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Emerging PET Radiotracers and Targets for Imaging of Neuroinflammation in Neurodegenerative Diseases: Outlook Beyond TSPO

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Abstract

The dynamic and multicellular processes of neuroinflammation are mediated by the nonneuronal cells of the central nervous system, which include astrocytes and the brain's resident macrophages, microglia. Although initiation of an inflammatory response may be beneficial in response to injury of the nervous system, chronic or maladaptive neuroinflammation can have harmful outcomes in many neurological diseases. An acute neuroinflammatory response is protective when activated neuroglia facilitate tissue repair by releasing anti-inflammatory cytokines and neurotrophic factors. On the other hand, chronic neuroglial activation is a major pathological mechanism in neurodegenerative diseases, likely contributing to neuronal dysfunction, injury, and disease progression. Therefore, the development of specific and sensitive probes for positron emission tomography (PET) studies of neuroinflammation is attracting immense scientific and clinical interest. An early phase of this research emphasized PET studies of the prototypical imaging biomarker of glial activation, translocator protein-18 kDa (TSPO), which presents difficulties for quantitation and lacks absolute cellular specificity. Many alternate molecular targets present themselves for PET imaging of neuroinflammation in vivo, including enzymes, intracellular signaling molecules as well as ionotropic, G-protein coupled, and immunoglobulin receptors. We now review the lead structures in radiotracer development for PET studies of neuroinflammation targets for neurodegenerative diseases extending beyond TSPO, including glycogen synthase kinase 3, monoamine oxidase-B, reactive oxygen species, imidazoline-2 binding sites, cyclooxygenase, the phospholipase A2/arachidonic acid pathway, sphingosine-1-phosphate receptor-1, cannabinoid-2 receptor, the chemokine receptor CX3CR1, purinergic receptors: $P2X_7$ and $P2Y_{12}$, the receptor for advanced glycation end products, Mer tyrosine kinase, and triggering receptor expressed on myeloid cells-1. We provide a brief overview of the cellular expression and function of these targets, noting their selectivity for astrocytes and/or microglia, and highlight the classes of PET radiotracers that have been investigated in early-stage preclinical or clinical research studies of neuroinflammation.

Keywords

astrocytes, microglia, neurodegenerative diseases, neuroinflammation, positron emission tomography (PET) imaging

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Introduction

Inflammation is an adaptive cellular response triggered by noxious stimuli and conditions, such as infection, tissue injury, or malfunction.[1,2] Famed immunologist Ruslan Medzhitov proposed that inflammation pathways evolved as an adaptive response for restoring homeostasis, as distinct from the chronic and maladaptive parainflammatory response leading to degenerative disease.[3] Initial prohomeostatic adaptive responses engage macrophages and other immune cells to release inflammatory mediators such as cytokines, whereas resolution reinstates the homeostatic state of the tissue, chronic parainflammatory conditions, can entail maladaptive changes in homeostatic set points leading ultimately to degenerative changes.

Neuroinflammation: Relevance to Neurodegenerative Disorders

Neuroinflammation is an inflammatory and adaptive response within the central nervous system (CNS).[4] Neuroinflammation is mediated by the production of small signaling proteins such as cytokines and chemokines, reactive oxygen species (ROS), and lipid second messengers that are produced in situ by glial cells resident in the CNS (ie, astrocytes and microglia), capillary endothelial cells, and immune cells arising in the periphery. These inflammatory factors provoke physiological reactions in relation to the context and duration of the primary stimulus or insult,[4] with self-limiting of the innate immune reactions upon successful resolution of the initiating condition.[5,6] In contrast, chronic neuroinflammation entails persistent activation of microglia and other immune cells in the CNS, with sustained release of inflammatory mediators leading to a feed-forward propagation of the inflammatory response.[7,8]

The blood–brain barrier (BBB) maintains the internal metabolic milieu of the brain in conjunction with glial cells, which can exert a concerted inflammatory process in response to stress.[9] Endogenous anti-inflammatory and neuroprotective responses normally keep transient inflammatory reactions in check and return the brain to homeostasis. However, as with chronic inflammatory conditions in peripheral organs, unregulated glial reactions can induce chronic neuroinflammation that promotes or propagates neurodegenerative diseases.[10-16] For example, in Alzheimer disease (AD), the neuroinflammatory cycle entails sustained activation of microglia and astrocytes in response to stimuli such as amyloid beta (Aβ) deposits.[17] Glia proinflammatory responses activated by Aβ include induction of cytokines (tumor necrosis factor α [TNF]-α, interleukin 1β [IL-1β], and S100 β), chemokines (macrophage inflammatory protein [MIP]-1β), and oxidative stress-related molecules, which can cause neuronal cell dysfunction and/or death and further propagate the inflammatory response.[17] Clinical evidence indicating neuroinflammation as a pharmacological target for chronic neurodegenerative diseases comes from epidemiological observations and immunostaining of postmortem brain tissue. For example, long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) may impart protection against AD.[18] Although prospective studies did not reveal a protective effect, a cross-sectional follow-up study showed that prior exposure to naproxen was associated with a significant reduction in the incidence of AD,[19] suggesting that NSAIDs may be neuroprotective only at the earliest stages of the AD process. Nonetheless, postmortem brain tissue from patients with AD exhibit overexpression of an array of inflammatory mediators in regions most affected by the primary pathologies, that is, Aβ deposition.[20] Taken together, these observations suggest that neuroinflammation constitutes a viable target for the discovery and development of both diagnostics and therapeutics for AD and, by extension, other neurodegenerative diseases.

Cellular Players of Neuroinflammation: Reactive Astrocytes and Reactive Microglia

Neuroinflammation in diseases such as AD, Parkinson's disease, and amyotrophic lateral sclerosis manifests in reactive morphology of astrocytes and microglia, accompanied by release of low to moderate levels of inflammatory mediators in the brain parenchyma. We now briefly summarize the key cellular players that regulate neuroinflammatory responses.

