

## Neuroinflammation PET imaging: Current opinion and future directions

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## **ABSTRACT**

Neuroinflammation is a pathological hallmark of numerous neurologic diseases. Positron emission tomography (PET) imaging enables a non-invasive means to investigate, quantify, and track the spatiotemporal dynamics of various immune cells in living subjects. Translocator protein 18 kDa (TSPO)-PET is a technique for detecting glial activation that has yielded valuable clinical data linking neuroinflammation to cognitive decline in neurodegenerative diseases and has also been used preliminarily as a therapy monitoring tool. However, considerable limitations of TSPO-PET have prompted identification of other more cell-specific and functionally relevant biomarkers. This review analyzes the clinical potential of available and emerging PET biomarkers of innate and adaptive immune responses, with mention of exciting future directions for the field.

## **INTRODUCTION**

Neuroinflammation typically involves activation of central nervous system (CNS) resident glia and infiltration of peripheral immune leukocytes into the brain and spinal cord. Microglia are the first line of defense and primary resident immune cells in the CNS. These cells surveil their surrounding microenvironment to maintain homeostasis and protect against infection and damage (1). Upon acute activation, microglia initiate inflammatory signaling cascades through release of cytokines/chemokines and generation of reactive oxidative species (ROS) to defend against pathogens and mitigate injury (1, 2). This activation is usually transient and resolves as basal physiology is restored. However, under aberrant conditions microglia may stay chronically active, turning a protective mechanism maladaptive (1).

Mounting data from rodent models of CNS diseases and genome wide association studies highlight the integral role of the immune system and its dysregulation in neurodegenerative

diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (2). In such diseases, microglia can take on both neurotoxic and neuroprotective roles and often lose the ability to function properly (e.g., ineffective and unregulated phagocytosis). Beyond microglia, a number of other cells are often involved in neurotoxic actions, including astrocytes, endothelial cells, and oligodendrocytes, in addition to infiltrating peripheral innate immune cells (e.g. macrophages, monocytes, and neutrophils) and adaptive immune cells (e.g. B and T lymphocytes) (1–3).

Immune cell activation states are context dependent (1), and therefore cannot be sufficiently studied through isolating/culturing cells, analyzing transcriptomic data, or biopsy sampling alone. There is a need for noninvasive techniques that enable longitudinal visualization of neuroimmune responses *in vivo*. Positron emission tomography (PET), is widely used in both research and clinical settings for studying biological processes in living subjects and is an indispensable tool for early detection/diagnosis of pathology, real-time therapy monitoring, and patient stratification in clinical trials. Imaging the CNS using PET requires active transport or passive diffusion of a radiolabeled molecule across the blood brain barrier (BBB). Thus, successful radiotracer design for imaging neuroinflammation typically employs the use of small molecules and requires striking a balance between achieving optimal binding characteristics (e.g. high affinity, high selectivity, and low non-specific binding), while maintaining properties conducive to transport across the BBB (e.g. molecular weight less than 500 Da, cLogP between 2 and 3, and total polar surface area between 20 and 120 Å<sup>2</sup>) (4). Apart from small molecules, biologics (e.g. monoclonal antibodies and minibodies) and other large molecules (e.g. adenoviruses and dendrimers) are increasingly being explored for CNS imaging. Given their larger sizes, these agents often require additional considerations and/or creative strategies to ensure adequate BBB

penetration (e.g. transferrin conjugation, active transport, or the use of focused ultrasound to temporarily disrupt the BBB).

A number of PET radiotracers have been developed for candidate biomarkers of neuroinflammation, including translocator protein 18 kDa (TSPO), cannabinoid receptor 2 (CB2R), and cyclooxygenase enzymes. However, many of these imaging targets face challenges that limit their overall success, such as suboptimal cellular specificity and low signal-to-noise ratios (SNR). Accordingly, there is an evolving shift in mindset to conduct rigorous and systematic validation of candidate biomarkers prior to engaging in time- and resource-intensive radiotracer development. Criteria for such validations include: (1) adequate concentration of available target binding sites ( $B_{\max} > 10\text{nM}$ ), (2) sufficient change in target expression in relevant CNS diseases (correlating with disease severity), (3) low basal expression in the CNS (ensuring reasonable SNR), (4) high specificity for a cell type of interest, and (5) functional relevance. This review briefly describes state-of-the-art approaches to neuroinflammation PET imaging and highlights recent key advancements in the field, while using the above criteria to critically analyze the potential clinical impact and foreseeable obstacles to widespread use of existing and emerging radiotracers.

