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# New and Old TSPO PET Radioligands for Imaging Brain Microglial Activation in Neurodegenerative Disease

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## Abstract

**Purpose of Review** We will discuss the developments in TSPO PET imaging and the contribution this technique has had to understanding neuroinflammation in vivo, as well as the limitations inherent to the currently available radioligands and the potential future direction.

**Recent Findings** Positron emission tomography (PET) imaging targeting the translocator protein 18 kDa (TSPO) has led to major advances in understanding the pathological role played by microglia activation and neuroinflammation in a diverse range of neurodegenerative conditions.

**Summary** The first-generation radioligand $^{11}$[C](*R*)-PK11195 has been the most widely studied and has led to considerable advancements in defining the role of neuroinflammation in neuronal degeneration and dysfunction. However, limitations including low signal-to-noise ratio and high nonspecific binding have led to the development of new TSPO-specific radioligands in an attempt to improve the quality of TSPO imaging. Unfortunately, these new radioligands have not been without their own problems, and the expected improvement in image quality has not been achieved.

**Keywords** Neuroinflammation · Microglial activation · TSPO · PET · Neurodegenerative diseases

## Introduction

Microglial activation has been implicated in the pathology underlying a diverse range of neurodegenerative conditions. Microglial cells represent approximately 10–15% of total white cells and are the intrinsic defensive cells of the brain. They normally exist in a quiescent state but become activated by changes in the local milieu due to injury releasing cytokines. In their activated state, microglia significantly upregulate the expression of the benzodiazepine receptor, now known as the translocator protein 18 kDa (TSPO), on their outer mitochondrial membrane [1]. TSPO has been implicated in a number of vital cellular functions; however, its exact function in metabolic homeostasis remains unclear [2••].

Increased microglial activation has been detected in a range of conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). For several years, there has been a great deal of interest in studying microglial activation in vivo using positron emission tomography (PET) in order to understand its role in the pathogenesis of neurodegeneration and determine whether altering microglial activation can lead to modification of disease processes.

The first-generation TSPO PET tracer, $^{11}$[C](*R*)-PK11195 (PK), an isoquinoline carboxamide, has been used extensively to study microglial activation. This tracer was developed in the 1980s and represents the first non-benzodiazepine-type compound shown to bind with high affinity to TSPO [2••]. The PET findings from PK have generally correlated well

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with pathological findings. However, the clinical usefulness of PK is somewhat limited due to several issues including low brain bioavailability, nonspecific binding and the relatively short half-life of carbon-11 [3, 4].

This has led to the development of second- and third-generation TSPO tracers over the past few years. Hundreds of new-generation radiotracers have been synthesised, and approximately 40 of these have undergone preclinical evaluation [4]. The most promising second-generation radioligands such as [$^{18}$F]-PBR06 [5], [$^{18}$F]-FEDAA1106 [6], [$^{18}$F]-FEPPA [7], [$^{11}$C]-DAA1106 [8], [$^{11}$C]-DPA-713 [9], [$^{18}$F]-PBR111 [10, 11], [$^{18}$F]-DPA-714 [12] and [$^{11}$C]-PBR28 [13] are being evaluated in clinical trials. While some carbon-11 radiolabelled tracers present better kinetics than PK [13, 14], their short half-life limits usage in PET centres without an on-site cyclotron. Hence, the current focus is on the development of fluorinated [$^{18}$F] radiotracers. These exhibit superior image quality and a longer half-life, thus improving accessibility to researchers and clinicians, besides significantly reduced costs [15•]. However, even some of these new radioligands demonstrate restrictions, including the slow accumulation of radiometabolites of [$^{18}$F]-PBR06, resulting in inaccurate estimations of TSPO [5, 6], along with slow kinetic behaviour of [$^{18}$F]-FEDAA1106 [6], necessitating longer scanning time and hereby limiting clinical usage.

The development of the third-generation TSPO tracers was as a consequence of the sensitivity of the second-generation tracers to a single nucleotide polymorphism (rs6971) in the TSPO gene [2••]. This was demonstrated in initial studies using [$^{11}$C]-PBR28, where it was observed that about 10% of participants did not show any binding to TSPO [13, 16]. This phenomenon was later noticed with all studied second-generation radiotracers [15•, 16, 17]. Owen and colleagues revealed that new TSPO radioligands show three patterns of binding affinity derived from this single polymorphism: high-affinity binders (HABs), mixed-affinity binders (MABs) and low-affinity binders (LABs) [18–20]. The different patterns of binding affinity vary in frequency in different ethnic groups [21]. The PET signal from MAB and LAB patients will significantly underestimate TSPO expression; therefore, determination of TSPO binding is required. Third-generation, or rs6971-insensitive, TSPO radioligands include flutriciclamide ($^{18}$[F]-GE180) and $^{11}$[C]-ER176; however, the clinical relevance of these compounds has not been determined [2••].

The use of PET imaging to determine TSPO density and quantify microglial activation in neurodegenerative processes poses a number of challenges as alluded to above. In this review, we will summarise and compare the findings of the different TSPO radiotracers and discuss their pros and cons in context of improving our understanding of the neuroinflammatory processes underlying a diverse range of neurodegenerative conditions.

## Alzheimer's Disease, Dementia and Mild Cognitive Impairment

AD was the first neurodegenerative disorder where microglia activation was detected in vivo by PET imaging. Cagnin and colleagues reported significant increases in PK binding in the entorhinal, temporoparietal and cingulate cortex in a group of eight patients with AD compared with 15 age-matched controls [22]. That study also included a patient with isolated memory impairment, but no evidence of dementia, who showed an increase of PK binding in the fusiform gyri, inferior temporal gyri and parahippocampus. Since then, a number of studies have confirmed the presence of activated microglia in cortical areas and grey matter in patients with AD and in patients with mild cognitive impairment (MCI). In MCI patients, increased cortical PK binding is generally observed in patients with raised cortical amyloid load measured by [$^{11}$C]-Pittsburgh compound-B ([$^{11}$C]-PIB) PET, which binds specifically to fibrillar β-amyloid plaques [23, 24•]. Only one study failed to detect any significantly raised PK binding in AD and MCI patients compared with controls [25]. However, the number of patients investigated was small (AD = 6, MCI = 6, control = 5), and therefore, the study was probably not powered to detect significant intergroup differences. Another study, performed on larger cohorts of AD and MCI patients, also failed to detect any significant differences between groups using the traditional region of interest (ROI) approach. However, voxel-wise statistical parametric mapping analysis did show small clusters of significantly increased PK binding potential in the occipital lobe of AD patients compared with healthy controls [26].