Astrocytes are abundant in the gray and white matter of brain, where they modulate neuronal metabolism and ion transit across the BBB.[21-24] Under pathological conditions, astrocytes undergo a phenotype change from a resting morphology to reactive form with hypertrophic cell bodies and processes.[25] In response to severe injury such as ischemic stroke, reactive astrocytes form a characteristic structure called an astrocyte scar, which prevents infiltration of peripheral inflammatory cells or molecules, thereby reinstating the BBB.[26-29] Although many reports have shown that reactive astrogliosis is a hallmark neuroprotective reaction of astrocytes against CNS disorders, complex mechanisms underlying reactive astrogliosis and induction of the neuroprotective effects suggest that they can both hinder and support CNS recovery.[25,30,31]

Microglia are resident immune cells in the brain, which continuously monitor and respond to the brain microenvironment.[32] Constituting about 10% to 20% of the total glial cells of the brain, microglial expression is highest in the gray matter, including neocortex, hippocampus, olfactory bulb, and the basal ganglia. Upon brain injury, microglia transit from the resting to activation state, which entails a pronounced change from ramified morphology to amoeboid structure with swollen cell bodies and short processes. The amoeboid form is capable of migration to the pathogen site[33] and presumably serves an immune surveillance function.[34,35] Typically, microglia have a very low threshold of activation and rapidly respond within 20 to 40 minutes of injury.[36] Although the causal sequence of microglial activation in neuroinflammatory disorders can be uncertain, the phenotype presents a reliable biomarker for brain injury.

Activated microglia are a double-edged sword, exerting toxic and beneficial roles depending on their polarization phenotype, activation status, and the cellular context.[37,38] Indeed, microglial activation may correspond to the binary activation profile of peripheral monocytes, characterized by M1/M2

phenotypes.[39] The M1-type microglia represent a proinflammatory phenotype releasing inflammatory mediators such as prostaglandins, TNF-α, IL-6, IL-1β, ROS, and glutamate, whereas the M2-type microglia serve to resolve the inflammatory response and express anti-inflammatory mediators such as IL-4, IL-13, IL-25, IL-1ra, insulin-like growth factor 1, and neurotrophic factors.[40]

Genetic profiling of reactive astrocytes from mice treated with a systemic injection of lipopolysaccharide (LPS) or upon middle cerebral artery occlusion to induce ischemia reveal 2 different types of reactive astrocytes, namely, A1 and A2,[41] as distinct from the microglia M1/M2 dichotomy noted above. A1 astrocytes upregulate many classical complement cascade genes previously shown to be destructive to synapses, whereas A2 astrocytes upregulate many neurotrophic factors that may restore or protect synaptic integrity. Activated M1 microglia induce the A1 astrocytes, which lose most normal astrocytic functions but gain a new neurotoxic function, rapidly killing neurons and mature, differentiated oligodendrocytes.[42] Importantly, A1-like reactive astrocytes are present in most major neurodegenerative diseases, raising the possibility that they are drivers for neurodegeneration.

Immunohistochemical techniques enable the detection of specific molecular markers and serve as essential tools for identifying and characterizing cells in healthy and pathological tissue.[43] Constitutive expression of ionized calcium-binding adapter molecule-1 (IBA1) is specific for microglia, irrespective of their activation state.[44,45] However, detection of IBA1 in conjunction with M1 or M2 markers can identify the microglial phenotype.[46] Commonly used M1 markers are CD68, a marker of microglial lysosomes indicative of phagocytic microglia, and major histocompatibility complex class II. On the other hand, M2-type microglia express the mannose receptor CD206.[46] Glial fibrillary acid protein (GFAP) is the prototypical marker for immunohistochemical identification of astrocytes. First isolated from postmortem brain of patients with multiple sclerosis (MS), GFAP was immunohistochemically localized within reactive astrocytes.[47,48] Although GFAP is immunohistochemically detectable in many astrocytes throughout the healthy CNS, double staining studies with multiple markers (including transgenic reporter proteins) show that many mature astrocytes in healthy CNS tissue do not express detectable levels of GFAP. Nevertheless, GFAP expression is a sensitive and reliable marker that labels majority of reactive astrocytes responding to CNS injuries.[43] These findings in vitro predict that detection of markers for activated microglia, and astrocytes in vivo should reveal neuroinflammation and associated neurodegenerative disorders.

Positron Emission Tomography Imaging: A Vital Diagnostic Tool for Tracking Neuroinflammatory Biomarkers and Diagnosing Neurodegenerative Diseases

In recent years, technical advances in imaging modalities such as positron emission tomography (PET) and magnetic resonance imaging (MRI) have encouraged their use for the evaluation of functional, neurochemical, and anatomical changes in the diseased brain.[49] PET is a noninvasive molecular imaging technique that enables quantification and visualization of physiological processes by recording the time-dependent distribution in living organs of tracer molecules labeled with positron-emitting isotopes. Development of novel radiotracers targeting diverse receptors, transporters, enzymes, and other molecular targets within the human brain has expanded the use of PET neuroimaging, specifically in neurodegenerative diseases, and has been reviewed by several groups.[50-57] Among its clinical research applications, PET can quantify neuropathological markers or reveal pharmacokinetic and pharmacodynamic properties of a drug candidate. Thus, it is not surprising that CNS PET imaging has become central for establishing proof of mechanism and optimal dosing for novel therapeutic agents, thereby allowing accelerated decision-making in clinical trials.

