Potential Impact of Amyloid Imaging in Vivo on Alzheimer’s Disease Treatment and Management

Beta-amyloid (Aß) modification therapies for Alzheimer’s disease (AD) are currently being developed that target Aß production, aggregation, and/or degradation. Some of these medications are already in Phase 3 studies. It will therefore be most relevant to be able to quantify the neurobiological target of such therapies directly in vivo in the brain. This could permit a reduction in the required sample size for future clinical trials and will allow a more individually tailored approach once such treatments become clinically available. This article reviews the prevalence of AD amongst other dementias, the Aß cascade, various Aß positron emission tomography (PET) tracers that are being developed, and the potential application of these tracers for Aß-modification therapies.

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Dementia is common in older adults and approximately doubles in frequency every five years, from about 1% of people aged 60 years to 30% to 40% of those aged 85 years of age and older.1,2 AD3 is the leading neurodegenerative disorder, accounting for approximately one third to two thirds of dementia cases.2,4 Improving early detection of AD and studying the effects of new treatments for AD are of epidemic importance. Studies indicate that, on average, acetylcholinesterase-inhibitor (AChEI) treatment delays cognitive decline in AD patients by nine to 12 months and the need for institutionalization by 18 months.5-9 Moreover, at one year, superior cognitive performance was observed in patients who started AChEI treatment at the beginning than in those who started six months after the beginning in trials with rivastigmine,10 galantamine11 and donepezil.12 Therefore, it would be prudent to apply functional neuroimaging within six months of identifying progressive cognitive decline that could represent incipient AD.

Although the accuracy of the clinical evaluation for AD can be further improved with [18F]fluorodeoxyglucose ([18F]FDG) glucose metabolism positron emission tomography (PET),13,14 or perfusion single photon emission computed tomography (SPECT),15 further improvements can be expected from imaging in vivo of more specific pathological processes for AD: extraneuronal Aß plaques,16-19 intraneuronal neurofibrillary tangles (NFT),16,20-22 and interneuronal synapse loss.23,24 To our knowledge, no NFT-specific or synapse-loss-specific tracers have been developed for imaging in vivo. Therefore, this article focuses on Aß-specific tracers.

The ß-amyloid Cascade

Mechanism of Aß production. The Aß1-40 and Aß1-42 (peptides of 40 to 42 amino acids) are derived from a transmembrane protein named amyloid precursor protein (APP).25 There are two APP cleavage pathways:18

• The non-amyloidogenic
The amyloidogenic pathway: APP is cleaved by \( \beta \)-secretase (or \( \beta \)-amyloid cleaving enzyme, [BACE]), followed by \( \gamma \)-secretase cleavage. BACE cleavage liberates \( \beta \)-APP that contains the A\( \beta \) peptide fragment, and \( \gamma \)-secretase cleavage liberates the A\( \beta \) peptide from \( \beta \)-APP. The A\( \beta \) peptides aggregate to senile plaques in the brain parenchyma and to cerebral amyloid angiopathy (CAA) in the blood-vessel walls.26

Aggregation of A\( \beta \) into senile plaques. Senile plaques are a form of A\( \beta \) accumulation and are one of the earliest pathological changes that appear before neuronal loss occurs in the aging and the AD brain.17 Senile plaques have two histologically different forms, which are thought to impact on disease symptoms and progression:17

- Diffuse plaques consist of amorphous A\( \beta \), lack the \( \beta \)-sheet structure and are not surrounded by dystrophic neurites.27 They are associated with normal aging.
- Dense-core (or neuritic) plaques consist of fibrillar A\( \beta \) and are found mostly in patients with AD, but also in a small amount in the normal aging brain.28

Fibrillar A\( \beta \) has the conformation of a \( \beta \)-sheet structure, which is specifically detected by Congo red or Thioflavin T staining.29 Most of the PET A\( \beta \) radioligands discussed below have been derived from these two dyes and are thought to be mainly binding to fibrillar A\( \beta \).