Several studies have also attempted to explore the relationship between levels of activated microglial, clinical symptoms and other pathological features of AD. A significant negative correlation between mini-mental state examination (MMSE) and PK binding in several cortical regions has been reported in some studies [27–29], but not in others [26]. Further studies are therefore required to clarify this issue.

In AD patients, Edison and colleagues [27] observed a significant 20–35% increase in microglial activation in the frontal, temporal, parietal, occipital and cingulate cortices, and a concomitant twofold increase in fibrillar β-amyloid deposition in these same cortical areas. More recently, in MCI patients, Parbo and colleagues have observed a positive correlation between levels of amyloid load and PK binding potentials in frontal, parietal and temporal cortices [24•]. These findings suggest that activated microglia occur in areas where amyloid plaques co-exist. However, some MCI cases with

increased PK binding and normal levels of [$^{11}$C]-PIB retention have been reported, suggesting that these two pathologies can occur independently [23].

A longitudinal study in AD patients with serial PK, [$^{11}$C]-PIB and [$^{18}$F]-flurodeoxyglucose PET scans demonstrated a longitudinal increase of cortical microglial activation along with progressive reduction of glucose metabolism in the majority of patients over a 16-month period. Additionally, voxel-wise correlation analysis revealed that microglial activation positively correlated with amyloid deposition and inversely correlated with regional cerebral metabolic rate at voxel level over time. These findings provide further evidence that neuroinflammation is not only associated with localised amyloid deposition but also glucose metabolism over time [29].

The expression of activated microglia in other forms of dementia has rarely been assessed in vivo. In frontotemporal dementia (FTD), for example, Cagnin and colleagues found enhanced PK binding in the typically affected frontotemporal regions [30]. Investigations using second-generation TSPO cohorts with AD and MCI have demonstrated varying results. While some studies found increased tracer uptake in patients [31–36], others did not [37–39]. Kreisl and colleagues investigated the relationship between neuroinflammation and disease severity in patients with AD and MCI compared with controls [33]. The authors revealed a consistent increase in TSPO expression throughout the grey matter cortex in 'PIB positive' AD patients compared with 'PIB negative' controls. However, [$^{11}$C]-PBR28 binding did not significantly differ between MCI patients and controls. Despite high [$^{11}$C]-PIB binding in some MCI patients, no correlation with [$^{11}$C]-PBR28 binding was observed. It has been suggested that TSPO upregulation may only occur after clinical conversion from MCI to AD, although, as already discussed, PET studies using PK have demonstrated the opposite. Hence, [$^{11}$C]-PBR28 might not be sensitive enough to detect a signal in MCI [33]. In a larger cohort, Hamelin and colleagues found elevated TSPO binding in AD patients compared with controls, as well as a positive correlation between PIB and TSPO binding [40•].

Another PET study using [$^{11}$C]-PBR28 in a cohort of AD patients, MCI patients and controls compared outcome measures such as total distribution volume ($V_T$), calculated using the two-tissue compartment model with arterial input function, as well as the standardised uptake volume ratio (SUVr) with cerebellum as pseudo-reference region [35]. The authors did not detect any difference in $V_T$ values amongst groups, but after correcting for the free fraction in plasma ($f_P$), AD patients showed increased $V_T/f_P$ values in the entorhinal and combined middle and inferior temporal cortices compared with MCI patients and controls. The cerebellum did not show any differences in both $V_T/f_P$ and SUV between patient groups. When using SUVr for [$^{11}$C]-PBR28, AD patients demonstrated higher PET signal than controls and MCI patients. Lyoo and colleagues suggested that SUVr could substitute for absolute quantification of [$^{11}$C]-PBR28 in distinguishing AD patients from MCI patients and controls, avoiding the need for arterial blood sampling [35]. Nevertheless, TSPO is widely expressed in the brain, including the cerebellum [41], so that there exists no true reference region devoid of specific binding to [$^{11}$C]-PBR28. Furthermore, the cerebellum seems to be affected in clinical progression of AD [42]; consequently substituting $V_T$ with SUVr would not be appropriate for all TSPO tracers.

Other studies have applied different methodological approaches to quantify TSPO in AD using the radioligand [$^{18}$F]-DPA-714 [37, 43]. Longitudinal studies detected increased tracer uptake in AD patients; however, methodological limitations including small sample sizes limit data interpretation [44, 45].

In summary, there are no consistent results regarding neuroinflammation in AD and MCI with TSPO PET imaging studies. This is likely due to methodological problems concerning the quantification of TSPO, small sample sizes and the limitations of the TSPO radioligands themselves, such as binding in the vasculature [46].

## Parkinson's Disease

Numerous studies have supported a role for microglia activation in the pathogenesis of PD. In PD brains, increased levels of activated microglia have been found in the substantia nigra pars compacta (SNc) [47], putamen, hippocampus, transentorhinal cortex, cingulate cortex and temporal cortex [48].

Two separate PET studies have reported significant increases in PK binding in the striatum of PD patients compared with normal controls [49, 50]. An increase in PK binding was also detected in the SNc of patients in both studies. However, the difference with the control group was significant in only one study, which was performed in patients with a recent diagnosis of PD [49]. In that study, midbrain PK binding potential values correlated positively with severity of motor symptoms (measured with UPDRS-III) and negatively with putaminal dopamine transporter availability (measured with [$^{11}$C]-CFT SPECT), supporting the hypothesis that activated microglia may contribute to dopamine neuronal loss in PD.

A third study found that PD patients demonstrated higher PK binding in the putamen contralateral to the most affected side and midbrain compared with controls [51]. These increases were larger in patients with more advanced disease ($N = 8$) than in patients with symptom duration less than 1 year ($N = 6$). However, the differences were not statistically significant as there was a large overlap between PD patients and healthy controls. It should be noted that several binding potential values had to be discarded due to fitting errors resulting in negative or unrealistically high values. This could have had

an impact on the results. Additionally, nigrostriatal dysfunction was not assessed in these patients to confirm diagnosis of PD.