Nonetheless, developing radioligands for PET imaging of molecular targets in the CNS is a demanding task, not least of all due to aspects of quantitation. In the first instance, a candidate tracer must cross the BBB, which can be an obstacle due to binding to plasma proteins, low intrinsic permeability, or active extrusion from the brain. In general, passive transfer across the BBB is promoted by low molecular weight <500 Da, low hydrogen-binding capability, lack of formal charge, and moderate lipophilicity (Log D range = 2.0-3.5).[58,59] Tissue uptake of PET tracers is often assessed as percentage of injected dose per gram of target tissue (standard uptake volume [SUV]). A peak uptake (SUV_{peak}) <2% ID/g generally indicates sufficient brain exposure for quantitation measurement of specific binding. Upon entering the CNS, an effective radiotracer must bind with sufficient affinity and specificity to its target binding site, which must be of adequate abundance to be detectable against a background of nonspecific binding. For reversibly binding tracers, specific binding will increase as a function of time until attaining an equilibrium defined by B_{max}, the concentration of the binding site, and K_D, the affinity of interaction between the tracer and its binding site,[60] where the ratio B_{max}/K_D represents binding potential (BP_{ND}). In practice, quantitation is difficult when BP_{ND} <1 due to low specific signal and when BP_{ND} >10 due to failure to attain equilibrium within the time constraints of a dynamic PET scan.[58,61] However, very high BP_{ND} can be an advantage for semiquantitative evaluation of diagnostic radiotracers in a clinical setting.[62] An ideal PET radiotracer to image neuroinflammation should not only have an adequate signal-to-noise ratio (specific vs nonspecific binding) but should also yield a good pathological versus baseline (normal) signal that is indicative of an inflammatory process. However, clinical translation of candidate radiotracer can fail due to deficiencies in animal models, which may not emulate key features of human neurodegenerative diseases.[63-65] The first phase of molecular imaging of neuroinflammation was led by PET studies with isoquinoline carboxamide [^{11}C]PK11195, the prototype ligand of translocator protein-18 kDa (TSPO), formerly known as the peripheral benzodiazepine site.[66,67] The TSPO is

a highly hydrophobic 5 transmembrane domain protein expressed in the outer mitochondrial membrane of microglia and other cells of macrophage lineage.[68] The TSPO is thought to mediate the transport of cholesterol into the inner compartments of mitochondria. While constitutively expressed in healthy brain, microglial TSPO expression is substantially upregulated in response to brain injury and in several neuroinflammatory diseases,[69,70] although there is also TSPO expression in reactive astrocytes.[71] Despite this ambiguity about its cellular origin, immense clinical interest has emerged in TSPO imaging as a biomarker for neuroinflammation in neurodegenerative diseases. We have recently summarized the binding properties as well as challenges for quantitation and interpretation PET studies of [^{11}C]PK11195 versus a dozen second- and third-generation TSPO tracers, including [^{18}F]FEDAA1106, [^{11}C]PBR28, [^{18}F]PBR06, [^{11}C]DPA-713, [^{11}C]ER176, and [^{18}F]DPA-714, belonging to 5 structural classes.[72] The new TSPO tracers generally have 2- to 4-fold higher BP_{ND} than [^{11}C]PK11195 in healthy brain, with the caveat that their binding affinity is often subject to a common TSPO allelic variant in human populations. Consequently, recent efforts have focused on developing high-binding TSPO radiotracers that are insensitive to genotype, thus obviating the need to stratify patients by allelic status.[73] Furthermore, available TSPO ligands do not distinguish between pro- and anti-inflammatory microglia, which might be differentially expressed at the onset and progression of a neuroinflammatory condition.[74] In any event, TSPO is just one aspect of inflammatory responses, and there is a need to develop PET tracers targeting specific biochemical markers of specific cell types involved in neuroinflammation; this is the focus of the present review.

Emerging Targets and Tracers for PET Imaging of Neuroinflammatory Biomarkers

Molecular targets for neuroinflammation imaging discussed herein can be broadly categorized into (1) enzymes and intracellular signaling molecules including glycogen synthase kinase 3 (GSK-3), monoamine oxidase-B (MAO-B), ROS, imidazoline-2 binding sites (I_2BS), cyclooxygenase (COX), and arachidonic acid; (2) G-protein coupled and ionotropic receptors such as sphingosine-1-phosphate receptor 1 (S1P1), cannabinoid-2 receptor (CB2), the chemokine receptor CX3CR1, and the $P2X_7$ and $P2Y_{12}$ purinergic receptors; and (3) members of the immunoglobulin receptor superfamily, including the receptor for advanced glycation end products (RAGE), Mer tyrosine kinase (MerTK), and triggering receptor expressed on myeloid cells-1 (TREM1). We present an overview of (1) the cellular expression and function of these targets, (2) their selectivity for microglia and/or astrocytes, and (3) PET radiotracers for the respective targets that have been investigated in preclinical models of neuroinflammation. The molecular targets are listed in Table 1, and the structures of corresponding lead PET radiotracers are shown in Figure 1.