## **TSPO-PET**

To date, the most extensively explored target for imaging neuroinflammation is translocator protein 18 kDa (TSPO) (1, 5). This mitochondrial protein is involved in numerous physiological processes including neuronal survival and steroidogenesis (1). Although TSPO is densely distributed in many peripheral tissues under homeostatic conditions, basal CNS expression is low. During neuroinflammatory states, TSPO expression becomes significantly upregulated

primarily in activated microglia, reactive astrocytes and CNS-infiltrating myeloid-lineage cells (e.g., macrophages, monocytes) (1, 5). Thus, TSPO can serve as an effective target for probing the temporal dynamics of glial and innate immune activation across diseases.

Historically,  $^{11}\text{C}$ -PK11195 has been the most widely utilized TSPO radiotracer. While PET studies using  $^{11}\text{C}$ -PK11195 have provided numerous valuable insights regarding glial/macrophage activation in different neurological disorders including dementia and stroke (1), its poor brain permeability and high plasma protein binding have limited its sensitivity and overall clinical utility. Thus, many 2<sup>nd</sup> generation TSPO tracers were developed to improve brain uptake and increase sensitivity for detecting subtle alterations in neuroinflammation. One of the most promising 2<sup>nd</sup> generation tracers is  $^{11}\text{C}$ -DPA-713, which displays favorable *in vivo* tracer characteristics (higher brain uptake) and superior sensitivity (e.g., improved SNR and specific binding) for detecting neuroinflammation in patients and rodent models of neurological disease compared to  $^{11}\text{C}$ -PK11195 and other TSPO tracers (6–8). Notably, Yokokura and colleagues identified multiple brain regions containing elevated  $^{11}\text{C}$ -DPA-713 binding in AD patients compared to healthy controls – a difference that was not detected with  $^{11}\text{C}$ -PK11195 (6). In addition, a negative correlation was identified between cognitive function and  $^{11}\text{C}$ -DPA-713 binding, further highlighting its sensitivity and potential clinical utility. Another promising 2<sup>nd</sup> generation tracer is  $^{11}\text{C}$ -PBR28 which has been applied clinically in a range of neurological disorders including dementia and chronic pain (5, 9). Importantly,  $^{11}\text{C}$ -PBR28 has been used to monitor immunomodulatory therapies in PD patients, wherein decreased binding of  $^{11}\text{C}$ -PBR28 was found after treatment with myeloperoxidase inhibitor AZD3241 (Figure 1). This provides proof-of-concept data that TSPO-PET could be used for clinical therapy monitoring (10). Similar to  $^{11}\text{C}$ -DPA-713,  $^{11}\text{C}$ -PBR28 exhibits greater affinity for TSPO and higher levels of specific

binding compared to  $^{11}\text{C}$ -PK11195 (5, 8). However, its binding potential is lower than that observed with  $^{11}\text{C}$ -DPA-713 in healthy humans (1.2 vs 7.3)(8).

An important limitation regarding clinical application of 2<sup>nd</sup> generation tracers is their universal sensitivity to the rs6971 TSPO polymorphism, which results in high-, mixed- and low-affinity binders (HABs, MABs and LABs) (5). Although the frequency of LABs is much lower than that of HABs and MABs, the lack of quantifiable TSPO signal in LABs limits the number of patients eligible for 2<sup>nd</sup>-generation TSPO-PET and necessitates time intensive genetic testing to validate patient eligibility prior to imaging. Recent advances in TSPO-PET have focused on the development of 3<sup>rd</sup>-generation tracers insensitive to the rs6971 polymorphism.  $^{11}\text{C}$ -ER176, a quinazoline analog of PK11195, was identified as a promising candidate with limited sensitivity to the rs6971 polymorphism *in vitro*, although *in vivo* investigation revealed lower brain uptake in LABs (vs. HABs and MABs) (1, 8). Nonetheless,  $^{11}\text{C}$ -ER176 exhibits sufficient  $\text{BP}_{\text{ND}}$  to permit imaging of LABs and appears to have enhanced specificity and SNR compared to previous tracers due to the absence of brain-penetrable radiometabolites.