In addition to the analysis of nigrostriatal structures, some studies have used a voxel-by-voxel analysis to assess the levels of PK binding in other brain regions. Gerhard and colleagues detected areas of significant increase in PK binding in the frontal and temporal cortices of PD patients compared with controls, suggesting that neuroinflammation also occurs in cortical areas in PD patients [50]. More recently, Edison and colleagues observed significant increases in microglial activation in temporal, parietal and occipital regions of non-demented PD patients compared with controls [52]. In the same study, PD patients with dementia presented more widespread cortical distribution of activated microglia, including anterior and posterior cingulate, striatum, frontal, temporal, parietal and occipital cortical regions. There was a large overlap between areas of microglial activation and areas of reduced glucose metabolism in several cortical regions, and both increased microglial activation and glucose hypometabolism correlated with MMSE score. Microglia activation could therefore be involved in the development of dementia in PD.

There are only a small number of PET studies using second-generation TSPO ligands in PD [53•, 54, 55••]. Terada and colleagues recruited 11 PD patients and 12 controls to undergo two [$^{11}$C]-DPA-713 scans 1 year apart [53•]. Binding potential ($BP_{ND}$), estimated by simplified reference tissue model (SRTM), was used to measure microglial activation and voxel-wise, as well as ROI analyses were conducted to compare regional $BP_{ND}$ amongst groups. Analyses revealed a significant increase in [$^{11}$C]-DPA-713 $BP_{ND}$ extrastriatally including the occipital, temporal and parietal cortices in PD patients. The degree of $BP_{ND}$ increased 1 year later, predominantly in the temporal and occipital cortices. However, elevated [$^{11}$C]-DPA-713 $BP_{ND}$ in the ROI analysis during follow-up could not be detected. The study presents several limitations, including small sample size, a short follow-up duration and the estimation of [$^{11}$C]-DPA-713 $BP_{ND}$ with SRTM. The second-generation TSPO radiotracer [$^{18}$F]-FEPPA, which has shown high affinity for TSPO, a suitable metabolic profile, high brain penetration and good pharmacokinetics [7, 56], was used in two other studies investigating microglial activation in the striatum [54], cortical and subcortical brain regions [55••]. Both studies detected a significant effect of genotype on [$^{18}$F]-FEPPA $V_T$ values, with a trend towards elevated TSPO binding predominantly in the PD-HAB group. However, there was no TSPO overexpression in PD patients or distinct correlations between disease severity, duration and $V_T$ values [54, 55••].

So far, only one study has reported a longitudinal assessment of the levels of activated microglia in PD. Eight PD patients were examined with serial PET scans over a 2-year period and PK binding remained stable in all patients [50], suggesting that microglial activation occurs early in the disease process. Unfortunately, longer follow-ups in large cohorts of patients are lacking.

## Idiopathic REM Sleep Behaviour Disorder

Over the last decade, there has been growing scientific interest in idiopathic rapid eye movement sleep behaviour disorder (iRBD), which seems to represent a prodromal phase of PD and other Parkinsonian disorders including dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). Longitudinal follow-up studies of these patients have shown that the majority of them develop PD or DLB later in life, with a smaller number developing MSA [57]. Several imaging studies have clearly shown that most of these patients, despite having no clinical evidence of parkinsonism and/or cognitive impairment, already demonstrate brain abnormalities typical of Parkinsonian syndromes. Using PK PET, Stockholm and colleagues have investigated the occurrence of activated microglia in a cohort of 21 patients with polysomnography-confirmed iRBD [58••]. They found that compared with age-matched controls, iRBD patients had increased levels of microglia in the SN, and to a lesser extent, in the putamen and caudate nuclei. Increased nigral PK binding correlated positively with increased binding of PK in the putamen and caudate nucleus. These changes occurred alongside the presence of nigrostriatal dysfunction measured with $^{18}$F-DOPA PET. However, no significant correlations were found between PK and $^{18}$F-DOPA uptake within the striatum. Activated microglia were also found in the visual associate cortex of the occipital lobe of these patients. The latter finding could explain the difficulties that iRBD patients seem to have performing tests of visuospatial skills.

These findings strongly support the presence of dopaminergic dysfunction and early inflammation in both subcortical and cortical areas in iRBD patients, providing further evidence that most iRBD patients are in a prodromal phase of a Parkinsonian disorder and microglial activation occurs early in the pathogenesis of PD. However, it remains to be determined if these imaging biomarkers can be used as early predictors of the clinical subtype of Parkinsonian disorders in iRBD patients. Interestingly, a PK PET study by Iannaccone and colleagues directly compared brain levels of activated microglia in PD patients and patients with DLB [59]. While PD patients had evidence of increased activated microglia only in the SN and putamen, patients with DLB had extensive additional increases in several associative cortical regions [59].

It remains to be understood if the presence of activated microglia at these early stages is detrimental and contributes to further neurodegeneration [60], or if it can have

neuroprotective properties as suggested by some recent studies [40•]. The latter knowledge is essential to help rationalise a role for anti-inflammatory agents as neuroprotective strategies in iRBD patents with high risk of developing Parkinsonism.

## Atypical Parkinsonism

Activated microglia have been found in the brains of patients with MSA [49], progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) [61]. Gerhard and colleagues found an increase in PK binding in the caudate, putamen and palladium of a small number ($N = 5$) of MSA patients compared with normal controls [62]. MSA patients also presented increased PK binding in extrastriatal regions such as the thalamus, pons, SNc and dorsolateral prefrontal cortex. These findings indicate that MSA patients have increased levels of neuroinflammation in brain areas typically involved in this condition and are in line with the distribution of microglial activation detected in the brains of MSA patients [63].

