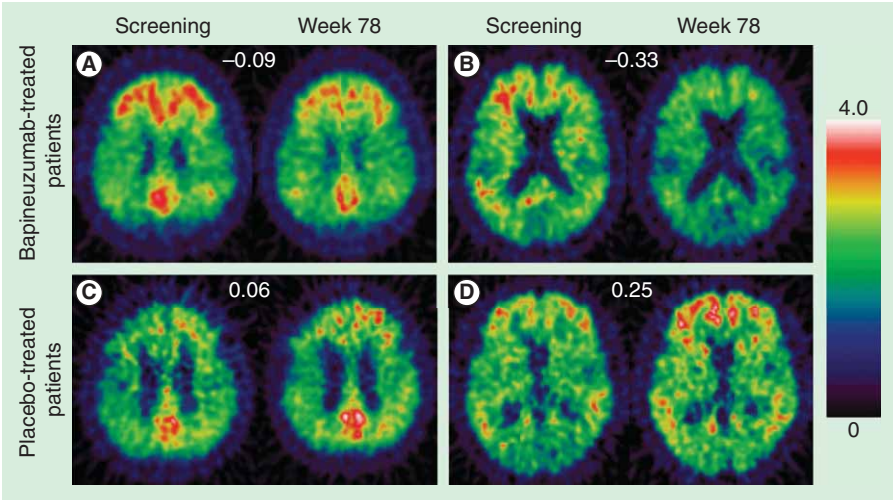

Figure 2. Example of using [^{11}C]Pittsburgh compound B amyloid PET imaging for therapy monitoring in Alzheimer's disease. Transverse PET slices on identical brain level at baseline screening and 78 weeks follow-up (**A** and **B**) in two dementia patients treated in a Phase II study with the humanized anti-amyloid β monoclonal antibody bapineuzumab and (**C** and **D**) in two patients of the placebo group. Scale bare shows uptake ratios as related to the cerebellum reference region.
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more importantly, expose those patients to the investigational study drug and procedures with the risk of experiencing adverse events.

This has created a unique role of PET imaging for amyloid burden as an eligibility criterion for enrollment into treatments designed to ameliorate the impact and burden of brain amyloid. Recent multicenter clinical trials incorporating amyloid PET eligibility schemas have, on average, shown that about 20% of patients recruited into the studies across the board do not have brain amyloid accumulation (Table 4). The percentage of negative scans is even higher in those trials recruiting patients earlier in their disease course, MCI and prodromal Alzheimer's patients. In addition to the clinical characteristics of patients (age, APO E status), other factors which influence the percentage of negative scans are related to where the PET imaging is performed in relation to other diagnostic assessments as part of screening, as well as recruiting physician threshold for diagnosing dementia related to AD even in the context of operationalized clinical eligibility criteria.

Studies have suggested a high degree of concordance between brain PET amyloid and CSF in within-subject comparison study designs [56,57]. Hence, it is not unusual to have as an enrollment criterion for clinical trials in AD spectrum patients the requirement for the determination of amyloid target for the treatment based on either a PET amyloid scan or CSF measure.

The question of how eligibility is determined for PET amyloid imaging has been subject to some interest. In particular, the issue is whether or not eligibility should be based on a visual interpretation by several readers or quantification of the PET image comparing it against some threshold value for positivity or even the combination of visual interpretation and quantification have all been suggested. Most clinical trials use a visual interpretation by more than one expert reader viewing images independently using the visual interpretation algorithm developed and approved for that particular amyloid radiotracer. Readers are required to be concordant in their interpretation of positive or negative for the scan, and if not, either a consensus process is performed or a third tiebreaking reader is used. Visual reads have the advantage of relatively fast turnaround time from the central core imaging lab where these assessments are typically performed. Speed is an important consideration as the screening period may be a limited number of days and the PET amyloid scan is usually one of the last assessments done given its cost relative to other screening assessments. Some have suggested a value in a more objective quantitative assessment for screening for eligibility. In particular, composite SUVr measured in key brain regions demonstrating cortical deposition of β-amyloid have been used in relatively fewer trials. One of the reasons for this is the dependence of the results obtained upon the image analysis algorithm, and makes it a little bit longer to develop a determination of eligibility or ineligibility based on the quantitative assessments.