Although TSPO-PET remains the most widely used technique for clinical neuroinflammation imaging, it is limited by its lack of cellular specificity (i.e., expression is also observed in platelets, endothelial cells and cancer cells) and inability to discriminate between beneficial versus toxic immune responses, which must be considered when interpreting results. Therefore, there is a need to discover new functionally relevant imaging biomarkers to specifically quantify different aspects of neuroinflammation.

## **ALTERNATIVE EXISTING AND EMERGING INNATE IMMUNE CELL IMAGING BIOMARKERS**

To overcome the limitations of TSPO-PET, other neuroimmune imaging biomarkers are being explored (Figure 2). Though not described here, MAOB, GSK3, RAGE, MERTK, and S1P1 are also being investigated as PET biomarkers and were recently reviewed (3).

## **P2X7R-PET**

P2X ligand-gated ion channel type 7 (P2X7R) is an ionotropic receptor activated by ATP and implicated in microglial proliferation, activation, and apoptosis (11). Under normal physiologic conditions, P2X7R is inactive and predominately expressed by innate immune cells (e.g. macrophages, monocytes, and microglia). Importantly, P2X7R is also found on astrocytes, oligodendrocytes, Schwann cells, and neurons, albeit to a lesser extent (11). Following injury, P2X7R expression increases significantly to promote inflammasome formation and release of proinflammatory cytokines and chemokines (3, 11). This upregulation and activation of P2X7R is associated with numerous neurodegenerative and neuroinflammatory conditions, including AD, PD, and MS (11, 12).

Development of radiotracers for P2X7R is a rapidly expanding area, and there are currently four promising radiolabeled P2X7R antagonists ( $^{11}\text{C}$ -GSK1482160,  $^{18}\text{F}$ -JNJ-64413739,  $^{11}\text{C}$ -JNJ-54173717, and  $^{11}\text{C}$ -SMW139) that have been validated in rodents and assessed in humans. First-in-man radiation dosimetry studies for  $^{11}\text{C}$ -GSK1482160 demonstrated low uptake in the CNS (2% ID/g) and moderate binding in peripheral organs of healthy controls (13).  $^{18}\text{F}$ -JNJ-64413739, on the other hand, showed significant brain uptake with favorable peripheral radiation dosimetry in healthy humans (12). However, high inter-subject variability for this tracer and the lack of an identifiable brain reference region may render image quantification challenging.  $^{11}\text{C}$ -JNJ-54173717 and  $^{11}\text{C}$ -SMW139 have both been used to image neuroimmune responses in patients.

Importantly, <sup>11</sup>C-JNJ-54173717 did not detect significant differences between PD or amyotrophic lateral sclerosis (ALS) patients compared to age-matched healthy controls (14, 15). This is possibly due to the suboptimal dynamic range of P2X7R expression in specific diseases and a reported single nucleotide polymorphism, rs3751143, which appeared to impact tracer binding in a subset of scanned PD subjects. On the other hand, first-in-man pharmacokinetic studies using <sup>11</sup>C-SMW139 demonstrated increased *in vivo* binding potential in patients with relapsing remitting MS compared to age matched healthy controls, warranting further investigation of this tracer (16).

Although P2X7R has a distinct role in inflammasome formation and has been implicated in inflammatory pathways in neurodegenerative diseases, certain factors may limit its overall utility as an imaging biomarker. Similar to TSPO, P2X7R lacks cellular specificity and has moderate basal expression in the CNS, thereby restricting SNR. Moreover, the rs3751143 polymorphism seems to effect binding affinity of certain P2X7R-PET tracers (14). Thus, further clinical investigation is needed in addition to detailed histological analysis of human brain tissue to better characterize P2X7R expression dynamics in human disease.