Minocycline is a tetracycline derivative with anti-inflammatory properties which appears to inhibit microglia activation. The effect of minocycline in MSA patients has been investigated with a prospective, randomised, double-blind, multinational clinical trial [64]. A small subgroup of patients received serial PK PET to assess the effect of minocycline on activated microglia. Unfortunately, the study failed to show a clinical effect of minocycline on motor function. However, in the PET subgroup, the three patients in the minocycline group showed an attenuated mean increase in microglial activation as compared with the placebo group (5 subjects, $P = 0.07$), and in two of them, PK binding was effectively decreased. This suggests that minocycline may interfere with microglial activation. These findings deserve further investigation.

PSP patients have also been reported to have high levels of activated microglia in several brain regions. In one study, a small group of four PSP patients demonstrated significantly increased mean PK binding in the basal ganglia, midbrain, frontal lobe and cerebellum compared with age-matched controls. Two of these patients underwent a follow-up scan after 6–10 months. The level of activated microglia remained stable over that time [57].

A more recent study utilised PK PET to assess levels of activated microglia in nine patients with PD, seven with MSA with parkinsonism, four PSP and 11 controls [61]. The aim was to explore the relationship between microglial activation and regional apparent diffusion coefficient (rADC, measured with diffusion-weighted MRI). The study was not powered to assess differences in levels of activated microglia amongst groups. Nevertheless, compared with controls, PK binding was increased in the striatum, midbrain and pons in patients with atypical parkinsonian syndromes, and in the pons of patients with PD. Putaminal binding was greater in atypical parkinsonian syndromes compared with PD. The main finding of this study, however, was that PK binding and rADC in the pons were positively correlated in the group of patients with atypical parkinsonism, suggesting that microglial activation may contribute to the changes in water diffusivity observed in the brainstem of these patients.

Finally, in CBD patients, increased PK binding has been found in the striatum, thalamus and SN reflecting the typical pathological pattern of this condition [65, 66].

## Amyotrophic Lateral Sclerosis

Cerebral microglial activation has been detected with PK PET during the evolution of ALS. Turner and colleagues observed significantly increased PK binding in the motor cortex, pons, dorsolateral prefrontal cortex and thalamus in ALS patients compared with controls [67]. These findings are in line with previous post-mortem observations and suggest that cerebral pathology is widespread in this condition. Interestingly, PK binding in the motor cortex correlated well with the clinical burden of upper motor neuron signs. The same authors have reported increased PK binding in the cortical motor areas of patients with clinically isolated upper motor neuron syndromes [68].

Studies using the second-generation TSPO tracers in ALS patients revealed a significant increase of tracer uptake in cortical [69], as well as corticospinal tracts, compared with controls [70]. Furthermore, disease severity correlated with tracer binding, suggesting clinical relevance of brain inflammation quantified in vivo [70].

## Huntington's Disease

In a post-mortem study, Sapp and colleagues found a significant and widespread accumulation of activated microglia in HD brains. This was particularly evident in the areas known to be targets such as the striatum, globus pallidus and frontal cortex [71]. The density of activated microglia correlated with the severity of neuronal loss. The pathological findings are replicated in a PK PET study by Pavese and colleagues, which found significant microglial activation in the striatum, cortex and other basal ganglia structures in HD patients [72]. The degree of microglial activation in the striatum also correlated with the severity of striatal dysfunction as measured with Unified Huntington's Disease Rating Scale motor scores.

Extending the study to premanifest HD (preHD) gene carriers, Tai and colleagues found elevated PK binding in the striatum and cortex of preHD carriers, and the striatal PK binding correlated with the likelihood of disease onset in

5 years [73]. This indicated that microglial activation is an early process in the pathogenesis of HD and that PK PET may play a role as a marker of disease progression, especially in preHD carriers. Elevated PK binding in the hypothalamus of HD patients and preHD carriers was also found in another study, and the extent of microglial activation correlated with the degree of hypothalamic $D_2$ receptor loss as measured by raclopride [74]. The hypothalamic dysfunction in turn may contribute to the altered metabolism and circadian rhythm observed in HD [75]. Furthermore, Politis and colleagues demonstrated increased peripheral cytokine IL-1ß in preHD compared with healthy controls [76]. Increased PK binding in the somatosensory cortex of preHD carriers correlated with the levels of several peripheral cytokines, suggesting an association between peripheral and central inflammatory responses in preHD.

Second-generation TSPO radioligands have not yet been fully explored in HD. A recently published study investigated the specificity and sensitivity of the [$^{18}$F]-PBR06 radiotracer in the HD mouse model, R6/2. The authors demonstrated that the [$^{18}$F]-PBR06-PET signal was increased in the striatum, cortex and hippocampus at late stages in the disease process compared with wild-type mice [77]. As already discussed, human TSPO PET studies using the PK ligand have shown microglial activation in the striatum, cortex, thalamus and hypothalamus, however not in the hippocampus. The authors postulate that [$^{18}$F]-PBR06 affords a greater signal-to-noise ratio compared with PK, which may account for the difference observed [77]. Further studies examining the utility of this TSPO ligand, as well as other second- and third-generation TSPO ligands, are required to define their role in HD.

## Limitations of TSPO Radioligands

Overall, the exploration and quantification of TSPO expression remain a challenge in spite of the development of new TSPO radiotracers. PK remains the most widely used and its sensitivity means that it is useful in clinical trials for new therapeutic agents targeting microglia. Despite this, its widespread use has been restricted for several reasons. The first challenge concerns the affinity of PK binding to a number of sites in the blood, such as plasma proteins, monocytes and platelets [78]. This in combination with a relatively low brain permeability results in a low signal-to-noise ratio, limiting its specificity and rendering it unable to detect subtle changes in neuroinflammation. Furthermore, the necessity for on-site cyclotron can render it an inaccessible ligand for many researchers and clinicians. As discussed, this consideration was a driving force in the development of the longer half-life $^{18}$F radioligands, which do not require an on-site cyclotron, thus, improving accessibility, cost-effectiveness and signal-to-noise ratio.