One very interesting eligibility algorithm is the hybrid of the visual interpretation and quantification employed in several trials including the A4 (National Institute on Aging/National Institute of Health, Eli Lilly) and Expedition 3 (Eli Lilly) trials. The former is a study in cognitively normal individuals older than 65 years, who happen to have a positive amyloid PET scan and thereby are invited to participate in a randomized, placebo-controlled trial of treatment as the first large-scale preventative Alzheimer's trial. In order to ensure the accuracy of

Table 4. Results of eligibility screening using amyloid PET in recent clinical Alzheimer's disease drug trials.

Study	Baseline PET scans	Positive scans	Negative scans	% Negative scans
1	152	132	20	13.2
2	180	153	27	15.0
3	149	99	50	33.6
4	112	93	19	17.0
5	91	87	4	4.4
6	230	179	51	22.0
Total	914	743	171	18.7

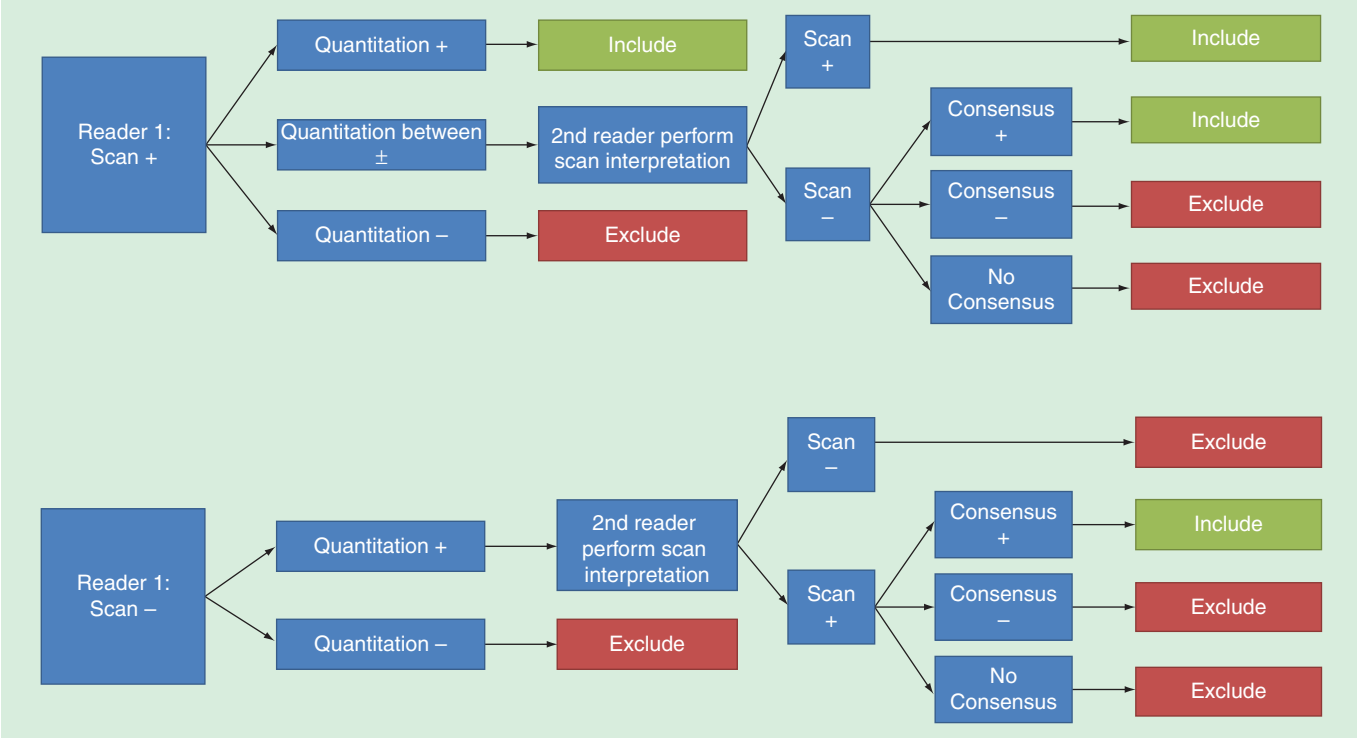

Figure 3. Eligibility schema for the A4 trial incorporating both visual assessment and quantitative evaluation for rigorous vetting of PET amyloid determination.

these clinical diagnoses for eligibility in this unusual population, an algorithm is employed which involves a single reader assessing a PET amyloid scan as positive or negative, assisted by quantitative information from the PET image, and an independent quantification performed by a core lab, and then matching up of the data done such that if both the reader and the core lab quantitative method agree that the subject is a positive, he/she is eligible for the trial (Figure 3). Likewise, if both the reader and the quantification agree on negative, the subject is ineligible for the study. In instances where there is disagreement between the reader and the quantification, the scan goes to a second reader who performs the same exercise determining a visual impression aided by adjunctive quantitative information. If the two readers agree on either positive or negative rating, the subject is eligible or ineligible, respectively. If the two readers disagree, then the process goes to consensus between the two who either determine a final positive or negative rating or are unable to agree, the latter case resulting in the subject being ineligible. This algorithm is described in Figure 3 and represents the most rigorous eligibility screening to date for AD clinical trials.

PET imaging for therapy monitoring in AD drug testing