### **Cyclooxygenase-PET**

Cyclooxygenase isoenzymes, cyclooxygenase-1 and cyclooxygenase-2, are targets of nonsteroidal anti-inflammatory drugs and are responsible for catalyzing the conversion of arachidonic acid into prostaglandins. These targets are moderately expressed throughout the healthy CNS (17, 18). Recent studies have identified elevated levels of cyclooxygenase-1 positive microglia within (and surrounding) sites of neurodegeneration (3, 19). Cyclooxygenase-2 expression is localized to neurons, microglia, and endothelial cells and primarily upregulated in neurons in response to inflammatory stimuli (3). Therefore, imaging cyclooxygenase enzymes may



illuminate unique aspects of immune pathophysiology and enable real time monitoring of immunotherapies.

Multiple cyclooxygenase-1 inhibitors and their analogs have been radiolabeled for PET imaging. One such analog,  $^{11}\text{C}$ -ketoprofen-methyl ester ( $^{11}\text{C}$ -KTP-Me), displayed promising cyclooxygenase-1 selectivity and brain uptake in rats with systemic inflammation (19). Studies in healthy humans demonstrated brain uptake (average peak SUV of 1.5 after 2 mins) of  $^{11}\text{C}$ -KTP-Me, as well as favorable stability and dosimetry for clinical translation (3). However, when assessed in AD patients, no differences in brain uptake or washout were observed, indicating possible low binding affinity or insufficient sensitivity of  $^{11}\text{C}$ -KTP-Me for detecting cyclooxygenase-1 alterations *in vivo* (19). More recently, the cyclooxygenase-1 specific radioligand, ( $^{11}\text{C}$ -1,5-bis-(4-methoxyphenyl)-3-(2,2,2-tri-fluoroethoxy)-1H-1,2,4-triazole ( $^{11}\text{C}$ -PS13), was assessed in rhesus monkeys and demonstrated marked uptake in regions containing high cyclooxygenase-1 expression, which was significantly blocked by cyclooxygenase-1 selective inhibitors, thereby warranting further evaluation (20).

A number of selective cyclooxygenase 2 inhibitors, including rofecoxib and celecoxib have been radiolabeled and assessed in animal models of neuroinflammation. Most have been unsuccessful due to low brain uptake, non-specific binding, low affinity, and/or metabolic instability. However, a recent study reported significant brain uptake of  $^{11}\text{C}$ -celecoxib in healthy baboons, indicating suitable BBB penetration (18). In parallel,  $^{11}\text{C}$ -3-(4-methylsulfonylphenyl)-4-phenyl-5-trifluoromethylisoxazole ( $^{11}\text{C}$ -TMI), another radiolabeled cyclooxygenase-2 inhibitor, was evaluated in healthy rhesus monkeys and also demonstrated sufficient brain uptake and high metabolic stability (17). These tracers have yet to be explored in humans, although preclinical toxicity and dosimetry data indicate that  $^{11}\text{C}$ -celecoxib is safe for exploratory human studies.

Further characterization of cyclooxygenase-targeted radiotracers in animal models of neuroinflammatory disease and patients is required to not only assess whether they can reliably track pathologic alterations of individual isoforms *in vivo*, but to also ascertain the clinical/diagnostic benefit of doing so.

## **CB2R-PET**

Cannabinoid type 2 receptor (CB2R) is moderately expressed on the surface of immune cells in the periphery and at low levels in the healthy CNS, predominantly on glia and endothelial cells, as well as certain subsets of neurons (21). Following CNS insult, CB2R expression is markedly upregulated on activated microglia, astrocytes, and neurons (21–23). CB2R upregulation has been implicated in multiple neurological disorders, and selective activation can attenuate inflammatory responses by reducing proinflammatory cytokine release (21).