Glycogen Synthase Kinase 3

Glycogen synthase kinase 3 is a serine/threonine kinase that exists in 2 closely related isoforms, namely, GSK-3α and GSK-3β.[75,76] While GSK-3α primarily regulates glycogen storage in the liver, the GSK-3β isoform is highly expressed in neural tissue, where it regulates brain development.[77,78] In rat cerebral cortex, GSK-3β is expressed in both astrocytes and microglia and is activated following exposure to LPS.[79] However, both messenger RNA (mRNA) isoforms are abundantly expressed in hippocampal and cortical neurons and in Purkinje cells. The GSK-3 regulates various complex biological processes, including glucose metabolism, cell signaling, cellular transport, apoptosis, proliferation, and intracellular communication.[80-82] Experimental autoimmune encephalomyelitis (EAE) is a T-helper cell-mediated autoimmune disease characterized by T cell and monocyte infiltration in the CNS associated with local inflammation.[83] Given that EAE leads to an approximation of the key clinical, immunological, and neuropathological features of MS, the rodent EAE model has been widely adopted for preclinical investigation of therapeutic targets and evaluation of drug candidates for MS.[83,84] Compared to wild-type mice, constitutively active GSK-3α/β knock-in mice had more rapidly progressing and severe EAE.[84] Furthermore, postmortem studies show increased GSK-3β activity in postmortem brain samples from patients with AD.[85] GSK-3 inhibition may, therefore, present a rational strategy for treating neuroinflammatory diseases, and GSK-3 PET could be used to measure inhibition by GSK-3 therapeutics. Given the role of GSK-3β in phosphorylation of tau and Aβ production,[86] in vivo imaging of GSK-3 might open a path to detecting increased tau hyperphosphorylation, Aβ aggregation, and glia-mediated inflammatory responses, all of which are hallmarks of AD and non-AD tauopathies.

Given the wide range of functions associated with GSK-3, it is unsurprising that GSK-3 has emerged as a target for CNS drug development and medical imaging in various disease settings.[53-55,87] For neurodegenerative diseases, GSK-3 PET imaging could indicate target engagement by GSK-3 therapeutics and offer a path to diagnostic agents that correlate with not only early cognitive impairment of AD but also increased tau hyperphosphorylation,[88,89] increased Aβ production, and local plaque-associated glial-mediated inflammatory responses,[90,91] all of which are hallmarks of AD and non-AD tauopathies. The greatest obstacle for molecular neuroimaging of GSK-3 has been to identify potent and highly selective small molecular weight ligands with sufficient brain penetration. We have developed the PET radiotracer for GSK-3 focusing on the synthesis of ^{11}C-labeled isotopologues of AR-A014418, and other laboratories have subsequently explored different scaffolds.[53,92] Only a handful of radiotracers for GSK-3 have been studied in vivo. Among these, [^{11}C]SB-216763 showed good brain uptake in rodents and nonhuman primates (NHPs) but was not selective for GSK-3 among structurally similar kinases.[93,94] [^{11}C]PyrATP-1- and ^{11}C-oxadiazole-based radiotracers failed to show appreciable uptake in vivo.[95,96] We also

Table 1. PET Imaging of Neuroinflammation: Molecular Targets and Potential Radiotracers.

Molecular Target	Cellular Expression	Expression in Neuroinflammation	Radiotracers Investigated
TSPO	Microglia and astrocytes	Upregulated	[^{11}C]PK11195, [^{18}F]FEPPA, [^{18}F]PBR06, [^{18}F]FEDAA1106, [^{11}C]PBR28, [^{11}C]ER176, [^{18}F]DPA-714
GSK-3	Microglia and astrocytes	Upregulated	[^{11}C]PF-367, [^{11}C]SB-216763,
MAO-B	Astrocytes >> microglia	Upregulated	[^{11}C]SL25.1188, [^{11}C]-*L*-deprenyl, [^{11}C]-*L*-deprenyl-D_2, [^{18}F]fluorodeprenyl-D_2, [^{18}F]fluororasagiline-D_2,
ROS	Microglia	Upregulated	[^{11}C]hydromethidine, [^{11}C]**1**, [^{18}F]ROStrace, [^{11}C]ascorbic acid
I_2BS	Astrocytes	Upregulated	[^{11}C]BU99008
COX-1	Microglia	Upregulated	[^{11}C]Ketoprofen-methyl ester, [^{11}C]PS13, [^{18}F]PS2
COX-2	Microglia	Upregulated	[^{11}C]MC1
Arachidonic acid	Microglia and astrocytes	Upregulated	[^{11}C]Arachidonic acid
S1P1	Microglia >> astrocytes	Upregulated	[^{18}F](R)-1-[[3-(6-fluorohexyl)-phenyl]amino-4-oxobutyl]phosphonic acid, [^{11}C]TZ3321, [^{18}F]TZ35110, [^{18}F]TZ43113, [^{18}F]TZ35104, [^{18}F]TZ4877, [^{18}F]TZ4881
CB2	Microglia and astrocytes	Downregulated	[^{11}C]NE40, [^{11}C]MA2, [^{18}F]MA3
CX3CR1	Microglia Lymphocytes	Upregulated	–
Purinergic receptor: $P2X_7$	Microglia >> astrocytes	Upregulated	[^{11}C]A-740003, [^{11}C]SMW139, [^{11}C]JNJ-54173717, [^{11}C]GSK1482160
Purinergic receptor: $P2Y_{12}$	Microglia (M2)	Downregulated	[^{11}C]**2**
RAGE	Microglia	fIRAGE: upregulated; sRAGE: downregulated	[^{18}F]RAGER, [^{18}F]FPS-ZM1
MerTK	Microglia and astrocytes	Upregulated	[^{18}F]JHU16907
TREM1	Microglia	Upregulated	[^{64}Cu]TREM1-mAb

Abbreviations: CB2: cannabinoid-2 receptor; COX: cyclooxygenase; GSK-3: glycogen synthase kinase 3; I_2BS: imidazoline-2 binding sites; MAO-B: monoamine oxidase-B; MerTK: Mer tyrosine kinase; RAGE: the receptor for advanced glycation end products; ROS: reactive oxygen species; S1P1: sphingosine-1-phosphate receptor 1; TSPO: translocator protein-18 kDa; TREM1: triggering receptor expressed on myeloid cells-1.

explored ^{18}F-labeled maleimide-based tracers for GSK-3.[97] Although preliminary PET imaging with these compounds showed moderate uptake, we saw no displaceable binding in rodent brain.

