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BACE1: Now we can see you

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Abstract

No *in vivo* imaging biomarker currently exists for BACE, a drug target for Alzheimer's disease (AD). A strategy aiming to find a novel brain-penetrant positron emission tomography (PET) radiotracer for BACE1 led to the discovery of a highly potent and selective aminothiazine inhibitor, PF-06684511. This scaffold has been now evaluated as BACE1 PET radiotracer, ([^{18}F]PF-06684511) after labelling with fluorine-18 (^{18}F) allowing its evaluation in non-human primates (NHP) as the first a brain-penetrant PET radiotracer for imaging BACE1 *in vivo.*

BACE1 inhibitor drug development for AD treatment has proven to be challenging due to the high attrition rate of drugs entering into clinics, however there are still some promising BACE1 drug candidates in on-going clinical trials.[1] BACE1, also known as β-site amyloid precursor protein cleaving enzyme 1, is a transmembrane enzyme which plays a crucial part in AD pathogenesis by initiating the production of the toxic protein amyloid-β which aggregates to form amyloid-β plaques.[2]

The production of a BACE1 PET radiotracer would help to address unmet needs in AD research such as: 1) monitoring and progression of brain BACE1 levels from presymptomatic to late stage AD; 2) establish a temporal relationship between BACE1 expression *versus* amyloid-β plaque formation, neurofibrillary tangles and neuroinflammation; 3) determination of target occupancy of therapeutic BACE1 inhibitors; and 4) the selection of non-heterogeneous AD cohorts for clinical trials. Therefore, the possibility of a non-invasive, high resolution, quantitative tool to gain information on BACE1 *in vivo* has fuelled a demand for BACE1 PET radiopharmaceuticals.

In this issue, Lei Zhang and collaborators at Pfizer and Karolinska Institutet put in place a rational, cost-efficient, four-step discovery process for the development of BACE1 PET radiotracers (**Figure 1**).[3] This involves: a) determination of target expression *in vitro*; b) radiotracer design and lead prioritisation through a combination of *in silico*, *in vitro* and *in vivo* approaches; c) PET radiotracer production; and d) PET radiotracer assessment *in vivo*.

In the first step, the maximum concentration (B_{max}) of BACE1 was determined in mouse brains. The authors identified a potent BACE1 inhibitor, **1** (IC_{50} = 12 nM, **Figure 2**), as a suitable candidate for tritium ([^{3}H]) radiolabelling to enable *in vitro* binding studies. The BACE1 specificity of compound **1** and BACE1 B_{max} was determined in frontal cortex regions of wild-type (WT), BACE1 heterozygous, and homozygous knockout (KO) mouse brain tissues. The brain binding of [^{3}H]**1** in WT mice was reduced by roughly 50% in heterozygous BACE1 KO mice and abolished in homozygous BACE1 KO mice, confirming that [^{3}H]**1** binds selectively to BACE1.

The determination of BACE1 B_{max} (approximately 7 nM) was essential for PET radiotracer triage, using the empirical formula B_{max}/K_d ratio ≥ 10,[4, 5] using this method the affinity (K_d) required for a successful BACE1 PET radiotracer was estimated to be ≤ 0.7 nM. Compound **1** was rejected as potential PET radiotracer because it was found to be a substrate of the P-glycoprotein (P-gp) transporter and a non-optimal B_{max}/K_d ratio of 3.8.

For the next step, sequential filters were applied to a 790 BACE1 inhibitor library in order to identify PET radiotracers able to enter into the brain, with strong BACE1 binding, and low non-displaceable binding. Preferred filters were: high passive permeability (measured by Ralph Russ canine kidney apparent permeability coefficient apical-to-basolateral, RRCK P_{app} AB $> 5 \times 10^{-6}$ cm/s), low drug efflux pump liability (measured by multidrug resistance protein 1 basolateral-to-apical/apical-to-basolateral ratio, MDR1 BA/AB ≤ 2.5), high potency to inhibit BACE1 ($IC_{50} < 5$ nM), and high brain free-fractions, as a predictor of low non-displaceable binding (Fu_b > 0.05) (**Figure 1**). Applying these filters, the library of 790 compounds was narrowed down to 16 candidates. It is noted that the authors used IC_{50} values instead of K_d values for B_{max}/K_d estimation. The validity of this approach is strictly speaking incorrect and would require further studies to validate the use of IC_{50} values in this context.

Subsequently, a multi-parameter optimization score[6] (CNS PET MPO score > 3) taking into account LogP, LogD, molecular weight, topological polar surface area, number of hydrogen bond donors, and pK_a, combined with a visual inspection of moieties amenable to ^{11}C- or ^{18}F-labeling generated two lead compounds, PF-06684511 (**2**) and **3** (**Figure 2**).