Many CB2R radiotracers have been developed and are reviewed in detail by Spinelli *et al* (21). One such radiotracer,  $^{11}\text{C}$ -NE40, has been characterized extensively in healthy mice, rats, and rhesus monkeys whereby it was shown to readily cross the BBB and bind to CB2R with high affinity and specificity. Recently, Yamagishi and colleagues demonstrated that  $^{11}\text{C}$ -NE40 can track neuroinflammation in a mouse model of accelerated aging more accurately than TSPO-PET (22). When assessed in healthy humans,  $^{11}\text{C}$ -NE40 exhibited promising brain uptake and washout, however, when used in AD patients,  $^{11}\text{C}$ -NE40 uptake was not associated with amyloid pathology, and in fact was reduced in patients compared to controls (24). This suggests that  $^{11}\text{C}$ -NE40 may have low *in vivo* selectivity for CB2R and/or could reflect a more complex expression profile of CB2R in clinical AD. Another CB2R tracer,  $^{11}\text{C}$ -A-836339, developed with higher affinity than  $^{11}\text{C}$ -NE40, has been evaluated in rodents. Increased  $^{11}\text{C}$ -A-836339 binding was observed in the

brains of transgenic APP<sub>swe</sub>×PS1<sub>dE9</sub> AD mice compared to wild-type controls (23). In contrast, no significant differences in <sup>11</sup>C-A-836339 brain uptake were reported in an ischemic rat model of stroke (21). Clinical assessment of <sup>11</sup>C-A-836339 has yet to be reported. Further evaluation is therefore necessary to assess the selectivity and kinetics of the most promising CB2R tracers and determine whether CB2R can be used as a biomarker to sensitively and reliably track neuroinflammation in human CNS diseases.

### CSF1R-PET

Colony Stimulating Factor 1 Receptor (CSF1R) is a cell surface tyrosine kinase receptor essential for myeloid cell survival, proliferation, and differentiation (25). High basal CSF1R expression in the healthy brain is primarily restricted to microglia and to a lesser extent neurons (with conflicting reports on neuronal expression) (25, 26). CSF1R levels are significantly increased in diseases associated with marked neuroinflammation, such as AD, MS, and Glioblastomas (26).

Initial efforts in CSF1R specific radiotracer development described the synthesis of <sup>11</sup>C-AZ683 which was hindered by the lack of BBB penetration *in vivo* (27). More recent work from Horti and colleagues led to synthesis of the high affinity CSF1R radioligand, 5-cyano-N-(4-(4-<sup>11</sup>C-methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide (<sup>11</sup>C-CPPC). *In vivo* evaluation of <sup>11</sup>C-CPPC in rodent (LPS and experimental autoimmune encephalomyelitis (EAE)) and non-human primate (LPS) models of disease, as well as *in vitro* autoradiography of human AD brain tissue (Figure 3) demonstrated significantly greater signal in the CNS of disease groups compared to controls. While early results appear promising, significant nonspecific binding (i.e., marked PET signal in the brains of CSF1R knockout and microglia-depleted mice) must be

addressed – perhaps by exploring other analogs for future tracer development (25). Moreover, the moderately high basal expression of CSF1R may limit accurate detection of subtle changes in microglial activation *in vivo*.

## **P2Y12R-PET**

Purinergic metabotropic 12 receptor (P2Y12R) is a chemoreceptor for adenosine diphosphate expressed peripherally on platelets but restricted to microglia within the CNS (28, 29). Due to its demonstrated role in microglial chemotaxis and cytokine/chemokine signaling, in addition to its absence on peripheral immune cells, P2Y12R is an attractive imaging target for visualizing the functional contribution of microglia in different neurological diseases *in vivo*. Histological data show that the specific role and expression patterns of P2Y12R in the CNS are dynamic and nuanced, depending on both disease type and stage. That is, P2Y12R expression can quickly change as microglia shift to adopt different activated phenotypes. For example, P2Y12R expression has been shown to decrease in human postmortem AD plaque associated brain tissue (30). However, regional changes in AD tissue appear to be quite dynamic and complex, with a recent study demonstrating that P2Y12R expression *decreases* in and around dense, mature plaques but *increases* in the midst of diffuse plaques (29).