In collaboration with Pfizer, our team recently discovered PF-367, one of the most potent and selective inhibitors of GSK-3 reported to date, with proven efficacy in vitro and in vivo efficacy in modulating tau phosphorylation.[98] [^{11}C]PF-367 readily crosses the BBB in NHP ($SUV_{peak} = 1$ at 5 minutes postinjection). Pharmacological blocking provoked relatively rapid washout of [^{11}C]PF-367 from the brain, indicating a high-binding signal. Due to its high BBB permeability and admirable selectivity for GSK-3 in a panel of protein kinases, [^{11}C]PF-367 represents a lead neuroimaging agent for GSK-3.

Monoamine Oxidase-B

Monoamine oxidase-B is a flavin–adenosine–dinucleotide enzyme of the outer mitochondrial membrane enzyme that catalyzes the oxidative deamination of dopamine and β-phenylethylamine but with lesser affinity for serotonin, noradrenaline, and tyramine; the suicide inhibitors pargyline and *L*-deprenyl evoke irreversible MAO-B inhibition.[99,100] The highest expression of the *MAO-B* gene in human brain is in the frontal cortex and locus coeruleus, but histological examination reveals focally high activities of MAO-B within serotonin and histamine neurons of murine brain.[101,102] Nonetheless, the great bulk of brain MAO-B apparently resides in astrocytes as revealed by microautoradiography with *L*-[^{3}H]deprenyl.[103] Furthermore, that study showed increased expression of MAO-B in spinal cord from patients diagnosed with motor neuron disease, which colocalized in reactive astrocytes double labeled for GFAP. During its catalytic cycle, MAO-B, like all amine oxidases, produces hydrogen peroxide, which may give rise to other ROS[104] in idiopathic neurodegenerative diseases. Increased astrocytic MAO-B activity due to astrocytosis in brain of patients with AD may impart oxidative stress, further exacerbating the ongoing neurodegeneration.[105,106] Therefore, MAO-B inhibition might be neuroprotective in diseases where ROS generation is implicated in neurodegeneration.[107]

Several radiotracers have been developed for MAO-B PET imaging, yet the majority of clinical research studies have been led using the propargyl compound *L*-[^{11}C]deprenyl to evaluate MAO-B in human neuropsychiatric studies.[54,108,109] In the past

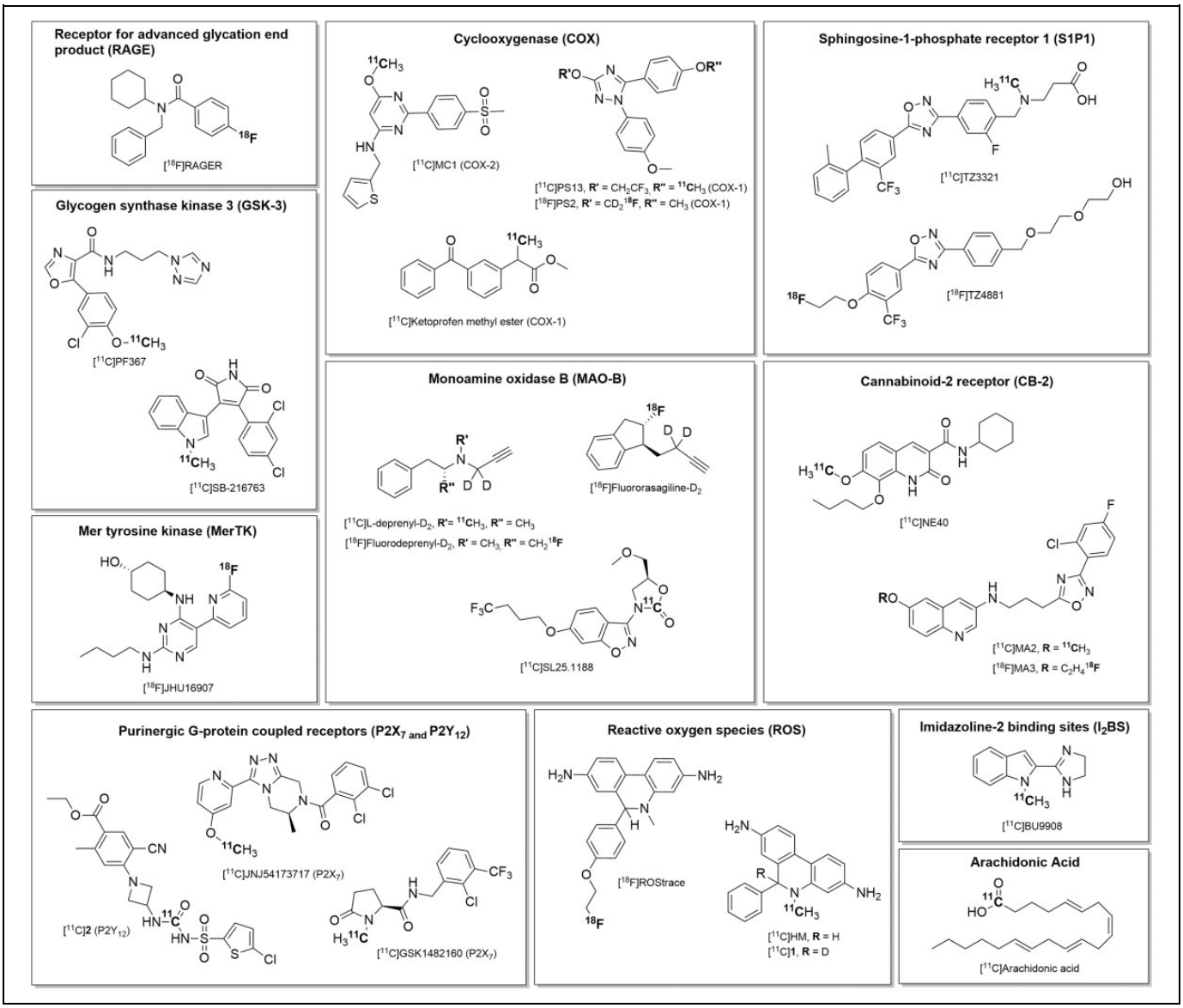

Figure 1. Chemical structures of selected PET radiotracers for PET imaging of neuroinflammation. PET indicates positron emission tomography.