However, second- and third-generation TSPO radiotracers are not without their own drawbacks, highlighted by three main confounding factors alluded to earlier [78]. The first factor concerns the rs6971 polymorphism. Although binding status can be easily identified and considered into data analysis, there remains no ideal TSPO PET radiotracer for LAB. The result is that the TSPO PET images obtained for LAB are of lower quality, thus limiting clinical usefulness. The second factor is the disproportion between the high signals from TSPO in the endothelial cells of the blood brain barrier (BBB) compared with that of the tissue, requiring appropriate kinetic correction [79]. The final factor is the difficulty in obtaining exact estimates of free plasma concentrations for appropriate quantification. The large and widespread TSPO component bound at the BBB masks various brain tissues such as grey matter, white matter and inflamed tissue. Hence, the identification of a suitable reference region is restricted and invasive arterial blood sampling is required [78].

Further challenges include the lack of specificity of TSPO binding for activated microglia, since TSPO is also expressed on astrocytes [80]. Furthermore, microglia exhibit pro- and anti-inflammatory properties, yet there is no suitable PET radiotracer for either phenotype of microglia [81•].

## Alternatives to Second-Generation TSPO Radiotracers

The development of more targeted biomarkers of other inflammatory components, such as monocytes, B cells, T cells and astrocytes, as well as molecular mediators of neuroinflammation, may offer greater insights into neuroinflammatory processes [13]. For instance, $^{11}$C-ketoprofen methyl ester specifically targets cyclooxygenase 1 and has been found to be safe with favourable imaging characteristics; however, additional investigations are necessary to evaluate its future use [82]. Likewise, $^{11}$C-RSR-056 and $^{11}$C-A-836339 may be promising radiotracers for the cannabinoid type 2 receptor, which is expressed mainly on immune cells and elevated expression is linked to neuroinflammatory conditions [83]. For more details about alternative neuroinflammation targets in PET imaging, please see recent reviews [80, 84••].

Another recent development is flutriciclamide ([$^{18}$F]-GE180), a third-generation TSPO radiotracer, which has previously been evaluated for optimum scan duration and kinetic modelling strategies in 10 healthy controls [85••]. This study cohort showed relatively low $V_T$ values; however, the authors noted that the tracer may be less sensitive to TSPO binding affinity status. Nonetheless, due to the small sample size and exclusion of low-affinity binders, further investigations are needed to evaluate this tracer for future use [85••].

## Conclusion

Despite numerous research studies and the development of new TSPO radiotracers, the exact role of neuroinflammation and the presence of activated microglia in neurodegenerative conditions remain unclear. For in vivo TSPO PET imaging, new radiotracers have been developed to overcome the limitations of PK. However, these have not been without their own obstacles, and we are yet to develop the ideal TSPO PET radiotracer to deliver on the promise of improved image quality compared with TSPO PET using the PK tracer.

Nonetheless, experience to date has confirmed TSPO as a sensitive and potent biomarker of microglial activity in the central nervous system. PK, in the absence of a more suitable candidate, continues to be the most reliable radioligand and facilitates the acquisition of TSPO PET imaging with the additional vantage that new data can be reliably compared with previously published studies.

### Compliance with Ethical Standards

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

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


1. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère J-J, Lindemann P, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci. 2006;27(8):402–9.
2. •• Dupont A-C, Largeau B, Santiago Ribeiro MJ, Guilloteau D, Tronel C, Arlicot N. Translocator protein-18 kDa (TSPO) positron emission tomography (PET) imaging and its clinical impact in neurodegenerative diseases. Int J Mol Sci. 2017;18(4) **The authors outline the *in vivo* exploration of neuroinflammation in neurodegenerative and neuropsychiatric conditions, including major advances and the clinical impact of TSPO impact to date.**
3. Le Fur G, Perrier ML, Vaucher N, Imbault F, Flamier A, Benavides J, et al. Peripheral benzodiazepine binding sites: effect of PK 11195, 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide. I. In vitro studies. Life Sci. 1983;32(16):1839–47.
4. Chauveau F, Boutin H, Van Camp N, Dollé F, Tavitian B. Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. Eur J Nucl Med Mol Imaging. 2008;35(12):2304–19.
5. Fujimura Y, Zoghbi SS, Simeon FG, Taku A, Pike VW, Innis RB, et al. Quantification of translocator protein (18 kDa) in the human brain with PET and a novel radioligand, 18F-PBR06. J Nucl Med. 2009;50(7):1047–53.
6. Fujimura Y, Ikoma Y, Yasuno F, Suhara T, Ota M, Matsumoto R, et al. Quantitative analyses of 18F-FEDAA1106 binding to peripheral benzodiazepine receptors in living human brain. J Nucl Med. 2006;47(1):43–50.
7. Wilson AA, Garcia A, Parkes J, McCormick P, Stephenson KA, Houle S, et al. Radiosynthesis and initial evaluation of [18F]-FEPPA for PET imaging of peripheral benzodiazepine receptors. Nucl Med Biol. 2008;35(3):305–14.
8. Ikoma Y, Yasuno F, Ito H, Suhara T, Ota M, Toyama H, et al. Quantitative analysis for estimating binding potential of the peripheral benzodiazepine receptor with [ $^{11}$ C]DAA1106. J Cereb Blood Flow Metab. 2007;27(1):173–84.
9. Boutin H, Chauveau F, Thominiaux C, Grégoire M-C, James ML, Trebossen R, et al. 11C-DPA-713: a novel peripheral benzodiazepine receptor PET ligand for in vivo imaging of neuroinflammation. J Nucl Med. 2007;48(4):573–81.
10. Fookes CJR, Pham TQ, Mattner F, Greguric I, Loc'h C, Liu X, et al. Synthesis and biological evaluation of substituted [18F]imidazo[1,2-a] pyridines and [18F]pyrazolo[1,5-a] pyrimidines for the study of the peripheral benzodiazepine receptor using positron emission tomography. J Med Chem. 2008;51(13):3700–12.
11. Van Camp N, Boisgard R, Kuhnast B, Thézé B, Viel T, Grégoire M-C, et al. In vivo imaging of neuroinflammation: a comparative study between [(18)F]PBR111, [(11)C] CLINME and [(11)C]PK11195 in an acute rodent model. Eur J Nucl Med Mol Imaging. 2010;37(5):962–72.
12. Arlicot N, Vercouillie J, Ribeiro M-J, Tauber C, Venel Y, Baulieu J-L, et al. Initial evaluation in healthy humans of [18F]DPA-714, a potential PET biomarker for neuroinflammation. Nucl Med Biol. 2012;39(4):570–8.
13. Fujita M, Imaizumi M, Zoghbi SS, Fujimura Y, Farris AG, Suhara T, et al. Kinetic analysis in healthy humans of a novel positron emission tomography radioligand to image the peripheral benzodiazepine receptor, a potential biomarker for inflammation. NeuroImage. 2008;40(1):43–52.
14. Endres CJ, Pomper MG, James M, Uzuner O, Hammoud DA, Watkins CC, et al. Initial evaluation of 11C-DPA-713, a novel TSPO PET ligand, in humans. J Nucl Med. 2009;50(8):1276–82.
15. • Vivash L, O'Brien TJ. Imaging microglial activation with TSPO PET: lighting up neurologic diseases? J Nucl Med. 2016;57(2):165–8 **This review summarises the recent developments in TSPO imaging, including the current limitations and possibilities for future direction in this field.**
16. Kreisl WC, Fujita M, Fujimura Y, Kimura N, Jenko KJ, Kannan P, et al. Comparison of [(11)C]-(R)-PK 11195 and [(11)C]PBR28, two radioligands for translocator protein (18 kDa) in human and monkey: implications for positron emission tomographic imaging of this inflammation biomarker. NeuroImage. 2010;49(4):2924–32.
17. Owen DRJ, Gunn RN, Rabiner EA, Bennacef I, Fujita M, Kreisl WC, et al. Mixed-affinity binding in humans with 18-kDa translocator protein ligands. J Nucl Med. 2011;52(1):24–32.
18. Owen DRJ, Matthews PM. Imaging brain microglial activation using positron emission tomography and translocator protein-specific radioligands. Int Rev Neurobiol. 2011;101:19–39.
19. Owen DR, Howell OW, Tang S-P, Wells LA, Bennacef I, Bergstrom M, et al. Two binding sites for [3H]PBR28 in human brain: implications for TSPO PET imaging of neuroinflammation. J Cereb Blood Flow Metab. 2010;30(9):1608–18.
20. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. J Cereb Blood Flow Metab. 2012;32(1):1–5.