To date, only one P2Y12R-PET radioligand has been developed, Ethyl6-(3-(3-((5-chlorothiophen-2-yl)sulfonyl)<sup>11</sup>C-ureido)azetidin-1-yl)-5-cyano-2-methylnicotinate; which is an isotopolog of a known high affinity P2Y12R antagonist. While autoradiography studies demonstrated utility of this tracer as a tool for studying P2Y12R *in vitro*, subsequent *in vivo* imaging studies in rats revealed low metabolic stability and lack of BBB permeability possibly due to PGP efflux (31). Of note, *ex vivo* biodistribution studies of this tracer in mice demonstrated

low binding in blood, addressing concerns regarding the possibility of high uptake in circulating platelets masking signal within the brain parenchyma. Although further work must be performed to better understand the relationship between P2Y<sub>12</sub>R expression patterns in different CNS disease states, the high specificity of P2Y<sub>12</sub>R for microglia in the CNS in addition to its dynamic functional role warrants investigation into CNS-penetrant P2Y<sub>12</sub>R-PET radioligands.

### **TREM1-PET**

Triggering receptor expressed on myeloid cells 1 (TREM1) is a transmembrane immunoglobulin receptor specifically localized to myeloid lineage cells. Interestingly, this receptor appears to be absent from microglia in mice (32), though it is present at relatively low levels in human microglia. TREM1 amplifies proinflammatory responses and has shown therapeutic potential in preclinical models of stroke and MS (32, 33). Although TREM1 expression is negligible in the healthy murine CNS, it is significantly upregulated on CNS-infiltrating peripheral myeloid cells during neuroinflammation (32). Thus, TREM1-PET could be useful for visualizing peripheral myeloid cell-driven immune responses in CNS disease.

Our group developed the first TREM1-targeted PET probe (<sup>64</sup>Cu-TREM1-mAb) by radiolabeling a selective anti-TREM1 monoclonal antibody (mAb). After validating its specificity, we demonstrated the ability of <sup>64</sup>Cu-TREM1-mAb to successfully detect CNS-infiltrating peripheral myeloid cells prior to symptom manifestation in EAE mice with greater sensitivity than TSPO-PET (32). Similarly, we showed that in a mouse model of stroke, TREM1-PET detected increased signal in ischemic brain tissue, spleen, and intestines of stroked mice (33). These results led to further investigation of TREM1 expression on intestinal macrophages, where it was shown to play a role in gut permeability and bacterial translocation post-stroke. This work highlights the

utility of TREM1-PET for characterizing systemic innate immune responses in the context of neurologic disease. Clinical translation of TREM1-PET and development of small molecule candidates is warranted for imaging TREM1 in pathologies with and without BBB dysfunction.

## **ADAPTIVE IMMUNE CELL IMAGING BIOMARKERS**

Adaptive immune cells (e.g. B and T lymphocytes) play a critical role in several neurological disorders. In recent years, B cell-targeted therapies have shown remarkable results in improving clinical symptoms of MS (34). Research has shown that CNS-infiltrating B cells are key drivers of maladaptive immune responses in MS by increasing antigen presentation and stimulating autoimmune T cells (34). Considering the promise of anti-B cell therapies, cell-specific *in vivo* imaging of B cells could be an essential tool for patient stratification and MS therapy monitoring.

Our group has pioneered methods to image the adaptive immune response in the CNS by radiolabeling antibodies specific for two B cell antigens, both of which have been targeted successfully in clinical trials for MS therapy: Cluster Differentiation Factor 20 (CD20) and Cluster Differentiation Factor 19 (CD19). CD20 and CD19 are transmembrane proteins involved in B cell immune responses. CD20 is not expressed on B cells during their earliest and latest stages of development, whereas CD19 is expressed across development on pre-B cells, plasmablasts, and a subset of long-lived plasma cells (34, 35). We developed a CD20-PET radiotracer, <sup>64</sup>Cu-rituximab, which was administered to transgenic mice expressing human CD20 induced with EAE to mimic MS autoimmune pathology. *In vivo* imaging and *ex vivo* studies confirmed increased binding of this tracer in spinal cords and brains of EAE mice compared to naïve controls (36). More recently, we also generated and assessed the first CD19-PET radiotracer, <sup>64</sup>Cu-CD19-mAB. Imaging in

EAE mice demonstrated significant binding in the CNS of diseased mice compared to naïve controls, correlating histologically with numbers of B cells (35). These studies lay the groundwork for future clinical investigations of B cell CNS imaging and exploration of possible small molecule tracers for these targets.