decade, several radiolabeled analogs of *L*-deprenyl (selegiline) have been developed and investigated for detection of reactive astrogliosis in neuroinflammation disorders. In autoradiographic studies with [^{11}C]-*L*-deprenyl, there was significantly higher binding in temporal lobe and the white matter of patients with AD compared to controls.[106] Moreover, increased regional radioligand binding was accompanied by an increased number of activated astrocytes as demonstrated by GFAP immunohistochemistry in adjacent brain slices. While the MAO-B suicide inhibitors *L*-deprenyl and rasagiline effectively blocked [^{11}C]-*L*-deprenyl binding in vitro, the MAO-A inhibitor pirlindole had not such effect.[106] The PET studies with [^{18}F]fluorodeprenyl showed highest specific binding in striatum, intermediate uptake in thalamus and cortex, and lowest binding in cerebellum of NHP.[110] However, the irreversible binding mechanism of the "suicide substrate" propargyl compounds can hinder quantitation in PET studies, since the trapping rate approaches the limit established by perfusion/delivery of the tracer, which led to development of the isotopologs *L*-[^{11}C]deprenyl-D_2 and [^{18}F]fluorodeprenyl-D_2. These compounds gave improved MAO-B quantitation through the deuterium isotope effect, which slowed the rate of irreversible binding to MAO-B.[109,111] Indeed, PET studies with [^{18}F]fluorodeprenyl-D_2 revealed favorable kinetic properties with relatively fast washout from NHP brain and improved sensitivity for MAO-B imaging, which was recapitulated in a comparison of [^{18}F]fluororasagiline-D_2 versus [^{18}F]fluororasagiline.[112] Nonetheless, the potentially brain-penetrating radioactive metabolites of propargyl compounds are problematic for quantitation.

An alternative approach for measuring MAO activity in vivo is the use of metabolic trapping, where the product of the catalytic activity of the enzyme is not covalently bound but is still retained within the target tissue.[113] To that end, [^{11}C]1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine ([^{11}C]PHXY) and [^{11}C]4-methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one ([^{11}C]COU)1-[^{11}C]methyl-4-aryloxy-1,2,3,6-tetrahydropyridines were investigated as metabolic trapping agents for monoamine oxidases using micro-PET imaging in rat and NHP brain.[113] Isozyme selectivity was determined after pretreatments with deprenyl (selective MAO-B inhibitor) or clorgyline (selective MAO-A inhibitor) or both irreversible inhibitors prior to scan. While both radiotracers evaluated failed to exhibit specificity for either isozyme in the rat brain, in vivo trapping of [^{11}C]PHXY was more sensitive to MAO-A inhibition, and [^{11}C]COU was more sensitive to MAO-B inhibition. Given the encouraging isozyme selectivity of [^{11}C]PHXY and [^{11}C]COU in the monkey brain, further evaluation of these radiotracers will be beneficial.

Our laboratory has been working over the past decade to develop several ^{11}C- and ^{18}F-labeled PET radiotracers for imaging MAO-B based on coumarin, pyrrole, and propargylamine scaffolds in preclinical studies.[114] A reversible MAO-B ligand [^{11}C]SL25.1188[115] has been synthesized by us via a novel [^{11}C]CO_2 fixation technology and validated for human use.[116] Preclinical characterization in NHP revealed very slow plasma metabolism, high brain uptake, and reversible binding kinetics in brain.[117] We and our collaborators have recently successfully translated [^{11}C]SL2511.88 for first-in-human clinical research studies, validating it as the first reversibly binding MAO-B radiotracer for human use.[118] It is presently being explored in several patient populations in clinical PET research studies, and we are working toward development of a fluorine-labeled derivative of SL25.1188. Our initial array of ^{18}F-labeled compounds showed fast binding kinetics and specificity to MAO-B ex vivo but imparted excessive cranial uptake due to defluorination in vivo.[119] Further optimization and evaluation of reversible MAO-B tracers in neuroinflammation are underway.

Aggregation and hyperphosphorylation of tau protein leading to the formation of pathological tau deposits are the hallmark of several neurodegenerative diseases including AD. Advances of molecular imaging in the recent years have led to the development of promising tau-PET tracers such as THK5317, THK5351, AV-1451, PBB3, and MK-6240.[120] Interestingly, off-target binding of tau-PET tracers are potentially attributed to MAO-A and/or MAO-B enzymes.[121] For example, binding to MAO-B in vivo in humans was recently demonstrated for another tau-PET tracer, [^{18}F]THK5351, in which selegiline (MAO-B inhibitor) reduced the cortical PET signal by 40% in patients with AD.[122] Taken together, tau-PET tracers devoid of affinity for MAO-A and MAO-B will need to be developed for diagnosis and monitoring of disease progression or treatment efficacy.