**Amyloid PET and SPECT Imaging Agents in Development (Table 1)**

There has been considerable interest in measuring regional cerebral A\( \beta \) levels in vivo with magnetic resonance imaging (MRI), PET or SPECT. MRI can provide high-resolution images, but necessarily requires large amounts of contrast agents, such as Gd[N-4ab/Q-malononitrile derivative \([^{18}F]2-(1-(6-[(2-fluoroethyl)methylamino]-2-naphthyl)ethylidend) malononitrile ([\( ^{18}F \)]FDDNP).34 The differences between nine AD patients and seven controls were demonstrated using the relative residence time (RRT) of the forebrain regions versus the pons. However, since the RRT is probably sensitive to peak and steady-state tracer levels, additional analyses have been used such as standardized uptake values at equilibrium normalized to the cerebellum and Logan distribution volume ratios with the cerebellum as reference region,35 providing similar results. The area of highest retention at equilibrium was the hippocampus, amygdala and entorhinal cortex region, while NFTs are mainly concentrated post mortem.20 In contrast, autopsy studies16 have shown that dense-core A\( \beta \) plaques are more densely concentrated in lateral temporal and occipital lobes while limbic areas, including the hippocampus, amygdala and entorhinal cortex region, contain the fewest dense-core A\( \beta \) plaques. Therefore, it has been hypothesized that \([^{18}F]FDDNP may be an in vivo marker for NFT as well as for diffuse and dense-core A\( \beta \) plaques.

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This could increase the ability of this tracer to detect presymptomatic AD, but it also suggests that \[^{18}F\]FDDNP is not a solely Aβ-specific radiotracer, complicating its use in monitoring the effectiveness of Aβ-reducing medication. Moreover, \[^{18}F\]FDDNP PET may also be positive for tau aggregation in frontal-lobe dementia dementia37 and for prion pathology.38,39 The use of \[^{18}F\]FDDNP as an Aβ PET tracer is further complicated by the intriguing finding that FDDNP competes with some but not all non-steroidal anti-inflammatory drugs (NSAIDs) for binding to Aβ fibrils in vitro and to Aβ plaques ex vivo,40 and by the fact that the conventional parameters for kinetic analysis of receptor binding (such as affinity \([1/K_d]\) and receptor density \([B_{max}]\)) may not be sufficient to accurately quantify Aβ binding.41

The second successful in vivo attempt to image Aβ plaques in the AD brain used the benzothiazole aniline derivative \[^{11}C\]2-(4’-(methylaminophenyl)-6-hydroxybenzothiazole (([^11]C)6-OH-BTA-1, also referred to as \[^{11}C\]PIB), which has been reported to bind specifically to fibrillar Aβ at tracer concentrations in vivo42 (Figure 1). Compared with nine healthy controls, 16 mild-AD patients typically showed marked retention of \[^{11}C\]PIB in areas of association cortex known to contain large amounts of amyloid deposits in AD, such as frontal, parietal, temporal, and occipital cortices and the striatum. \[^{11}C\]PIB retention was equivalent in AD patients and healthy controls in areas known to be relatively unaffected by amyloid deposition, such as subcortical white matter, pons, and cerebellum. Of note, significant and high correlations were observed between in vivo \[^{11}C\]PIB PET and postmortem \[^{3}H\]PIB and Aβ Enzyme-
Linked Immunosorbent Assay (ELISA) uptake in 14 brain regions examined, in one 63-year-old female severe-AD patient.\textsuperscript{43} A significant negative correlation between \textsuperscript{[18]F}FDG and \textsuperscript{[11]C}PIB retention was observed in the parietal cortex but not in the frontal cortex at initial and two-year follow-up evaluations.\textsuperscript{44,45} This is interesting as in vitro studies have suggested that the neurotoxicity of fibrillar Aβ is related to impaired glucose transport\textsuperscript{46} and is enhanced under conditions of reduced glucose metabolism,\textsuperscript{47} while in vivo \textsuperscript{[18]F}FDG PET and postmortem neuropathology data only suggested correlations with NFT but not with Aβ deposition.\textsuperscript{48}

The relationship between glucose metabolism and Aβ pathology may be different in distinct brain regions of AD patients, and Aβ plaque formation may not be directly responsible for neuronal dysfunction\textsuperscript{45} in all brain regions. Simplified quantification methods have been validated for \textsuperscript{[11]C}PIB against kinetic modeling using arterial input data and graphical and compartmental approaches,\textsuperscript{49,50} and parameters derived from 60 minutes may be similar to those from 90 minutes acquisition time.\textsuperscript{51} Voxel-based analyses of \textsuperscript{[11]C}PIB PET data have confirmed the previously obtained region-of-interest data\textsuperscript{52,53} and have shown that \textsuperscript{[11]C}PIB PET was superior to \textsuperscript{[18]F}FDG PET in discriminating mild-to-moderate AD patients from healthy controls.\textsuperscript{45,53} Also, \textsuperscript{[11]C}PIB provided better contrast between three AD patients and three controls than \textsuperscript{[18]F}FDDNP.\textsuperscript{54}

