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ADVANCES IN IMAGING (HADASSAH CONFERENCE)

The application of positron emission tomography (PET) imaging in CNS drug development

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Abstract

As drug discovery and development in Neuroscience push beyond symptom management to disease modification, neuroimaging becomes a key area of translational research that enables measurements of the presence of drugs and downstream physiological consequences of drug action within the living brain. As such, neuroimaging can be used to help optimize decision-making processes throughout the various phases of drug development. Positron Emission Tomography (PET) is a functional imaging technique that allows the quantification and visualization of biochemical processes, by monitoring the time dependent distribution of molecules labelled with short-lived positron-emitting isotopes. This review focuses on the application of PET to support CNS drug development, particularly in the early clinical phases, by allowing us to measure tissue exposure, target engagement, and pharmacological activity. We will also discuss the application of PET imaging as tools to image the pathological hallmarks of disease and evaluate the potential disease-modifying effect of candidate drugs in slowing disease progression.

Keywords PET · Biodistribution · Target engagement · Occupancy · Disease progression · Biomarker · Neurodegenerative disease

Introduction

The productivity of pharmaceutical research and development as measured by new drug approvals per dollar spent has declined in the past two decades, despite advances in scientific and technological practices (Scannell et al. 2012). Setting aside issues relating to business models (Schuhmacher et al. 2016), the development of drugs to treat central nervous system (CNS) disorders is considered to be particularly challenging. Part of the problem is the absence of a good understanding of the human biology underlying certain diseases (Plenge 2016), and the poor predictive value of preclinical models (National Academies of Sciences 2017). However, CNS drug development is subject to the additional inherent challenge of very limited access to the target organ, both for therapeutics to cross the blood brain barrier (Begley 2004), and our ability to predict or directly measure the presence of drug in the brain, and the biochemical/physiological consequences of that presence (Deo et al. 2013). This review will focus on application of positron emission tomography (PET) to support CNS drug development, particularly in the early clinical phases, by allowing us to measure tissue exposure, target engagement, and pharmacological activity.

PET is a functional imaging technique that allows the quantification and visualisation of biochemical and physiological processes, by monitoring the time dependent distribution of molecules labelled with short-lived positron-emitting isotopes. The labelled molecules, referred to collectively as radiotracers, may be drugs themselves, molecules of biological interest which are substrates for a physiological process, or tool compounds which have been developed to bind with a high degree of selectivity to a specific molecular target (e.g. receptor, transporter, enzyme, or aggregated protein). Radiotracers in the latter category are also referred to as PET ligands.

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A common question regarding imaging in drug development is, "What can we do with imaging?" This can unfortunately lead to a long list of things that can be imaged, with little regard for why one might do so, or what if any impact the data will have on the development of a particular drug. A more fruitful approach is to focus on the question arising in the drug development process, and choose a suitable method to answer it. Within such a framework PET can be used to help answer questions such as: does the drug cross the blood brain barrier?; does the drug bind to its molecular target, and what is the relationship between dosing/plasma exposure and that binding (occupancy); is there evidence of biological activity (in humans) consistent with the proposed mechanism of action?; can we select patients more likely to benefit from/respond to treatment?. The often linear nature of the drug development process means that such questions tend to be asked, hopefully answered, in a particular order, with knowledge gained in one experiment/study being used to guide the direction of future research. As such PET imaging can play a role at different stages of drug development, as described in Table 1. The main features and distinctions of the different applications of PET are described in Table 2.

Table 1 The role of PET imaging at different stages in the drug development process

Phase 1

- Characterize the tissue distribution of a drug in humans [biodistribution].
- Provide evidence of biological activity in humans [target engagement].
- Quantify the interaction of a drug with its molecular target in vivo [target occupancy].
- Measure pharmacodynamic (PD) responses at the target or downstream [proof of disease relevant pharmacology].

Phase 2

- Select patients for clinical trials [by confirming presence of the target].
- Demonstrate mechanism of action [PD response which may only be observable in patients].
- Evaluate potential surrogate markers [link PD response to clinical observations].
- Explore variability in response.

Phase 3 and beyond

- Produce data to support disease modification claim.
- Support product differentiation (e.g. by comparing the fate of two radiolabelled drugs).
- Increase awareness of a condition through high impact research.
- Support product line extensions (e.g. time course of pharmacodynamic effect with a new formulation)

Biodistribution

Perhaps the most obvious prerequisite to drug action is target exposure. If a drug cannot cross the blood brain barrier and enter the brain parenchyma in an adequate quantity, desired CNS effects will not be achieved. Methods exist to predict blood brain barrier permeability and liability to efflux transporters, based on a drug's physicochemical properties, but PET remains unique in its ability to provide a direct measurement of drug concentration in the living human brain, as well as information on the rate of delivery into the brain (Bergstrom et al. 2003).