Another role of PET amyloid imaging in clinical trials is to assess the longitudinal effects of treatment on brain amyloid burden and to correlate with the clinical outcomes. This is a difficult task at best given the rather slow amyloid accumulation over time, on the order of 1–4% per year, with significant variability between subjects. There has been interest in understanding the source of this variability, whether it represents true biological differences or mostly technical noise inherent in PET imaging, particularly in large multicenter data sets utilizing different instrumentation. Quite naturally, the focus of this has been on the outcome measure used in these large multicenter trials, the SUVr, a simple target to background ratio obtained during secular equilibrium of the radiotracer. Given the multiplicity of sites and experiences in performing brain PET imaging, these simple measures have been preferred, although they are biased in certain ways. It would be ideal if a more rigorous pharmacokinetic model of the outcome measure could be obtained. In order to achieve this, however, serial dynamic PET acquisitions, ideally with blood measures would be required. As a result, the field has lived with what is known to be a biased quantitative outcome measure.

Recently, there has been some increased interest in improving the SUVr for within-subject longitudinal assessment. A relook at the ideal reference regions for determining longitudinal change has taken place in several imaging laboratories. For cross-sectional data, the cerebellum, either cortical gray matter alone or whole cerebellum, has been used as a reference tissue based on the belief that there is low likelihood of amyloid deposition in this region. Studies using the Alzheimer's disease neuroimaging initiative longitudinal PET amyloid data set have assessed the impact of other reference regions including the white matter in the centrum semiovale [58]. While being preliminary, these data suggest that there is more stability and less variability, when

doi: 10.1586/14737175.2015.1023296

using white matter as reference tissue, or as a correction to SUVrs using a cerebellar reference. The impact of this is actually quite significant insofar as it can demonstrate more subtle statistical findings in extant amyloid data sets based on lower variability and the same signal-to-noise properties. The optimization of reference regions for longitudinal assessment as well as other technical corrections including partial volume error corrections are the source of continued investigation.