In order to select a single lead compound, *in vivo* non-displaceable binding assessment studies were performed on **2** and **3** using a liquid chromatography–mass spectroscopy/mass spectroscopy (LC-MS/MS) method. The non-displaceable binding was determined in four brain regions: cerebellum, frontal cortex, hippocampus, and striatum. Compounds **2** and **3** were injected into mice at tracer concentrations (iv, 10 μg/kg) and evaluated by comparing a control group with a blocking group pre-treated with a high dose of a selective fused aminodihydrothiazine BACE1 inhibitor. Of the two compounds, **2** displayed BACE1 specific binding *in vivo*. The selectivity of **2** for BACE1 was confirmed by demonstrating a significant reduction of binding in BACE1 KO *versus* WT mice brain. In summary, compound **2** was shown to be a potent inhibitor of BACE1, highly selective over BACE2 and other targets, possesses low lipophilicity and good physiochemical parameters, and finally it has low efflux and high passive permeability across the blood-brain barrier. All these factors are "go criterion" important for successful PET radiotracers and therefore **2** is a promising candidate to be translated into a valuable diagnostic tool to be evaluated in preclinical mammalian models.

A distinguishing feature of this paper is that the researchers carefully characterised the *in vitro* and *in vivo* profile of the lead compound **2** before advancing to PET radiotracer assessment, increasing their likelihood of finding a successful PET radiotracer, minimising the effort and cost of PET radiotracer development.

PET radiotracer production started by selecting fluorine-18 (^{18}F) as the radionuclide with which to functionalise **2** for PET imaging applications, based on the assessment of potential radiosynthetic strategies starting from [^{18}F]fluoride. [^{18}F]**2** ([^{18}F]PF-06684511) was obtained in high radiochemical purity (>99%) and with high molar radioactivity of 84-175 GBq/μmol, by displacement of the tosyl moiety of **4** (**Figure 2**) with [^{18}F]fluoride/K2.2.2/K_2CO_3, followed by the removal of the BOC protecting group, HPLC purification and reformulation. The radiosynthetic strategy suffers of a low radiochemical yield for the production of [^{18}F]**2** (4-7%) and multiple step synthesis to obtain precursor **4**, factors that need to be considered for routine productions of [^{18}F]**2**.

Lately, *in vivo* PET assessment of [^{18}F]**2** was performed in NHPs (i.v. 10 μg/kg). [^{18}F]**2** demonstrated good brain uptake and widespread distribution in various brain regions, with increased signal in hippocampus and striatum. The high selectivity of [^{18}F]**2** towards BACE1 was shown by pre-administration of BACE1 inhibitor LY2886721 (5 mg/kg, PO, 120 min prior to PET scanning). Blocking studies showed a markedly reduced signal in the brain. These results suggest that [^{18}F]**2** is a promising radiotracer for BACE1 PET imaging in humans, although the metabolic profile of [^{18}F]**2** has yet to be elucidated and toxicology safety assessment studies are required

before beginning clinical trials. Furthermore, *in vivo* assessment in NHP was only performed in one healthy animal, limiting the statistical significance of the findings, which ought to be replicated in increased subject number and in other research institutes.

The CNS PET ligand discovery process reported by L. Zhang *et al.* is a logical and efficient method, which successfully led to the identification of a novel BACE1 PET radiotracer. The process is based on a comprehensive PET ligand triage that includes: determination of BACE1 density, prioritisation of BACE1 inhibitor guided by *in silico*/*in vitro*/*in vivo* parameters, and the design of a radiosynthetic approach.[3] Following this protocol, a single lead compound **2** was selected from a library of 790 candidates leading to the evaluation of [^{18}F]**2** which showed *in vivo* specificity to BACE1.

Although at present the AD progression cannot be halted or reversed, the availability of a BACE1 radiotracer for PET imaging will allow the *in vivo* disposition of BACE1 and pharmacological characterisation of BACE1 inhibitors to be studied in clinical trials. Furthermore, BACE1 PET imaging could positively affect the study of AD aetiology, and thus assist the discovery of new AD therapies. In the long term, BACE1 PET radiotracers may allow the development of early diagnostic tools for AD, giving patients and physicians the possibility of better lifestyle management and evaluation of disease modifying treatments.