A typical CNS biodistribution involves radiolabeling a drug molecule by isotopic substitution of 12C with 11C or of 19F with 18F, avoiding chemical modification, which may alter the behavior of the molecule. The radiotracer is then administered intravenously to a healthy subject at a tracer dose (usually <10 micrograms; allowing studies to be supported by pre-clinical safety studies proportionate to the nature and scope of the human exposure (ICH 2009). The radiotracer's distribution is then tracked over time by acquiring dynamic PET data. At the same time arterial blood samples are drawn from a cannula inserted into the radial arterial. The radioactivity concentration in the plasma (or blood) describes the concentration of the unchanged tracer available for delivery to the brain (the input function). Kinetic analysis allows the PET signal to be corrected for intravascular radioactivity in the brain, and for the delivery of the drug and its partition ratio between the brain and the plasma to be estimated (Gunn et al. 2001). Biodistribution studies may be conducted in the presence of a therapeutically relevant dose of unlabeled compound (given as an oral dose) if it is thought that the brain entry of the drug may be affected by drug dose in a non-linear manner, for example by the saturation of a transporter or induction of an enzyme. Because the passage of a molecule across the blood brain barrier can be assumed to be determined by physical factors, not under conscious control, studies can be conducted in an unblinded fashion without the need for a placebo control. The design of such a study is summarized in Table 3.

Once biodistribution data has been collected a number of parameters can be derived, ranging from the total concentration of radioactivity in the brain through to (in some cases) the inferred occupancy of the drug at its target. In the context of drug development, it is useful to compare these parameters to those commonly used to describe BBB penetration as obtained by in vitro and pre-clinical in vivo studies, often referred to as Drug Metabolism and Pharmacokinetics (DMPK) (Table 4). Having obtained a result, expressed for example as the total volume of distribution (V_T; equilibrium partition coefficient of between brain tissue and plasma (Innis et al. 2007)), one can ask: how much drug in the brain is sufficient for clinical efficacy? This simple question, rarely leads to a simple answer. If we knew the free fractions of drug in plasma

Table 2 Types of imaging study classified by application

	Biodistribution	Occupancy	Pathology	Downstream pharmacodynamics
Measurement	Measures the brain uptake of a radiolabelled drug.	Measures blockade of the binding of a radioligand to a drug target.	Measures binding of a radioligand to a molecular hallmark of pathology.	Measures downstream consequence of target modulation
Requirements	Requires the drug to be radiolabelled with carbon-11 or fluorine-18, which is almost always feasible.	Requires the existence, or development, of a PET ligand (usually different to the drug), which is not always feasible. • ligand development is problematic. • Target density too low (<1 nM or 10 fmole/mg protein). • molecular target is unknown.	Requires the existence of a PET ligand, though the target of the ligand need not be the same as the target of the drug.	May use a PET ligand, or a tracer that is a substrate for a biological process of interest.
Value	Target exposure	Target engagement	Patient identification (presence of target) Proof of mechanism pharmacodynamics	Measured physiological response often closely linked to behavioral or clinical outcomes.

and tissue, the amount of the target available to the drug, the in vivo affinity of the drug, the required occupancy for therapeutic effect, the pharmacokinetic behavior of the drug, and the likely therapeutic window, then it would be possible to estimate the V_T required, however, such information is rarely available..

As a rule of thumb, very low V_T measured in the course of a PET experiment (< 0.2) indicates very low levels of brain entry, if any, that may not be confidently distinguished from experimental noise, intravascular radioactivity, or scatter from extra-cranial radioactivity. Such molecules are often considered to have a poor profile for a CNS drug and further development is assumed to carry a higher risk of failure. However, under certain circumstances further development may be justified, for example, where the drug is well tolerated and high plasma concentrations may be achievable, leading to efficacious brain levels; or in the case of drugs that have very high affinity for their target such that they illicit pharmacodynamic effects at very low levels of receptor occupancy (e.g. agonist of opioid receptor).

The interpretation of in vivo PET data, particularly with regard to CNS biodistribution can be supported by equilibrium dialysis measures of the free fraction of radioligand in plasma (f_p) and the non-displaceable compartment tissue (f_{ND}) as described by (Gunn et al. 2012), which can provide additional insights into the mechanisms of brain entry/efflux. In the absence of a measurable displaceable or specific binding component (i.e. reference region, where V_s is assumed to be 0), V_T equals to total non-displaceable volume distribution in the brain (V_{ND}), the f_{ND} is the free fraction of the non-displaceable compartment (free and non-specific) in the brain, while the f_P is the free fraction of the radioligand in the plasma (Innis et al. 2007).