## **CONCLUSIONS AND FUTURE DIRECTIONS**

Considering the growing emphasis on developing immunomodulatory therapies for neurological diseases, there is a clear need for companion diagnostic tools that can monitor responses to such therapies and stratify eligible patients for clinical trials. Despite its limitations, TSPO-PET remains the most widely used tool for assessing neuroinflammation in clinical studies and has provided valuable insight into the pathophysiology of immune responses in relation to cognitive decline across many neurologic disorders. Informed by the shortcomings of TSPO-PET, new biomarkers should be selected and validated according to the characteristics needed to answer critical biological/clinical questions of interest. For example, questions such as, “Are activated microglia driving cognitive decline in AD?” or, “What is the relative contribution of microglia versus peripheral myeloid cells to MS pathology?” require highly specific biomarkers that delineate between CNS resident microglia and peripheral CNS-infiltrating myeloid cells. TSPO does not fulfill this requirement. Thus, it remains necessary to identify biomarkers with high cell-type specificity and reflect specific immune cell functional phenotypes. This is in addition to having an adequate dynamic range to enable detection of such phenomena *in vivo*. A few of the emerging PET imaging biomarkers mentioned in this review, including CSF1R and TREM1, have the potential to overcome some of the limitations of TSPO-PET and provide more precise measures of specific immune cell function, however further validation is still necessary.

In addition to the biomarkers discussed, other innate immune targets are also being explored, including CD11b, found exclusively on activated myeloid lineage leukocytes. To date, this target has primarily been explored in the periphery, but one study recently demonstrated favorable brain uptake of the  $^{89}\text{Zr}$ -anti-CD11b Ab radiotracer in a murine glioblastoma model (37). Additionally, considering the known infiltration and neurotoxicity of adaptive immune lymphocytes in both autoimmune and neurodegenerative disorders, further work must consider development and evaluation of additional adaptive immune imaging agents for CNS applications. T cell imaging, for example, has only been utilized in peripheral cancer detection and immunotherapy monitoring, primarily via targeting CD4, CD8, and OX40 (38, 39).

Here, we summarized the potential of various neuroinflammation imaging biomarkers and emphasized a growing need to *extensively validate* them as clinically informative tools *prior to* developing radiotracers. Future work will require a more detailed discussion around the nuanced microglial phenotypes when evaluating neuroimmune imaging biomarkers. While previously assigned to the binary pro- or anti-inflammatory axis, it is now clear that microglia are significantly more complex than the previously prescribed M1/M2 paradigm, where “pro-inflammatory” or “anti-inflammatory” can refer to a continuum of activation states with both maladaptive and beneficial phenotypes. Thus, there is a call to action for the field of neuroimmune PET imaging to identify and validate biomarkers that better capture these complex and dynamic microglial activation states in order to more accurately investigate disease progression and treatment response (1, 2). In addition, tracer development and validation using computational BBB permeability modeling tools like the Multiparameter Optimization or artificial intelligence algorithms in conjunction with high-throughput screening is needed to increase efficiency of tracer design and development. Cutting edge technologies like human BBB microfluidic devices and BBB organoids



could also provide critical cost-effective tools to systematically evaluate tracer BBB permeability *in vitro*. Furthermore, *in vitro* testing using postmortem human tissue remains critically important (although tissue can be limited and/or difficult to obtain for certain pathologies) to better understand the utility of new biomarkers/radiotracers in disease prior to *in vivo* clinical evaluation.

In parallel, the development of new clinical imaging systems including time-of-flight PET/MRIs and the groundbreaking total-body PET will substantially improve image resolution and sensitivity, thus enabling enhanced detection of targets with low endogenous expression, subtle alterations, and/or diffuse expression in the CNS. This innovation will also increase translation and accessibility of CNS PET imaging by reducing the required radioactive dose by orders of magnitude, shortening scan times, and eliminating the need for invasive arterial plasma sampling for input function determination. Moreover, total body imaging will, for the first time, enable simultaneous monitoring of peripheral and central changes in immune pathology to visualize *in vivo* the contribution and interplay of systemic pathology to the CNS (40).