Figure 1

\textbf{[11]C}PIB Binding: Control vs. Mild AD

\textbf{A.} Parametric maps of Logan’s invasive model Distribution Volume (a measure for Aβ binding potential) from data 0-120 minutes after IV injection of 10.4 mCi \textsuperscript{[11]C}PIB in a 74-year-old female healthy control subject.\textsuperscript{121}

\textbf{B.} Parametric maps of Logan’s invasive model Distribution Volume (a measure for Aβ binding potential) from data 0-120 minutes after IV injection of 9.5 mCi \textsuperscript{[11]C}PIB in a 74-year-old female mild-AD patient.\textsuperscript{121}

The third successful in vivo attempt to image Aβ plaques in the brain of AD patients compared the novel stilbene derivative \textsuperscript{[11]C}4-Methylamino-4’-hydroxystilbene (\textsuperscript{[11]C}SB-13) with \textsuperscript{[11]C}PIB in five female AD patients versus six matched healthy controls\textsuperscript{121} (Figure 2). The two radiotracers demonstrated similar binding properties with respect to regional distribution of retention (increased retention in the frontal and posterior temporal-inferior parietal association cortices in the AD patients, but not in
the controls). The data indicated that $^{[11]}$C$SB^{-13}$ may be similar to $^{[11]}$C$PIB$ in discriminating AD patients from healthy controls.

The fourth successful in vivo attempt to image Aβ plaques in the brain of AD patients used the benzoxazole derivative $^{[11]}$C$2$-[2-(2-dimethylaminothiazol-5-yl)ethenyl]-6-[2-(fluoro)ethoxy]benzoxazole ($^{[11]}$C$BF^{-227}$) and showed retention in the frontal, temporal and parietal cortices in 10 AD patients, who could be distinctly differentiated from 11 healthy controls.55

The fifth successful in vivo attempt to image Aβ plaques in the brain of AD patients was made with a SPECT radioligand: $^{[123]}$I$6$-iodo-2-(4’-dimethylamino)phenyl-imidazo[1,2-$\alpha$]pyridine ($^{[123]}$I$IMPY$).56,57 In one study, the average cortical:cerebellar equilibrium distribution volume ratios were 1.25 in eight AD patients versus 1.06 in seven healthy controls,57 and 1.22 in four AD patients versus 0.85 in three healthy controls in another study.56 However, $^{[123]}$I$IMPY$ may not be selective for Aβ only, as it has also been reported to bind to prion deposits in scrapie-infected mice.58 Additional studies are in progress to more fully validate $^{[123]}$I$IMPY$ as a potential tool for assessing AD onset and progression. Given the longer radioactive half-life of $^{123}$I (13.2 hours), such tracers could be synthesized at one location and transported to a nuclear-medicine facility with a SPECT scanner at another location, greatly increasing the accessibility of this Aβ imaging method.

### Aβ Modification Therapies

Aβ modification therapies target amyloid production, amyloid aggregation, and/or amyloid degradation. Some of them are being tested in on going clinical trials.61-64 Alpha-secretase activators include statins and estrogen. It has been suggested that some AChEIs may stimulate the non-amyloidogenic α-secretase cleavage of APP as well.65 Beta-secretase inhibitors include TAK-070.66 Gamma-secretase inhibitors include LY45-0139,67 nonpeptidic isocoumarin compounds (JLK inhibitors),68 STI571 imatinib mesylate,69,70 and NSAIDs.70 Gamma-secretase modulators include R-flurbiprofen (MPC-7869 or tarenflurbil).71,72 Especially, tarenflurbil has finished its Phase 2 trials in Canada and England in 2005, and presently Phase 3 trials in AD patients are ongoing in the U.S. and Canada. Tarenflurbil has shown apparent effect on activities of daily living, CDR score, and the Alzheimer’s Disease Assessment Scale-cognitive items (ADAS-cog) test.73

Amyloid-aggregation-targeting therapies by antifibrillization...
include the glycosaminoglycan mimetic NC-531 (tramiprost), PBT-2, PPI-1019, and TTP-448. Tramiprost was originally developed by Neurochem in Montreal, Canada. The results of a Phase 3 trial have been collected but—to our knowledge—not yet been reported.