In this situation, If V_T is:

- > f_P/f_{ND}, brain entry exceeds that expected from passive diffusion alone, i.e. active or facilitated transport into the tissue is present, and at equilibrium a higher free drug concentration will be present in the brain than in the plasma
- ~ f_P/f_{ND}, the drug is entering via passive diffusion and at equilibrium the brain free drug concentration will be the same as that in plasma
- < f_P/f_{ND}, brain entry is lower than that expected from passive diffusion alone, i.e. active or facilitated transport out of the brain is present (e.g. via the PgP transporter), and at equilibrium a lower free drug concentration will be present in the brain than in the plasma.

Table 3 CNS biodistribution design parameters

- 4–6 healthy subjects
- T1 weighted MRI scan to provide a structural image and full out confounding pathology
- Unlabelled oral dose of study drug [optional]
- Dynamic PET acquisition following injection of a microdose of C-11 or F-18 labelled drug
- Arterial blood samples to correct for radiolabelled metabolites
- Tracer kinetics analysis including blood volume correction.

One application of biodistribution studies which warrants further discussion is the evaluation of the brain penetration of large molecular weight biological molecule such as antibodies. Recombinant antibodies have shown some promise (based upon in vitro systems and pre-clinical models) in treating aspects of CNS diseases (Neves et al. 2016), however, under normal conditions their passage from the blood across the BBB is restricted. Intraparenchymal, intracerebroventricular, and intrathecal injections can be used to deliver large molecules directly to the CNS compartment, but these routes

Table 4 The relationship between parameters obtained in PET biodistribution studies and in vitro measures of BBB penetration

PET term	DMPK equivalent(s)	Description	Comments
C_T (Bq mL^{-1})	$C_{(brain)}$ (μM, ng mL^{-1})	Total conc. in brain. In PET this is radioactivity not drug.	Describes CNS exposure, but not pharmacologically active concentrations.
V_T (mL cm^{-3})	Brain:plasma ratio (K_p)	Total brain:total plasma ratio at equilibrium (note for V_T mostly V_{ND} i.e. volume distribution of non-specific and free).	Reflects transport, plasma protein binding, and non-specific binding in the tissue, and can vary widely between centrally active compounds. Advantage of PET is that it is measured in human.
K_1 (mLcm^{-3} min^{-1}) or k2	PS product (also known as K_{in} or Cl_{in} (μL min^{-1} g brain^{-1}) or K_{out} or Cl_{out}	Clearance from arterial plasma to tissue, or from tissue to plasma	Describes BBB permeability i.e. rate of transport across BBB
C_{FT} (Bq mL^{-1})	$C_{u(brain)}$ (μM, ng mL^{-1})	Free concentration in brain	Predicted using PET data and free fractions obtained by equilibrium dialysis (specific volume of distribution (V_s) is assumed to be 0 and V_T is assumed to be equal to V_{ND}). $C_{FT} = V_{ND} * f_{ND} / f_p * C_{FP}$
C_{FT}/C_{FP}	Approx. $Kp_{,uu}$? [in PET C_{ISF} and C_{ICF} will be pooled]	Free concentration in brain:free concentration in plasma, concentration gradient at BBB.	Describes the extent of equilibrium across the BBB. 1 = passive diffusion; <1 = active efflux; >1 = active influx
Occupancy (%)	Displacement of radioligand binding [in PET normally blocking]	Drug occupancy at the molecular target	If you assume passive diffusion, and that the in vitro K_D equals the in vivo K_D, free concentration in the brain can be used to estimate occupancy. Occupancy = $C_{FT}/(C_{FT}+ K_D)$

are invasive and often impractical. Therefore, understanding influx and efflux of such molecules may be critical to identifying efficacious drugs, and designing dosing regimens that can achieve therapeutic brain levels. Evaluating the CNS distribution of antibodies is, however, challenging. The long plasma half-life of antibodies means that even low rates of blood-brain barrier penetration may be sufficient to allow the achievement of therapeutic drug concentrations in the brain, and studies should examine drug distribution into the brain over periods much longer than feasible with most common PET radioisotopes. Labelling with radionuclides such as the radiometal zirconium-89 (half-life, 78.4 h) may provide better time scale for such studies, but high radiation burden, and poor imaging characteristics of zirconium-89 limit the populations or locations in which macromolecule biodistribution studies can be conducted (ICRP 1992), and the time scale available may still not be sufficient to evaluate molecules that achieve equilibrium distribution in weeks or months. In cases when human biodistribution of a macromolecule is considered, chelation and labelling with a radiometal means that further testing is required to ensure chelation and labelling has not changed important properties of the drug such as the immunoreactivity (Tolmachev et al. 2014). Antibodies are also prone to target mediated drug disposition (the so called antibody sink effect) resulting in non-linear pharmacokinetics, meaning that peripheral uptake may influence the amount of tracer available to the brain. Finally, it should be noted that pharmacokinetic models used to quantify PET data obtained with small molecule radiotracers cannot be easily applied to antibodies, and in addition to aforementioned factors, antibodies may also enter the brain by different mechanisms, such as transcytosis (Broadwell 1989) and extracellular pathways (Banks 2004) as opposed to passive diffusion or active transport.