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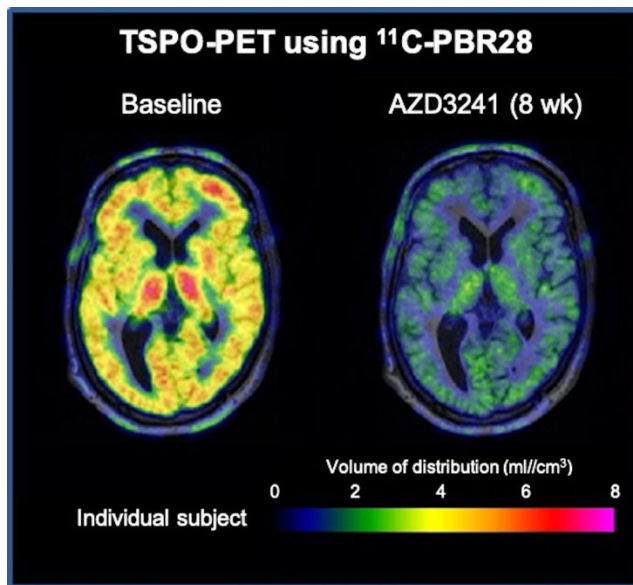
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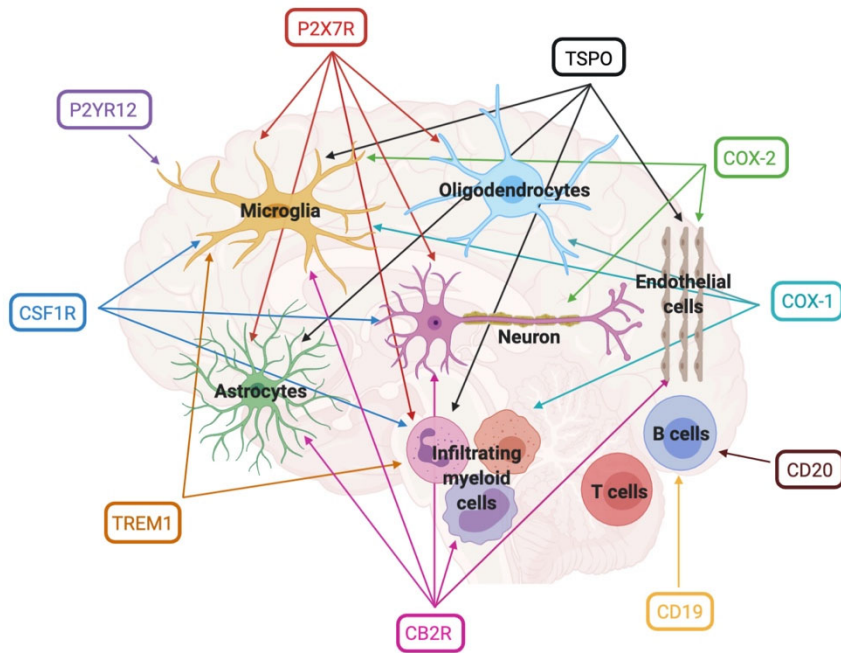
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**FIGURES:**

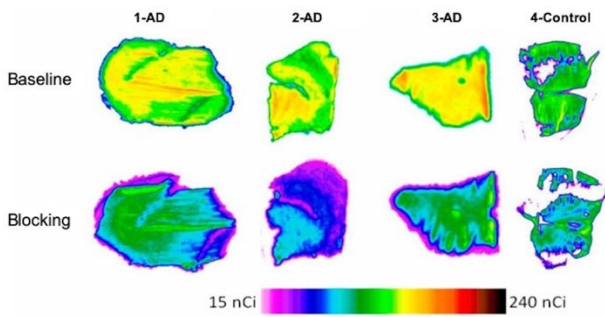


*Figure 1: TSPO-PET imaging shows response to immunomodulatory therapy, myeloperoxidase inhibitor AZD3241, in a representative PD patient. Modified from (10)*



**Figure 2:** Immune cell expression in humans of highlighted imaging biomarkers.





**Figure 3:** *In vitro* autoradiography examining postmortem human AD brain tissue versus control using  $^{11}\text{C}$ -CPPC. Modified from (25).