Reactive Oxygen Species

Oxidative stress is produced by various enzymatic reactions and chemical processes when ROS generation exceeds the capacity of antioxidant defense mechanisms, which include a network of compartmentalized antioxidant enzymic and nonenzymic molecules usually distributed within the cytoplasm and various cell organelles.[123] The ROS particularly implicated in oxidative stress include superoxide anion (O_2 $\cdot-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot$OH).[124,125] Under normal physiological conditions, microglial cells (like all living cells) express low ROS levels due to tight regulation by antioxidant pathways.[126] Some basal expression of oxidative stress molecules is essential for many physiological functions such as cell survival, proliferation, differentiation, and apoptosis.[123] After an inflammatory challenge, microglial cells differentiate into the activated M1 phenotype that evoke a rapid increase in ROS levels, with positive feedback further upregulating the proinflammatory M1 gene profile. Although this may be an advantageous acute cellular response, excessive and sustained ROS production may be responsible for tissue injury in neurodegenerative diseases with an inflammatory component. Uncontrolled ROS production can damage DNA and react with lipids, proteins, and carbohydrates, thus impairing cellular functions while further exacerbating inflammatory reactions.[123] Several reports have shown that cerebral ROS levels tend to increase with age, perhaps in conjunction with increased MAO-B activity. Moreover, elevated ROS levels are detected in the brain of patients with AD as well as cellular and animal models of AD.[127] Overproduction of ROS is considered a contributor to the degeneration of nigrostriatal dopamine neurons in Parkinson disease,[128] as noted in "Monoamine Oxidase-B" section.

A new PET radiotracer, [^{11}C]ascorbic acid, exhibits potential to measure ROS-dependent cellular accumulation.[129] [^{11}C]ascorbic acid was applied to a model of endogenous ROS production, namely, stimulated neutrophil-lineage cells undergoing oxidative burst. For this study, HL60 human promeylocyctic leukemia cells and freshly isolated human neutrophils were employed. During phagocytosis, neutrophils undergo Nox-mediated generation of ROS to destroy bacteria and simultaneously oxidize extracellular ascorbic acid. To investigate tracer uptake via this mechanism, cells were incubated with 10 μCi [^{11}C]ascorbic acid in the presence or absence of phorbol 12-myristate 13-acetate (2 μmol/L) and blocking agent, cytochalasin B (20 μg/mL).[129] For both HL60 cells and neutrophils, a significant increase in cell-associated activity and approximately 2-fold increase in tracer uptake was noted with activation. These results suggest the potential role of [^{11}C]ascorbic acid, more specifically the ascorbate recycling mechanism, to image ROS-driven neuroinflammatory disease states.

Recent studies report on 2 potential radiotracers for PET imaging of ROS. Hydromethidine (HM) is the closely related analogue of hydroethidine that has been studied extensively as a fluorescent probe for the detection of ROS.[130] The underlying

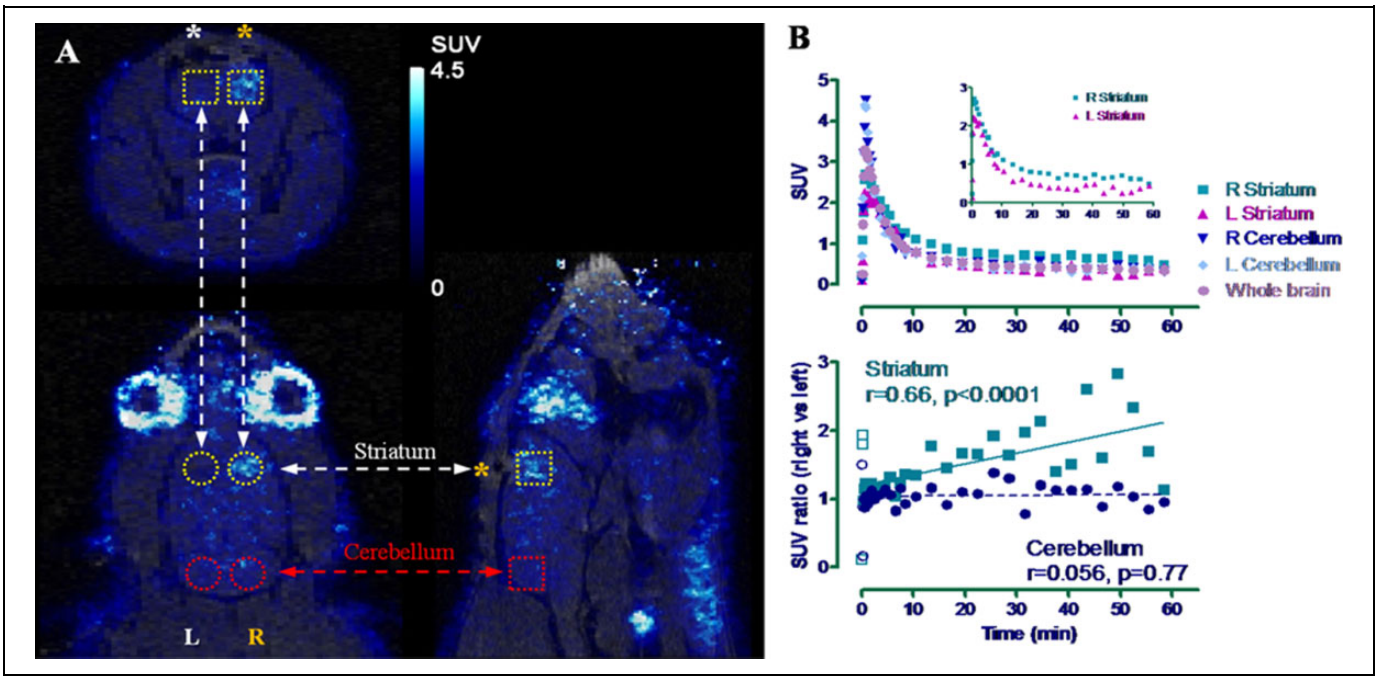

Figure 2. PET image of rat brain with [^{11}C]HM following unilateral intrastriatal (right) injection of sodium nitroprusside (saline in left striatum).[131] (A) Average PET images (0-60 minutes, transverse, coronal, and sagittal views) are aligned with MRI (the surgery sites; * are indicated in the transverse and sagittal MRI), with cylindrical ROS placed in right and left striatum and right and left cerebellum; (B, top panel) time–activity curves (TACs) between right and left side striatum or cerebellum and whole brain; (B, bottom panel) SUV ratio of right to left striatum increased linearly with time, whereas SUV ratio of right to left cerebellum displayed no significant change with time. PET indicates positron emission tomography; MRI, magnetic resonance imaging; ROS, reactive oxygen species; SUV, standard uptake value.