In principle any study in which PET data is acquired over the brain can be considered to be a CNS biodistribution study, however, the number of published studies explicitly investigating the CNS biodistribution of drug candidates is limited (Christian et al. 1996; Bauer et al. 2006; Huiban et al. 2017). This is perhaps explained by the fact that if a drug candidate fails to cross the BBB its development interest is low, whereas if a drug does cross the BBB publication may be delayed until information concerning chemistry and pharmacology has been disclosed.

In summary biodistribution studies can provide data on the delivery of drugs to the brain in humans, including: brain: plasma ratio; Influx and efflux rates; free concentration in brain (in combination with equilibrium dialysis). These data can be obtained early: microdose studies require a reduced toxicology package and can in theory take place before traditional Phase 1 studies. Positive data can be used to: Prioritize one molecule over another (around Phase 1); differentiate a molecule from the competition; predict occupancy in the absence of a PET ligand. However, making a decision based on negative data may not be easy. CNS biodistribution studies with macromolecules are particularly relevant due to their low BBB permeability, but are technically challenging.

Target occupancy

Basic pharmacological principles indicate that drug clinical efficacy is a function of their binding to their molecular targets. Fractional target occupancy is a function of free drug concentration in the brain, which in turn is a function of its plasma concentration and transport kinetics. Thus linking of drug plasma concentration to target occupancy allows the understanding of the drug concentration-occupancy relationship that once established, can be used in all future studies in the drug development process.

A PET scan with a suitable radioligand can quantify the density of a molecular target in the brain. By measuring the effect of different doses of a drug on the number of available targets, and comparing these with the target concentration at baseline, occupancy studies can demonstrate brain penetration and target engagement, as well as allow us to evaluate the relationship between plasma exposure and target occupancy. In addition to providing a high degree of confidence that a drug is binding to its intended target in vivo in human, occupancy studies can refine the likely efficacious dose range. These studies require the availability of a well characterized PET ligands for the target of interest. Such a ligand must not only be appropriately selective for the target, but must also possess physicochemical and pharmacological characteristics that allow them to be radiolabelled, safely administered to humans, and for their target binding to be quantified (in vivo) (Honer et al. 2014). In addition to target occupancy PET ligands may also be used to measure changes in target availability (density) in health and disease, and are therefore of interest not just to those working directly on drug development. A representative list of PET ligands and tracers with CNS applications that have been shown to be useful in human studies is given in Table 5.

PET measurements of brain target occupancy in drug development should be conducted as early as possible in the development process to allow this information to be used in the majority of clinical studies. As such, occupancy studies should be combined with single ascending dose first in human (SAD FIH) Phase I studies, to provide information that combined with the safety and tolerability data can determine the doses to be used in multiple ascending dose (MAD) safety and tolerability studies and Phase II proof-of-concept (POC) trials. PET occupancy as part of SAD FIH studies, will by necessity involved occupancy measurement after single dose administration, which may present a problem when applying these data to situations involving repeat dose drug administration. If the drug follows "direct kinetics" where the exchange between various compartments is rapid, changes in target occupancy will be driven primarily by changes in plasma concentration, and hence the relationship between drug plasma concentration and target occupancy will be the same during single and repeat dose regimens. This is the situation for the majority of existing CNS drugs. However, some compounds follow "indirect kinetics", where there is a significant delay in the kinetics between blood and brain, and a drop in drug plasma concentration can occur without a corresponding change in target occupancy (see (Abanades et al. 2011) for an example). In such a situation, the relationship between plasma concentration and target occupancy is time-dependent, and the translation between single dose and repeat dose occupancy is more complicated, and requires information collected in a time-occupancy study design (Abanades et al. 2011).

Answers to additional frequently asked questions and a guide to the interpretation of dose-occupancy relationships are provided in Supplementary Material (Box 1) and Table 6.

Imaging the pathological hallmarks of disease

Throughout the pharmaceutical industry the application of neuroimaging is increasingly focused on imaging the so called pathological hallmarks of disease, particularly for neurodegenerative disorders, this is in part driven by the advent of biologic based drugs designed to remove or arrest the buildup of toxic misfolded forms of proteins such as β-amyloid (Aβ), tau protein (τ-protein) and α -synuclein (α-SYN). This change in focus has changed the way in which molecular imaging is applied in CNS drug development, as described in Table 7.

Imaging of misfolded protein pathology can complement clinical endpoints in several ways. At screening, confirmation of pathology may be used to enrich and stratify the study population and confirm the presence of pathology. As a pharmacodynamic endpoint, misfolded protein imaging can also be used to evaluate the hypothesis that a treatment is disease modifying as opposed to purely symptomatic, with the assumption being that such direct pathological measures will provide an advantage over clinical endpoints such as cognitive tests in differentiating symptomatic treatment effects from true modification of the underlying pathology.