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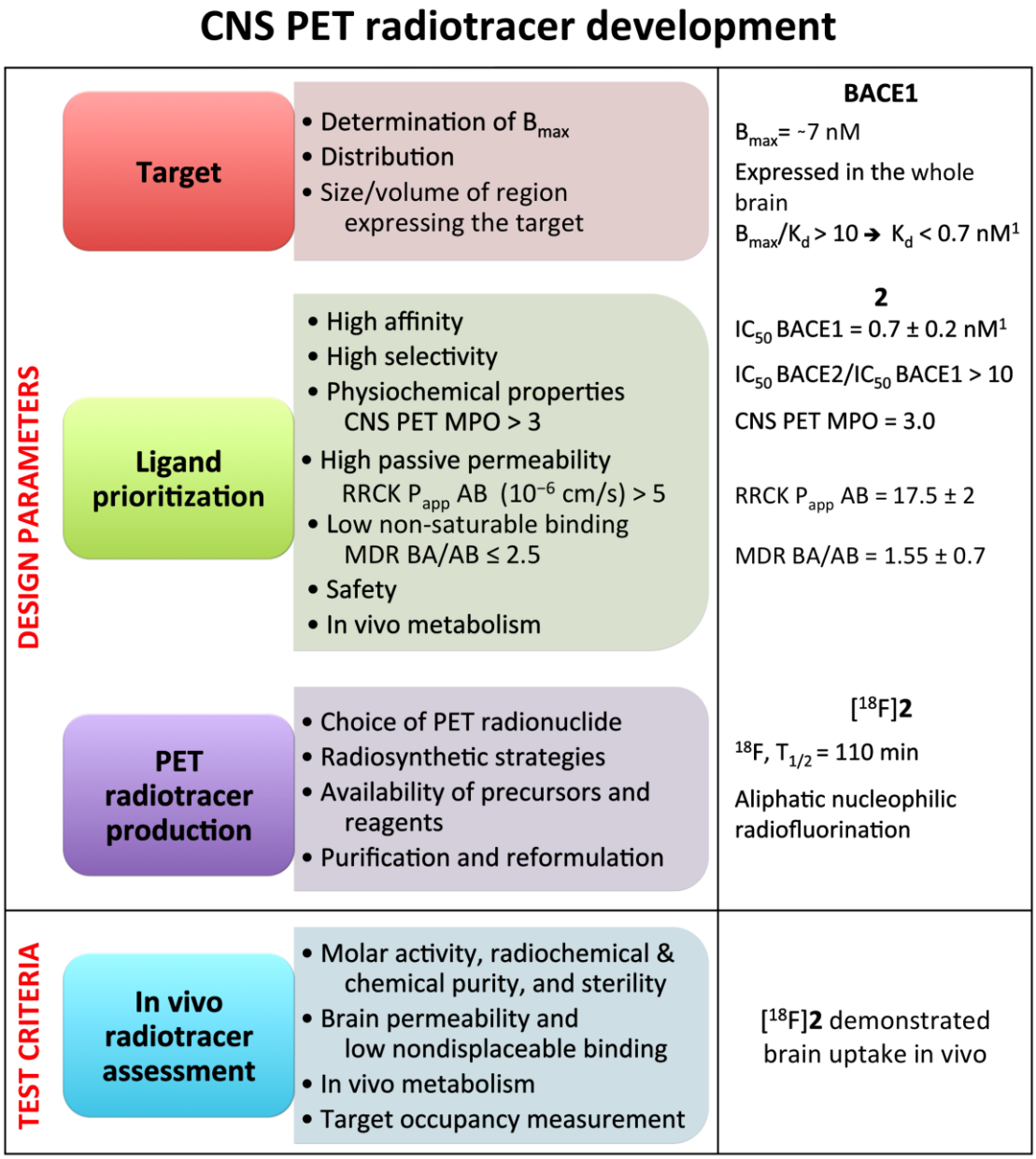
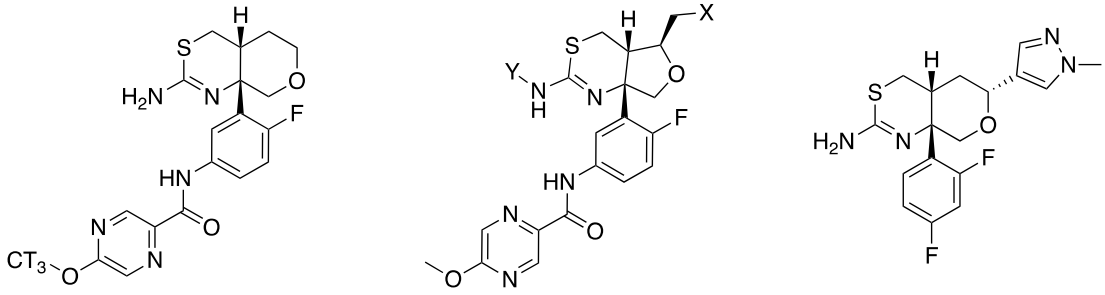


Figure 1 Design parameters and test criteria for CNS PET ligands ([1]See text for discussion of IC_{50} and K_d values).



[^{3}H]**1**

2: X = ^{19}F, Y = H (PF-06684511)
[^{18}F]**2**: X = ^{18}F, Y = H
4: X = OTs, Y = Boc

3

Figure 2 Chemical structure of tritiated compound [^{3}H]**1**, BACE1 inhibitors (**2** and **3)**, BACE1 PET radiotracer [^{18}F]**2** and its precursor **4**.