21. Guo Q, Owen DR, Rabiner EA, Turkheimer FE, Gunn RN. Identifying improved TSPO PET imaging probes through biomathematics: the impact of multiple TSPO binding sites in vivo. NeuroImage. 2012;60(2):902–10.
22. Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, et al. In-vivo measurement of activated microglia in dementia. Lancet. 2001;358(9280):461–7.
23. Okello A, Edison P, Archer HA, Turkheimer FE, Kennedy J, Bullock R, et al. Microglial activation and amyloid deposition in mild cognitive impairment: a PET study. Neurology. 2009;72(1):56–62.
24.• Parbo P, Ismail R, Hansen KV, Amidi A, Mårup FH, Gottrup H, et al. Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. Brain J Neurol. 2017;140(7):2002–11 **This study found a positive correlation between amyloid load and PK binding potentials, revealing increased neuroinflammation in the majority of amyloid-positive cases of MCI.**
25. Wiley CA, Lopresti BJ, Venneti S, Price J, Klunk WE, DeKosky ST, et al. Carbon 11-labeled Pittsburgh compound B and carbon 11-labeled (R)-PK11195 positron emission tomographic imaging in Alzheimer disease. Arch Neurol. 2009;66(1):60–7.
26. Schuitemaker A, Kropholler MA, Boellaard R, van der Flier WM, Kloet RW, van der Doef TF, et al. Microglial activation in Alzheimer's disease: an (R)-[$^{11}$C]PK11195 positron emission tomography study. Neurobiol Aging. 2013;34(1):128–36.
27. Edison P, Archer HA, Gerhard A, Hinz R, Pavese N, Turkheimer FE, et al. Microglia, amyloid, and cognition in Alzheimer's disease: an [11C](R)PK11195-PET and [11C]PIB-PET study. Neurobiol Dis. 2008;32(3):412–9.
28. Yokokura M, Mori N, Yagi S, Yoshikawa E, Kikuchi M, Yoshihara Y, et al. In vivo changes in microglial activation and amyloid deposits in brain regions with hypometabolism in Alzheimer's disease. Eur J Nucl Med Mol Imaging. 2011;38(2):343–51.
29. Fan Z, Aman Y, Ahmed I, Chetelat G, Landeau B, Ray Chaudhuri K, et al. Influence of microglial activation on neuronal function in Alzheimer's and Parkinson's disease dementia. Alzheimers Dement. 2015;11(6):608–621.e7.
30. Cagnin A, Rossor M, Sampson EL, Mackinnon T, Banati RB. In vivo detection of microglial activation in frontotemporal dementia. Ann Neurol. 2004;56(6):894–7.
31. Yasuno F, Ota M, Kosaka J, Ito H, Higuchi M, Doronbekov TK, et al. Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [11C]DAA1106. Biol Psychiatry. 2008;64(10):835–41.
32. Yasuno F, Kosaka J, Ota M, Higuchi M, Ito H, Fujimura Y, et al. Increased binding of peripheral benzodiazepine receptor in mild cognitive impairment-dementia converters measured by positron emission tomography with [$^{11}$C]DAA1106. Psychiatry Res. 2012;203(1):67–74.
33. Kreisl WC, Lyoo CH, McGwier M, Snow J, Jenko KJ, Kimura N, et al. In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease. Brain J Neurol. 2013;136(Pt 7):2228–38.
34. Varrone A, Oikonen V, Forsberg A, Joutsa J, Takano A, Solin O, et al. Positron emission tomography imaging of the 18-kDa translocator protein (TSPO) with [18F] FEMPA in Alzheimer's disease patients and control subjects. Eur J Nucl Med Mol Imaging. 2015;42(3):438–46.
35. Lyoo CH, Ikawa M, Liow J-S, Zoghbi SS, Morse CL, Pike VW, et al. Cerebellum can serve as a pseudo-reference region in Alzheimer disease to detect neuroinflammation measured with PET radioligand binding to translocator protein. J Nucl Med. 2015;56(5):701–6.
36. Suridjan I, Rusjan PM, Kenk M, Verhoeff NPLG, Voineskos AN, Rotenberg D, et al. Quantitative imaging of neuroinflammation in human white matter: a positron emission tomography study with translocator protein 18 kDa radioligand, [18F]-FEPPA. Synapse. 2014;68(11):536–47.
37. Golla SSV, Boellaard R, Oikonen V, Hoffmann A, van Berckel BNM, Windhorst AD, et al. Quantification of [18F]DPA-714 binding in the human brain: initial studies in healthy controls and Alzheimer's disease patients. J Cereb Blood Flow Metab. 2015;35(5):766–72.
38. Gulyás B, Vas A, Tóth M, Takano A, Varrone A, Cselényi Z, et al. Age and disease related changes in the translocator protein (TSPO) system in the human brain: positron emission tomography measurements with [11C]vinpocetine. NeuroImage. 2011;56(3):1111–21.
39. Varrone A, Mattsson P, Forsberg A, Takano A, Nag S, Gulyás B, et al. In vivo imaging of the 18-kDa translocator protein (TSPO) with [18F]FEDAA1106 and PET does not show increased binding in Alzheimer's disease patients. Eur J Nucl Med Mol Imaging. 2013;40(6):921–31.
40.• Hamelin L, Lagarde J, Dorothée G, Leroy C, Labit M, Comley RA, et al. Early and protective microglial activation in Alzheimer's disease: a prospective study using 18F-DPA-714 PET imaging. Brain J Neurol. 2016;139(Pt 4):1252–64 **This study compared AD patients with different rates of progression and found that TSPO binding was higher in patients with slower clinical progression, suggesting that neuroinflammation may play a protective role in the early stages.**
41. Doble A, Malgouris C, Daniel M, Daniel N, Imbault F, Basbaum A, et al. Labelling of peripheral-type benzodiazepine binding sites in human brain with [3H] PK 11195: anatomical and subcellular distribution. Brain Res Bull. 1987;18(1):49–61.
42. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1(1):a006189.
43. Golla SSV, Boellaard R, Oikonen V, Hoffmann A, van Berckel BNM, Windhorst AD, et al. Parametric binding images of the TSPO ligand 18F-DPA-714. J Nucl Med. 2016;57(10):1543–7.
44. Fan Z, Okello AA, Brooks DJ, Edison P. Longitudinal influence of microglial activation and amyloid on neuronal function in Alzheimer's disease. Brain J Neurol. 2015;138(Pt 12):3685–98.
45. Kreisl WC, Lyoo CH, Liow J-S, Wei M, Snow J, Page E, et al. (11)C-PBR28 binding to translocator protein increases with progression of Alzheimer's disease. Neurobiol Aging. 2016;44:53–61.
46. Turkheimer FE, Edison P, Pavese N, Roncaroli F, Anderson AN, Hammers A, et al. Reference and target region modeling of [11C]-(R)-PK11195 brain studies. J Nucl Med. 2007;48(1):158–67.
47. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology. 1988;38(8):1285–91.
48. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol. 2003;106(6):518–26.
49. Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann Neurol. 2005;57(2):168–75.
50. Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiol Dis. 2006;21(2):404–12.
51. Bartels AL, Willemsen ATM, Doorduin J, de Vries EFJ, Dierckx RA, Leenders KL. [11C]-PK11195 PET: quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? Parkinsonism Relat Disord. 2010;16(1):57–9.
52. Edison P, Ahmed I, Fan Z, Hinz R, Gelosa G, Ray Chaudhuri K, et al. Microglia, amyloid, and glucose metabolism in Parkinson's disease with and without dementia. Neuropsychopharmacology. 2013;38(6):938–49.