Both [^{18}F] FDG and Aβ PET imaging have been proposed as biomarkers to evaluate the effect of drug treatment on disease progression in Alzheimer's disease (AD). Sample size estimates using longitudinal natural history data from ADNI indicate that 66 AD patients or 217 patients with mild cognitive impairment (MCI) per treatment group would be required to detect a 25% AD-slowing treatment effect in a 12-month, multi-center RCT with 80% power and two-tailed alpha = 0.05 (Chen et al. 2010). This is comparable to the sample size estimated using MRI-based measurements of regional and whole brain atrophy and it is a fraction of the approximately 600 completers per group estimated to detect a 25% treatment effect using a clinical endpoint, such as the AD Assessment Scale- Cognitive (ADAS-Cog). Although promising, it should be noted that these estimates were based on data from a limited number of subjects and have large confidence intervals.

Table 5 Representative examples of radiotracers for CNS applications that have shown utility in humans

Targets Misfolded proteins	Carbon-11 labelled	Fluroine-18 labeled	Comments
β-Amyloid	[11C]PIB	[18F]Flutemetamol [18F]Florbetapir([18F]AV-45) [18F]AZD 4694 [18F]FBM [18F]FDDNP [18F]-SMIBR-W372 ([F-18]-W372) [18F]Florbetaban [18F]MK3328	
Tau		[18F] T807 (AV1451; Flortaucipir) [18F]GTP1 [18F]RO6958948 [18F]MK6240 [18F]PI-2620	Relative sensitivity to 3-repeat to 4-repeat tau isoforms remains to be confirmed.
Enzymes			
Aromatic amino acid decarboxylase (AADC).		6-[18F]-L-DOPA (FDOPA)	Used to assess dopamine synthesis capacity and storage; providing an indirect measure of functional integrity of the nigrostriatal dopaminergic pathway.
AChE	[11C]MP4A		
Aromatase	[11C]VOR		
FAAH	[11C]CURB		
MAO-A	[11C]Harmine [11C]Clorgyline [11C]Befloxatone		
MAO-B	[11C]Deprenyl-d2		May be used as a marker of astrocytes
PDE4	[11C](R)-Rolipram		
PDE10A	[11C]IMA107 [11C]MP-10 [11C]Lu AE92686	[18F]MNI659	
Receptors			
Adenosine A1		[18F]CPFPX	
Adenosine A2A	[11C]SCH442416	[18F]MNI444	
GABAA	[11C]Flumazenil	[18F]Flumazenil	
GABAA (alpha 5 preferring)	[11C]Ro15 4513		
CB1	[11C]MePPEP [11C]OMAR [11C]SD5024	[18F]FEMMEP-d2 [18F]MK-9470	
D1	[11C]NNC 112 [11C]SCH 23390		
D2/D3	[11C]Raclopride [11C]FLB 457 [11C]MNPA (agonist) [11C](+)PHNO (agonist) [11C]NPA (agonist)	[18F]Fallypride	
H1	[11C]Doxepin		
H3	[11C]GSK189254 [11C]GR 103545	[18F]FMH3	
5-HT1A	[carbonyl-11C]WAY [carbonyl-11C]DWAY [11C]CUMI (agonist)	[18F]FCWAY [18F]MefWAY [18F]MPPF	
5-HT1B	[11C]AZ10419369 [11C]P943		
5-HT2A	[11C]MDL 1000907	[18F]Altanserin [18F]Altanserin-d2	
5-HT4	[11C]SB-207145		
5-HT6	[11C]GSK-215083		

Table 5 (continued)

Targets Misfolded proteins	Carbon-11 labelled	Fluroine-18 labeled	Comments
mGluR1		[18F]FITM	
mGluR5	[11C]SP 203 [11C]ABP688	[18F]SP 203 [18F]F-FPEB	
Nicotinic ($\alpha4\beta2$)		2-[18F]F-A-85380 (2-[18F]FA) 6-[18F]FA [18F]Nifene (agonist) [18F]AZAN	
Nicotinic ($\alpha7$)		[11C]CHIBA-1001 [18F]ASEM	
NK1		[18F]SPA-RQ [18F]MK-0999 ([18F]FE-SPA-RQ)	
NMDA		[18F]GE-179	
NOP	[11C]NOP-1A		
Opiate (DOR)	[11C]Methylnaltrindole		
Opiate (MOR)	[11C]Diprenorphine [11C]Carfentanil (agonist) [11C]CLY2795050 (antagonist)	[18F]Fluoroethyl-diprenorphine	
Sigma 1	[11C]SA4503		
Transporters			
DAT	[11C]PE2I [11C]Methylphenidate	[18F]FP-CIT [18F]FE-PE2I [18F]FECNT	
Glycine T1	[11C]CFpyPB [11C]GSK 931145 [11C]RO5013853	[18F]CFPyPB	
NET	[11C]MeNER-d2	[18F]FMeNER-d2	
SERT	[11C]DASB [11C]MADAM [11C]AFM [11C]HOHMADAM		
TSPO	[11C](R)-PK 11195 [11C]PBR28 [11C]DAA1106 [11C]DPA-713 [11C]ER176	[18F]FBR [18F]FEPPA [18F]PBR111 [18F]DPA-714	Commonly referred to as a marker of microglia activation, but target sensitivity to changes in cell number versus activation state remain unclear.
VMAT2	[11C]DTBZ [11C]MTBZ	[18F]florbenazine [18F]AV-133 [18F]FP-DTBZ	
Other			
		[18F]FDG	Glucose utilization
	[15O]Oxygen		Oxygen utilization
	[15O]water		Blood flow
	[11C]leucine		Protein synthesis
SV2a	[11C]UCB-J		Marker of synaptic density
MC1		[18F]BCPP-FE	Mitochondrial complex 1 density

