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Imaging Alzheimer's disease pathology: one target, many ligands

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Over the past five years there has been a surge of interest in using positron emission tomography (PET) to determine the *in vivo* density of the senile plaque, a key pathological feature of Alzheimer's disease. The development of the tracers [^{11}C]-PIB, [^{11}C]-SB13 and [^{18}F]-FDDNP has coincided with drug strategies aimed at altering the brain metabolism of amyloid-β peptides. The evolution of these novel ligands serves not only as an excellent example of how rapidly imaging technologies can progress but also as a reminder that the fundamental biological knowledge, which is necessary to fully interpret the PET data, can be left trailing behind.

At their best, *in vivo* imaging modalities such as positron emission tomography (PET) and magnetic resonance imaging (MRI) have the ability to provide quantitative, high-resolution data that detail biological processes and structures, thus ensuring that these complementary imaging techniques have the ability to influence key decision points in the drug discovery process [1]. For example, with preclinical PET, biodistribution studies provide quantitative information on the penetration of the labelled drug into different tissue compartments, and receptor-occupancy studies can aid dose selection for future clinical trials. In the clinical setting, and especially in early-phase trials, the readouts from imaging techniques can be useful in enriching patient populations or acting as surrogate or direct markers for drug activity (i.e. pharmacodynamic markers). Imaging has the greatest potential to provide the greatest impact on neurodegenerative and psychiatric disease research owing to the present inability to non-invasively and routinely sample the brain.

Alzheimer's disease (AD), the most common form of dementia, slowly destroys cognitive regions of the brain. Despite advances in clinical screening since its first characterization a century ago [2], the method for the definitive diagnosis of AD is still neuropathological examination of the brain [3]. The gross pathology of the AD brain at postmortem is typically characterized by narrowing of the gyri and widening of the sulci, thinning of cortical ribbon, hippocampal atrophy and ventricular enlargement; however, none of these features are sufficient to define AD [4]. Instead, the key pathological features of AD – senile plaques (SPs) and neurofibrillary tangles (NFTs) – are identified using either histopathological dyes (e.g. Congo Red or thioflavin S) or immunohistochemistry. SPs and NFTs are mainly composed of aggregated (polymeric) forms of amyloid-β (Aβ) peptide (both 1–40 and 1–42) and hyperphosphorylated Tau (phospho-Tau) proteins, respectively. The presence of these lesions at sufficient densities with a defined neuroanatomy is considered diagnostic for AD [3]. In addition, characteristic AD pathology includes neuronal loss or atrophy, gliosis and, in many cases, the presence of cerebral amyloid angiopathy (CAA) and Lewy bodies (LBs) [5].

AD is primarily a disorder that affects aging individuals, with accompanying risk factors that are associated with an APOE4 genotype [6] and that overlap with those of cardiovascular disease [7]. Familial AD cases are rare, accounting for ~5% of total cases, and are linked to mutations either in the gene encoding Aβ peptides [e.g. the amyloid precursor protein (APP)] or genes linked with Aβ cleavage from APP (e.g. those encoding Presenilin 1 and Presenilin 2) [8]. These familial cases, in particular, have provided a persuasive argument for the amyloid hypothesis of AD, which places the Aβ peptide as the central, causative disease agent [9]. In response to this theory, a range of interventional strategies aimed at either reducing the production of Aβ peptides (i.e. use of γ- and β-secretase inhibitors [10]) or modulating their clearance (i.e. 'active' and 'passive' immunization [11]) from the brain are at various stages in the drug-development process. These treatments

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will provide a crucial, and perhaps definitive, test of the importance of Aβ in disease onset and progression.

However, to progress these Aβ-modulating drugs into the clinic rapidly, reliable (and, perhaps less importantly, specific) markers of disease activity and progression are required. As with all neurological disorders, detecting peripheral (blood) markers is challenging because the blood–brain barrier prevents the free diffusion of potential analytes from the central nervous system compartment. Cerebrospinal fluid (CSF) provides a more attractive biofluid than plasma for marker identification because its composition is anatomically more reflective of the interstitial fluid that bathes the parenchymal cells. However, the necessity to collect CSF via lumbar puncture (in living individuals) means that there is still a considerable spatial factor (and a cellular barrier) between the fluid withdrawn by the needle and the neuronal cells. This is echoed in the observation that, at present, a CSF measure of soluble Aβ1–42 combined with a Tau:phospho-Tau ratio is the only specific clinical marker of AD [12,13].

The employment of an imaging modality that could track disease progression and, in addition, be sensitive to therapeutic intervention is extremely attractive. One such technology that has attracted extensive attention in the AD field is PET, which employs imaging agents (radiotracers) with high affinity for the aggregated forms of Aβ found in SPs. PET is a non-invasive imaging technique that enables *in vivo* quantification of biochemical and physiological processes [14]. Clearly, the low resolution of PET (compared with MRI) does not enable the visualization of individual SPs. Instead, regional maps (i.e. tomograms) that reflect areas of ligand retention, and hence plaque density, are constructed.