The very latest drug is a cyclohexanehexol stereoisomer, which blocks the accumulation of Aß oligomers and reduces AD-like behavioral deficits, AD-like neuropathology, and accelerated mortality in a transgenic mouse model of AD. Because this drug is able to alter Aß pathology even after the symptoms appear, it seems very useful to be applied not only to preclinical-AD subjects but also to AD patients.

Immunization is one Aß modification therapy option that has been studied in transgenic mice and AD patients. Although immunization improves cognitive function in APP transgenic mice and may slow cognitive decline in AD patients, and although it reduces Aß plaques in APP transgenic mice and possibly also in AD patients, the studies in AD patients had to be terminated prematurely owing to brain hemorrhage and/or meningoencephalitis. Pathological evidence of the post-immunization patients showed that, although there is no effect on the frequency and severity of CAA per se, hemorrhages could clearly be attributed to amyloid-laden blood vessels, and bleedings only occurred in brain areas affected by CAA. Antibody responders to active immunization with AN1792 had better cognitive function but more brain volume loss. Passive immunization has been tested in PDAPP transgenic mice but to our knowledge, no clinical studies have been performed.

**Potential Impact of Aß Imaging on AD Management**

As cerebral fibrillar Aß deposition may occur decades before the manifestation of the clinical AD syndrome, imaging of this pathology in vivo may gain considerable amount of time for therapies that intend to prevent Aß accumulation, (e.g., by inhibiting fibrillar Aß production or aggregation). A syndrome of amnestic mild cognitive impairment (MCI) has been identified for which subjects are at an increased risk of progression to AD. MCI subjects have—depending on the definition and group from which the subjects have been recruited—an annual incidence of about 12% progressing to AD in contrast to 1% to 2% for cognitively normal subjects from the same community. These increases in [11C]PIB uptake in non-demented subjects may be related to performance decline in cognitive tests that are highly sensitive to AD-like memory changes.

Of interest, the pattern of Aß deposition in subjects with autosomal dominant PS1 mutations, predisposing to early onset familial AD, is different from sporadic late onset AD, with earlier and higher [11C]PIB retention in the striatum.

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Contraindication to A\textsubscript{\beta} immunization therapies to be fully effective. Amnestic MCI for these preventive therapies to be fully effective.

Since CAA is assumed to be a contraindication to A\textsubscript{\beta} immunization (vide supra),\textsuperscript{81} screening for this prior to immunization therapy might improve its results. The gradient-echo (GE) or T2-weighted MRI technique and A\textsubscript{\beta} imaging could be performed in AD patients to assess for CAA and cerebral \textbeta-amylloidosis, respectively, prior to immunization therapy to prevent post-immunization brain hemorrhage.\textsuperscript{26,81} Moreover, \textglb\textsuperscript{11}C\textsubscript{\beta} PET might also be able to detect CAA in the absence of cerebral \textbeta-amylloidosis.\textsuperscript{102}

Given the fact that there appeared to be no change in A\textsubscript{\beta} binding measured with \textglb\textsuperscript{11}C\textsubscript{\beta} PET over two years in mild-AD patients,\textsuperscript{44} and that the test-retest reliability of \textglb\textsuperscript{11}C\textsubscript{\beta} PET is about 3\% to 7\%,\textsuperscript{44,50} it has been estimated that anti-A\textsubscript{\beta} therapy needs to induce at least a 15\% decrease in A\textsubscript{\beta} load before its effect can be detected.\textsuperscript{103}

Incorporating in vivo A\textsubscript{\beta} PET may make clinical trials more efficient, as the target patient population group can be better defined and a relevant neurobiological outcome measure can be assessed that may be more sensitive than, and predictive of, assessments of clinical outcome.

**Conclusions**

A\textsubscript{\beta} PET can contribute to the management of AD by helping to:

- establish whether there is a cerebral \textbeta-amylloidosis underlying the dementia syndrome, which can help with the differential diagnosis of the potential cause(s) of the dementia;
- identify patients at risk of developing AD, who would be suitable candidates for anti-A\textsubscript{\beta} therapies (particularly medications that target A\textsubscript{\beta} production or aggregation);
- select patients for anti-A\textsubscript{\beta} therapies that could have serious adverse effects, such as A\textsubscript{\beta} immunization; and
- monitor the efficacy of anti-A\textsubscript{\beta} therapies.

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