Nevertheless, these studies suggest that FDG PET is viable surrogate end point in the evaluation of potential disease modifying therapy in AD.

Attempts to use Aβ PET as a pharmacodynamic marker to evaluate novel AD drug treatment effects have yielded mixed results. A dose-dependent reduction in brain amyloid load, as measured by [^{11}C]PIB PET, was reported following Gantenerumab treatment, although changes in PET signal did not correlate with changes in clinical severity (Ostrowitzki et al. 2012). There was no effects of treatment on either Aβ PET or clinical endpoints following either Bapinezumab (Vandenberghe et al. 2016) or Solanezumab treatment (Doody et al. 2014). In patients with prodromal or mild AD, one year treatment with aducanumab reduced Aβ PET signals in a dose- and time-dependent manner, where the reduction is accompanied by a slowing of clinical decline (Sevigny et al. 2016). Aβ

Table 6 Interpreting dose (or PK)- receptor occupancy (RO) relationships

Case	Interpretation
PK-RO shows a non-linear (sigmoidal) relationship	As expected based on Michaelis-Menten kinetics/receptor theory
Occupancy shows a linear relationship with dose	Higher and lower doses should be examined to describe the occupancy curve.
Generalized low occupancy (<10%) across doses	This indicates low brain uptake (of the drug), possibly due to: • Poor CNS penetration • Physiochemical properties • Substrate for efflux transporter • Subject to metabolism (saturable or non-saturable) • Lower than expected affinity for receptor in vivo compared with ex vivo or in vitro model. To investigate further consider a biodistribution study with the labelled drug to see if the drug is entering the brain.
One subject shows low occupancy, despite a plasma exposure consistent with other subjects who show a much higher occupancy.	Possible explanations include a lower brain entry of the drug, perhaps due to saturation of an active transport mechanism; higher efflux perhaps due to a difference in transporter expression in the subject concerned, or a difference drug-target Kd in that subject (e.g. receptor polymorphism).
Occupancy shows a curvilinear relationship with dose	This suggests: • Multiple binding sites/receptors or non-linear metabolism of the drug is affecting occupancy measurements.

PET is often implemented in a sub-study of a larger multi-center clinical trial, used as secondary or exploratory endpoints in a sub-population of the whole study. As such it is crucial to understand the within-subject variability and to keep the study sufficiently powered to detect the desired treatment effect on the imaging endpoints. Finally, an extended follow-up period should be used to allow sufficient time to detect significant change from baseline in the placebo group.

Recent therapies for AD have focused on slowing down τ-protein accumulation and spread. Post-mortem studies show that the density of neurofibrillary tangles is strongly associated with cognitive impairment and neurodegeneration. τ-PET imaging is potentially useful for evaluation of drug candidates in clinical trials and as a biomarker for selecting participants who are more likely to progress and provide surrogate biomarker for disease modification effects. A number of τ-targeting radiotracers have been developed and used in human studies: ^{11}C-PBB3, ^{18}F-THK5351, ^{18}F-THK5317, ^{18}F-THK5117, ^{18}F-T808, ^{18}F-FDDNP, ^{18}F-AV1451. ^{18}F-AV-1451 has been the tracer that is most widely in clinical studies so far, including as a secondary outcome measure in AD phase 3 clinical trials. Longitudinal studies in cognitively normal elderly subjects are also underway to evaluate the amount and rate of change in tau signal over a 3 year period (18F-AV-1451 PET Imaging in Participants Enrolled in the LEARN Study 2017). However, the use of these 1st generation τ tracers may be limited by technical issues, including off-target binding, and poor signal to noise ratios. Some of these technical issues appear to have been addressed by the development of a 2nd generation of radiotracers recently developed (including ^{18}F-GTP-1, 18F-RO-948, ^{18}F-MK6240 and ^{18}F-PI2620), although further studies in larger sample are still required.