53.• Terada T, Yokokura M, Yoshikawa E, Futatsubashi M, Kono S, Konishi T, et al. Extrastriatal spreading of microglial activation in Parkinson's disease: a positron emission tomography study. Ann Nucl Med. 2016;30(8):579–87 **Using the [$^{11}$C]DPA713 ligand, the role of microglial activation in the early stages of PD was analysed and the authors concluded that extrastriatal spreading of neuroinflammation occurs early in the pathophysiological process.**

54. Koshimori Y, Ko J-H, Mizrahi R, Rusjan P, Mabrouk R, Jacobs MF, et al. Imaging striatal microglial activation in patients with Parkinson's disease. PLoS One. 2015;10(9):e0138721.

55.•• Ghadery C, Koshimori Y, Coakeley S, Harris M, Rusjan P, Kim J, et al. Microglial activation in Parkinson's disease using [18F]-FEPPA. J Neuroinflammation. 2017 [cited 2018 Dec 4];14(1). Available from: http://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-016-0778-1. **This study investigated patients of HAB for the rs6791 polymorphism with MAB patients to assess regional differences in [$^{18}$F-FEPPA binding in PD patients and HCs. The results demonstrated a significant main effect of genotype on radioligand binding in all brain regions, but no effect of disease or disease *and* genotype interaction in any brain region.**

56. Rusjan PM, Wilson AA, Bloomfield PM, Vitcu I, Meyer JH, Houle S, et al. Quantitation of translocator protein binding in human brain with the novel radioligand [18F]-FEPPA and positron emission tomography. J Cereb Blood Flow Metab. 2011;31(8):1807–16.

57. Iranzo A, Fernández-Arcos A, Tolosa E, Serradell M, Molinuevo JL, Valldeoriola F, et al. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients. PLoS One. 2014;9(2):e89741.

58.•• Stokholm MG, Iranzo A, Østergaard K, Serradell M, Otto M, Svendsen KB, et al. Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. Lancet Neurol. 2017;16(10):789–96 **This study investigated the potential role of the PK ligand as a clinical marker of short-term conversion to a synucleinopathy in patients with iRBD.**

59. Iannaccone S, Cerami C, Alessio M, Garibotto V, Panzacchi A, Olivieri S, et al. In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. Parkinsonism Relat Disord. 2013;19(1):47–52.

60. Smith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull. 2012;87(1):10–20.

61. Kobylecki C, Counsell SJ, Cabanel N, Wächter T, Turkheimer FE, Eggert K, et al. Diffusion-weighted imaging and its relationship to microglial activation in parkinsonian syndromes. Parkinsonism Relat Disord. 2013;19(5):527–32.