Out of the myriad of agents that have been reported to target Aβ polymers, only three have so far moved forward into evaluation studies using cohorts of AD patients: [^{18}F]-FDDNP [15], [^{11}C]-PIB [16] and [^{11}C]-SB13 [17] (full chemical nomenclature is provided in Box 1). The results from initial studies are encouraging; all three ligands display uptake and retention in areas of the brain that are known to contain high densities of plaques [18]. Here, I focus on: (i) the occurrence of amyloid structures in AD (and other neuropathologies); (ii) the chemical evolution of the amyloid imaging tracers; (iii) models of ligand binding to Aβ fibrils; (iv) *in vivo* imaging studies using amyloid imaging agents; and (v) the clinical relevance of amyloid in AD.

BOX 1

Chemical nomenclature of compounds relevant to this review

6-Me-BTA-1	2-[4′-(methylamino)phenyl]-6-methylbenzothiazole
BTA-1	2-[4′-(methylamino)phenyl]benzothiazole
6-OH-BTA-1 (PIB)	2-[4′-(methylamino)phenyl]-6-hydroxybenzothiazole (Pittsburgh compound B)
DDNP	1,1-dicyano-2-[6-(dimethylamino)naphthalene-2-yl]propene
FDDNP	2-(1-{6-[(2-fluoroethyl(methyl)amino]-2-naphthyl} ethylidene)malononitrile
FEM-IMPY	6-iodo-2-[4′-*N*-(2-fluoroethyl)methylamino] phenylimidazo[1,2-*a*]pyridine
SB13	4-*N*-methylamino-4′-hydroxystilbene
BSB	(trans,trans)-1-bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene
X34	1,4-bis(3-carboxy-4-hydroxyphenylethenyl)benzene
Methoxy-X04	1,4-bis(4′-hydroxystyryl)-2-methoxybenzene
FENE	1-{6-[(2-fluoroethyl)(methyl)amino]naphthalen-2-yl}ethone
TZPI	2-[4′-(4″-methylpiperazin-1-yl)phenyl]-6-iodobenzothiazole

The occurrence of amyloid structures in AD

Fundamental to the understanding of the *in vivo* signal generated by any PET tracer is a detailed knowledge of the receptor complex to which the ligand binds. In the case of AD, the 'receptor' is a polymer composed of Aβ peptide subunits that are arranged in an ordered, hierarchical manner into a mature structure termed an amyloid fibril [19]. The polypeptide backbone of each Aβ peptide in the fibril is folded into the same basic motif, termed a β-pleated sheet or a cross β-structure [20]. It is the unique stereochemistry of this fold that confers the ability of the fibrils to bind specifically to the histological dyes Congo Red and thioflavins S and T [19]. These stains are important because they serve as initial starting templates for two of the chemical series developed as potential Aβ PET ligands (see later section about the chemical evolution of the amyloid imaging tracers).

Within the AD brain there are two topologically distinct extracellular pools of fibrils composed of Aβ peptides: (i) parenchymal SPs; and (ii) deposits in the walls of arteries and arterioles of the cerebral cortex (and the leptomeninges) that give rise to cerebrovascular amyloid angiopathy (CAA) [21] (Table 1). There are also distinct biochemical differences between these two Aβ pools. SPs usually have high concentrations of Aβ1–42 species and, in addition, contain a number of post-translational modifications including oxidation, isomerizations and N-terminal truncations [22,23]. Vascular Aβ deposits are, by comparison, spared from post-translational modification [24] and tend to contain high concentrations of Aβ1–40 peptides [25,26]. Understanding the relative tracer-binding characteristics of these two amyloid pools *in vivo* is fundamental to

TABLE 1

Summary of amyloid pathologies in AD, tauopathies (TauP) and Lewy body dementias (LBD)

Pathological feature	Polypeptide	Disease[a]			Amyloid location	Post-translational modifications
		AD	TauP	LBD		
Senile plaques	Aβ peptides 1–40	+	–	(+)	Extracellular	+ (truncation, racemization or oxidation)
Cerebral amyloid angiopathy	and 1–42	(+)	(+)	(+)	Abluminal[b]	–
Neurofibrillary tangles	Tau	+	+	(+)	Intraneuronal	+ (phosphorylation or truncation)
Lewy bodies	α-Synuclein	(+)	(+)	+	Intraneuronal	+ (phosphorylation)

[a] +, pathology always present; (+), variable pathology; –, no pathology.
[b] in cerebral blood vessels.