In addition to AD, another area of focus in the neuroscience drug development community is idiopathic Parkinson's disease (PD). Idiopathic PD is characterized by a progressive loss of dopamine neurons in the substantia nigra (SN), with clinical symptoms manifesting when ~80% of striatal dopamine content or 50% of SN dopaminergic neurons are lost. Pathologically PD is characterized by the present of α-SYN deposition in Lewy bodies and dystrophic neurons in the striatal and cortical regions (Braak and Braak 2000). A major goal of PD drug development is to develop a disease modifying therapy that slows the progressive degeneration of nigrostriatal dopaminergic system. The use of clinical measures of disease progression is challenging for drug developers as they tend to focus on motor symptoms of PD and are confounded by the symptomatic effects of standard dopaminergic therapy. Importantly in PD, the link between α-SYN aggregation and synaptic dysfunction opens up a completely new approach towards understanding these diseases and treatment options (Schulz-Schaeffer 2010). As such PET imaging using radiotracer that directly targets α-SYN would be of particular

Table 7 Practical consequences of an increased focus on pathology and opposed to occupancy

Occupancy	Disease progression / pathology
Population	
• Targets expressed in healthy tissue	• Targets effectively absent in healthy tissue
• New ligands can be evaluated in healthy volunteers, and Occ50 estimates can be extrapolated to patients.	• Most studies, including evaluation of novel tracers, need to be conducted in patients.
	• What kind of subjects should be enrolled?
	• Data on natural history is in many cases lacking, does the disease have a dynamic phase? (early stage – low load/rapid change?; late stage – high load/slow or no change?)
Duration	
• Studies often involve a single dose (or limited repeat dosing)	• Diseases typically progress slowly, If we want to detect a slowing of progression we either need a long study, or a sensitive method, robust to off-target brain changes.
Sample size	
• Since occupancy is a fractional within-subject measure, and PK-RO data from different subjects is typically well described by standard dose-response models, small sample sizes can be used.	• Progression is still poorly characterised or unknown; linear models are almost certainly not appropriate across all stages of a disease.
	• Covariates such as age, age at onset, gender, genotype, duration of symptoms, and disease severity at baseline, will add to variability in baseline binding, and potentially rate of progression.
	Treatment comparisons are likely to involve looking for a difference between two differences; so within and between subject variability have an impact.

interest in the evaluation of disease modifying treatments. However, despite intense activity, no α-SYN radioligand is currently available as such, presynaptic dopaminergic markers arguably provide the best alternative imaging endpoint.

PET imaging with radiotracers that target the dopaminergic system in PD has been employed for the past two decades. Dopamine synthesis, storage and reuptake can be assessed with PET imaging using tracers that targets presynaptic and postsynaptic dopaminergic integrity. We will review the application of PET imaging in PD clinical trials as a biomarker to support patient selection, track disease progression and justify disease modification effect. Dopamine transporter (DAT) imaging with SPECT is widely used to support patient selection in PD clinical trials. DAT imaging provides an indirect measure of axonal degeneration and loss of presynaptic terminals. Several DAT tracers have been developed and reached phase 3 and 4 clinical applications; including [^{11}C]-cocaine, [^{11}C]/[^{18}F]-FP-CIT, [^{11}C]/[^{18}F]-CFT, [^{11}C]-PE2I, [^{18}F]-FE-PE2I, and [^{11}C]methylphenidate. Up to 80% of patients with a normal DAT scan continued to have normal scans and showed no clinical progression (Batla et al. 2014; Marshall et al. 2006), leading to the use of such scans in clinical trials to exclude patients with essential tremor or those who present similar clinical features as PD but exhibit no evidence of dopaminergic deficit (Scans Without Evidence of Dopamine Deficit (SWEDD)) (Antonini et al. 2001).

^{18}F-FDOPA PET provides measures of presynaptic dopamine synthesis capacity and storage. Postmortem and animal studies showed that striatal ^{18}F-FDOPA uptake correlates with both nigrostriatal dopamine cell counts and dopamine concentrations in the striatal terminals (Snow et al. 1993). Abnormal ^{18}F-FDOPA uptake relative to healthy controls has been demonstrated in early PD patients (Whone et al. 2003) and high risk genetic carries, including asymptomatic heterozygous Parkin and PARK6 carriers (Khan et al. 2005, 2002; Adams et al. 2005), asymptomatic LRRK2 mutation carriers (Adams et al. 2005), and monozygotic twins of PD patients (Piccini et al. 1999).

PET imaging with [^{18}F] FDG in PD patients showed a characteristic PD related pattern (PDRP), which consists of increased metabolism in the putamen, Globus pallidus, thalamus, cerebellum, pons, and sensorimotor cortex, with reduced metabolism in the lateral frontal, paracentral, and parieto-occipital areas. PDRP precedes onset of motor symptoms by 2 years and can differentiate PD from clinically similar conditions, such as MSA and PSP (Eckert et al. 2007), suggesting that [^{18}F]FDG PET may be used to support patient selection in clinical trials (Eckert et al. 2005; Tang et al. 2010).