To date, very few studies have reported the results of longitudinal amyloid PET measures in treatment studies. As these data become available, there will be more clarity as to the role of amyloid PET as a longitudinal biomarker of amyloid burden and whether alternative strategies including serial PET assessment of tau deposition may be more useful, by virtue of having greater dynamic signal change allowing it to be used preferentially for this role. Insofar as tau PET is now just being incorporated into multicenter treatment trials and there is limited data of longitudinal nature up to this point, this remains an objective for the future.

Expert commentary

In AD, PET imaging with tracers targeting disease pathology (amyloid and tau aggregates) or neuronal injury ([^{18}F]FDG) has gained wider acceptance over the last years in clinical routine use as a valuable biomarker for early diagnosis. PET is also of great use in differential dementia diagnosis, especially with the recent possibility of non-invasively studying AD onset and progression at the prodromal or even asymptomatic disease stage leading to a potential progress in understanding AD pathogenesis. Apart from clinical routine use, in AD drug testing, these PET techniques are of major use to streamline the clinical trials by enriching the study populations with individuals in whom the drug target/actual AD disease is present, to monitor drug effects on a biological basis and for other purposes. PET imaging is also an important tool for studying the amyloid hypothesis of AD pathophysiology. There is evidence supporting the primacy of β-amyloid deposition in brain as an early event in AD and it remains to be seen whether or not agents targeting amyloid administered to the right individuals will result in positive therapeutic response.

Open questions for PET imaging in AD, especially for drug testing, are related, for instance, to the biomarker data finding acceptance as trial endpoints, like in pre-symptomatic populations where a clinical phenotype is not yet manifest, and to whether a combination of different PET tracer information (e.g., amyloid and tau or amyloid and cholinergic neurotransmission tracers) has a potential to improve stratification and therapy monitoring.

Five-year view

One expectation about the future of PET imaging in AD is to broaden the current portfolio of specific tracers by candidates that are able to visualize and quantify cholinergic neurotransmission or neuroinflammation. By that, it will hopefully be possible to gain a wider picture of the different underlying pathological processes in this disorder, potentially providing evidence for/against the amyloid cascade hypothesis. Another future potential lies in the wider use of PET imaging in AD as a biomarker to support clinical testing in symptomatic subjects and, potentially, as the decisive diagnostic test in pre-symptomatic subjects. Furthermore, it is estimated that as soon as effective anti-amyloid/tau drugs are identified to treat AD, usage of PET imaging will find a massive increase and it will become a standard test to confirm the presence of the disease/drug targets and to non-invasively monitor treatment efficacy on an individual basis.

Financial & competing interests disclosure

H Barthel and O Sabri have served as consultants and received honoraria and travel expenses from Bayer Healthcare and Piramal Imaging. J Seibyl has served as a consultant to Pirimal Imaging, Navidea Biopharmaceuticals and GE Healthcare, and has equity in Molecular Neuroimaging. 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.

Key issues

- Alzheimer imaging by PET has recently gained increased interest mainly due to newly developed amyloid and tau tracers.
- PET imaging of amyloid has provided insights into the nature and time course of brain amyloid accumulation.
- PET detection of brain amyloid has consistently been demonstrated to be either indicative or prognostic of future cognitive impairment, although longitudinal studies remain to be performed in healthy populations in particular.
- Genotypes resulting in greater brain amyloid burden have been studied with amyloid PET to confirm the phenotype; greater PET standard uptake value ratio scores are associated with cognitive impairment.
- Especially for more holistic disease severity evaluation, estimation of glucose consumption by PET imaging using [^{18}F]fluorodeoxyglucose and (in the future) tau PET imaging are valuable add-ons to amyloid imaging.
- Amyloid (and potentially in the future tau, neuroinflammation, cholinergic neurotransmission) PET imaging is a valuable tool to improve Alzheimer drug testing, mainly as eligibility criterion for patient inclusion and for therapy monitoring.

doi: 10.1586/14737175.2015.1023296

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