62. Gerhard A, Banati RB, Goerres GB, Cagnin A, Myers R, Gunn RN, et al. [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. Neurology. 2003;61(5):686–9.

63. Schwarz SC, Seufferlein T, Liptay S, Schmid RM, Kasischke K, Foster OJ, et al. Microglial activation in multiple system atrophy: a potential role for NF-kappaB/rel proteins. Neuroreport. 1998;9(13):3029–32.

64. Dodel R, Spottke A, Gerhard A, Reuss A, Reinecker S, Schimke N, et al. Minocycline 1-year therapy in multiple-system-atrophy: effect on clinical symptoms and [(11)C] (R)-PK11195 PET (MEMSA-trial). Mov Disord. 2010;25(1):97–107.

65. Gerhard A, Watts J, Trender-Gerhard I, Turkheimer F, Banati RB, Bhatia K, et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in corticobasal degeneration. Mov Disord. 2004;19(10):1221–6.

66. Henkel K, Karitzky J, Schmid M, Mader I, Glatting G, Unger JW, et al. Imaging of activated microglia with PET and [11C] PK 11195 in corticobasal degeneration. Mov Disord. 2004;19(7):817–21.

67. Turner MR, Cagnin A, Turkheimer FE, Miller CCJ, Shaw CE, Brooks DJ, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol Dis. 2004;15(3):601–9.

68. Turner MR, Gerhard A, Al-Chalabi A, Shaw CE, Hughes RAC, Banati RB, et al. Mills' and other isolated upper motor neurone syndromes: in vivo study with 11C-(R)-PK11195 PET. J Neurol Neurosurg Psychiatry. 2005;76(6):871–4.

69. Corcia P, Tauber C, Vercoullie J, Arlicot N, Prunier C, Praline J, et al. Molecular imaging of microglial activation in amyotrophic lateral sclerosis. PLoS One. 2012;7(12):e52941.

70. Zürcher NR, Loggia ML, Lawson R, Chonde DB, Izquierdo-Garcia D, Yasek JE, et al. Increased in vivo glial activation in patients with amyotrophic lateral sclerosis: assessed with [(11)C]-PBR28. NeuroImage Clin. 2015;7:409–14.

71. Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol. 2001;60(2):161–72.

72. Pavese N, Gerhard A, Tai YF, Ho AK, Turkheimer F, Barker RA, et al. Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology. 2006;66(11):1638–43.

73. Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain J Neurol. 2007;130(Pt 7):1759–66.

74. Politis M, Pavese N, Tai YF, Tabrizi SJ, Barker RA, Piccini P. Hypothalamic involvement in Huntington's disease: an in vivo PET study. Brain J Neurol. 2008;131(Pt 11):2860–9.

75. Morton AJ, Wood NI, Hastings MH, Hurelbrink C, Barker RA, Maywood ES. Disintegration of the sleep-wake cycle and circadian timing in Huntington's disease. J Neurosci. 2005;25(1):157–63.

76. Politis M, Lahiri N, Niccolini F, Su P, Wu K, Giannetti P, et al. Increased central microglial activation associated with peripheral cytokine levels in premanifest Huntington's disease gene carriers. Neurobiol Dis. 2015;83:115–21.

77. Simmons DA, James ML, Belichenko NP, Semaan S, Condon C, Kuan J, et al. TSPO-PET imaging using [18F]PBR06 is a potential translatable biomarker for treatment response in Huntington's disease: preclinical evidence with the p75NTR ligand LM11A-31. Hum Mol Genet. 2018;27(16):2893–912.

78. Turkheimer FE, Rizzo G, Bloomfield PS, Howes O, Zanotti-Fregonara P, Bertoldo A, et al. The methodology of TSPO imaging with positron emission tomography. Biochem Soc Trans. 2015;43(4):586–92.

79. Rizzo G, Veronese M, Tonietto M, Zanotti-Fregonara P, Turkheimer FE, Bertoldo A. Kinetic modeling without accounting for the vascular component impairs the quantification of [(11)C]PBR28 brain PET data. J Cereb Blood Flow Metab. 2014;34(6):1060–9.

80. Lavisse S, Guillermier M, Hérard A-S, Petit F, Delahaye M, Van Camp N, et al. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J Neurosci. 2012;32(32):10809–18.

81.• Janssen B, Vugts DJ, Funke U, Molenaar GT, Kruijer PS, van Berckel BNM, et al. Imaging of neuroinflammation in Alzheimer's disease, multiple sclerosis and stroke: recent developments in positron emission tomography. Biochim Biophys Acta (BBA) - Mol Basis Dis. 2016;1862(3):425–41 **This review summarises the clinical and pre-clinical research using TSPO PET in these conditions, as well as discussing new molecular targets for imaging.**

82. Ohnishi A, Senda M, Yamane T, Sasaki M, Mikami T, Nishio T, et al. Human whole-body biodistribution and dosimetry of a new

PET tracer, [(11)C] ketoprofen methyl ester, for imagings of neuroinflammation. Nucl Med Biol. 2014;41(7):594–9.

83. Mu L, Slavik R, Müller A, Popaj K, Cermak S, Weber M, et al. Synthesis and preliminary evaluation of a 2-Oxoquinoline carboxylic acid derivative for PET imaging the cannabinoid type 2 receptor. Pharmaceuticals (Basel). 2014;7(3):339–52.

84.•• Tronel C, Largeau B, Santiago Ribeiro MJ, Guilloteau D, Dupont A-C, Arlicot N. Molecular targets for PET imaging of activated microglia: the current situation and future expectations. Int J Mol Sci. 2017;18(4) **The authors discuss the progress made in TSPO PET imaging, as well as the current limitations and pitfalls faced. They provide an overview of alternative molecular targets and how they may enhance our understanding of microglial activation in neurodegenerative conditions.**

85.•• Fan Z, Calsolaro V, Atkinson RA, Femminella GD, Waldman A, Buckley C, et al. Flutriciclamide (18F-GE180) PET: first-in-human PET study of novel third-generation in vivo marker of human translocator protein. J Nucl Med. 2016;57(11):1753–9 **The first published study to demonstrate the kinetic properties of a third-generation TSPO radioligand.**

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