Longitudinal assessment of disease progression by multitracer PET indicated that the neurodegenerative process in PD follows a negative exponential course and slows down with increasing symptom duration and progression of illness (Hilker et al. 2005; Nandhagopal et al. 2009). Longitudinal

changes in three presynaptic dopaminergic markers (vesicular monoamine transporter type 2 - VMAT2, DAT and FDOPA radiotracers) were similar over 25 years, although the slope for FDOPA uptake revealed a faster rate of decline for disease duration less than 15 years (Nandhagopal et al. 2009).

Several longitudinal studies have demonstrated striatal DAT decline although the rate of change does not always correlate with changes in motor symptoms (Simuni et al. 2018). Findings from clinical trials also highlight the inconsistency between drug-related changes in motor symptoms and changes in striatal DAT binding. Across studies, the annual rates of decline for DAT as measured with SPECT (123-I Ioflupane) range between 4 and 8% per year in the striatum. In contrast to DAT, the rate of decline for ^{18}F-FDOPA was 6–12% (Kaasinen and Vahlberg 2017). To our knowledge, DAT *PET* has never been used as a pharmacodynamic marker in clinical trials. In a recent study, DAT imaging using ^{11}C-PE2I was shown to be more sensitive to disease progression that ^{18}F-FDOPA. Stronger negative correlations were shown between motor severity and striatal ^{11}C-PE2I BP_{ND} relative to those with FDOPA Ki (Li et al. 2018). Moreover, longitudinal changes in motor severity were not associated with changes in ^{18}F-FDOPA uptake, but did correlate with changes in ^{11}C-PE2I (Li et al. 2018).

The variability in the reported changes is undoubtedly influenced by differences in analysis methods and patient characteristics including duration of disease, severity of motor symptoms and concomitant medications, as illustrated by. a recent study using the large PPMI cohort (Tinaz et al. 2018; Zubal et al. 2007) suggests that different analytical methodologies provide different sensitivity to assess change of DAT signals over time and influence the strength of correlation between outcome measures and motor symptoms. The ability to detect the greatest change over time for DAT SPECT was the greatest when change over time was calculated as a percent loss in area under the curve and non-linearly fitted to a mono-exponential curve. Using this method, the greatest rate of change was observed in caudate, followed by anterior putamen and dorsal putamen. At a later time, the rate of change converges and becomes similar across striatal sub-regions. There was a moderate correlation between the change in AUC and motor UPDRS scores. These findings provide some support for the use of DAT imaging as an outcome measure in the evaluation of disease modifying drugs in prodromal patients or those at early phase of the disease.

Of note that due to the sensitivity of presynaptic markers to compensatory mechanisms; it may be useful to combine presynaptic markers, with tracers that assess postsynaptic dopaminergic function, such as [^{18}F] FDG PET (Eckert et al. 2005). Indeed evidence from a limited number of studies suggested that abnormal PDRP pattern can be recovered by successful treatment with dopaminergic replacement therapy or deep brain stimulation (Feigin et al. 2001; Lin et al. 2008; Hilker et al. 2004). However, there is also evidence to suggest that PDRP patterns can be altered by symptomatic therapies, so the utility of this approach remains to be confirmed.

Finally the choice of dopaminergic imaging biomarker should take into account whether the drug under study is intended to prevent disease expression/onset or arresting disease progression once symptoms become clinically manifested. The sample size required to demonstrate a significant neuroprotective effect would decrease with the length of follow-up period and increases with patients' symptom duration at the time of study inclusion (Hilker et al. 2005). Another important consideration is the effect of age on presynaptic dopamine tracer progression. For example, it has been shown that the rate of progression in the loss of presynaptic dopaminergic function was significantly slower in younger patients (de la Fuente-Fernandez et al. 2011).

Conclusion

PET imaging is unique in providing the ability to directly quantify drug concentration, target engagement and pharmacodynamics effects of drug treatment in the living human brain. PET methods are non-invasive, translatable, and the outcome parameters are quantifiable. Sophisticated study designs and the combination of PET with other technologies (such as in vitro equilibrium dialysis) allow a robust determination of novel drug distribution in the human brain, and quantification of the relationship between drug plasma concentration and target occupancy over time. An ever increasing range of PET ligands makes more and more CNS targets accessible to evaluation.

Although appropriate application of PET methods requires a sophisticated multidisciplinary team, and is therefore relatively expensive, the information it provides can significantly reduce the size and duration of early phase clinical trials, saving multiples of the cost of the PET study. In certain cases, PET studies can prevent the progression of unfavorable molecules to Phase II and III clinical trials. For these reasons, PET studies have become an indispensable part of CNS drug development.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent This is a review article and therefore does not contain any new data from studies with human participants or animals performed by any of the authors.

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