hypothesis is that HM, being neutral, readily crosses cell membranes including those of the BBB, while oxidized species are trapped due to their charge and/or by interchelation with cellular DNA.[130] In one report, [^{11}C]HM and its deuterated isotopolog, [^{11}C]**1**, were evaluated by autoradiography ex vivo and in preclinical PET.[131] Both tracers demonstrated good brain uptake in rats, but [^{11}C]**1** displayed relatively faster washout compared to [^{11}C]HM. Pretreatment of rats with LPS significantly increased the brain retention of both radiotracers. Furthermore, ex vivo autoradiography as well as PET imaging in rats unilaterally treated with microinjections of sodium nitroprusside (which increases ROS levels due to inhibition of oxidative phosphorylation) demonstrated increased retention of both radiotracers in the treated cerebral hemisphere (Figure 2). Overall, [^{11}C]HM possesses favorable characteristics as a radiotracer for imaging ROS using PET, exhibits fast washout from the brain of untreated rats, and increased brain retention in the LPS animal model of oxidative stress. The faster washout of [^{11}C]**1** from rat brain is attributable to reduced basal oxidation (and hence reduced retention of radiotracer) due to the stronger carbon–deuterium bond.[131] However, further studies including quantitation of the amounts of oxidized and reduced forms of these radiotracers in brain are needed. A recent study has reported in vivo evaluation of a fluorine-labeled ROS radiotracer, [^{18}F]ROStrace, for PET imaging of superoxide in an rodent LPS model of neuroinflammation.[132] [^{18}F]ROStrace rapidly crossed the BBB and was extensively trapped in brain of LPS-treated animals but had rapid washout from brain of healthy rats. The basis of quantitation of ROS by PET remains to be established, but we see great promise in emerging methods for measuring superoxide levels in neuroinflammation and neurodegenerative disease.

Imidazoline-2 Binding Sites

The imidazoline derivative idazoxan was originally developed as a selective antagonist for α_2-adrenoceptor.[133] However, binding studies showed that [^{3}H]idazoxan had affinity for non-adrenoceptor sites that were termed imidazoline-binding sites or imidazoline-preferring receptors.[134,135] Although the endogenous ligand is unknown, the idazoxan-preferring subtype, I_2BS, is expressed in several tissues, including the brain, where it is located on astroglial cells and seem to be involved in the regulation of GFAP expression.[135] At a subcellular level, I_2BS is also expressed in mitochondrial membranes of astrocytes. The density of I_2BS-binding sites is elevated in AD brain postmortem. Alterations in I_2BS density have been seen in neurodegenerative diseases as evidenced by immunohistochemical studies conducted on human postmortem brains from patients with AD and Huntington disease.[135,136] In cortical membranes of patients with AD, I_2BS density ([^{3}H]idazoxan binding) was markedly increased (63%) in comparison to control patients. By contrast, I_2BS density was markedly decreased (56%) in the

putamen of patients with Huntington disease. In addition, I_2BS is a potential imaging marker for human glioblastomas.[137] Increased I_2BS density was found in glial astrocytic tumors than in normal healthy brain specimens as evidenced by [^{3}H]idazoxan-binding studies conducted in surgical specimens of human glial tumors and normal healthy tissues.[137] Moreover, enhanced I_2BS density was more specific for glial than nonglial tumors, suggesting the potential role of PET tracers for I_2BS as a potential imaging marker for human glioblastomas.

Recent efforts directed toward elucidation of the role of I_2BS in disease states inspired the development of [^{11}C]BU99008, a PET radiotracer for I_2BS imaging.[138] In vitro characterization of BU99008 demonstrated its selectivity and nanomolar affinity for I_2BS in rodent and cynomolgus brain. Further, in vitro competition studies with the selective I_2BS blocker BU224 against [^{3}H]BU99008 binding revealed a 2-site fit in membranes from rat brain, whereas BU224 displacement indicated a single-site fit in cynomolgus brain. BU99008 apparently has affinity for a second binding site in rodent brain, unrelated to I_2BS. On structural grounds, BU99008 might be expected to have off-target binding to MAO, and indeed the I_2BS is recognized to be a modulatory site present in a subpopulation of MAO-B enzymes.[139] While MAO-A and MAO-B inhibitors evoked little displacement of I_2BS-binding sites in cynomolgus brain membrane preparations, coincubation with an MAO-B inhibitor displaced the lower affinity binding component of [^{3}H]BU99008 in rodent brain.[138] Despite the higher MAO-B activity in NHP compared to rodents, [^{3}H]BU99008 lacked significant off-target binding to MAO-B in cynomolgus brain membranes, suggesting a species difference in the off-target binding.

In vivo evaluation of [^{11}C]BU99008 showed rapid uptake in the NHP brain, with highest uptake in the globus pallidus, caudate, and thalamus; moderate uptake in the putamen and parts of cerebral cortex (cingulate, frontal, and insula); and lowest uptake in the cerebellum and occipital cortex.[138] In vivo competition studies revealed that [^{11}C]BU99008 displayed reversible binding kinetics and specificity for the I_2BS site. Importantly, in vivo blocking studies using the MAO-A and MAO-B inhibitors, moclobemide and lazabemide, respectively, did not cause any significant change in binding signal of [^{11}C]BU99008.[138] A recent comprehensive PET imaging study of [^{11}C]BU99008 in rats showed great sensitivity to minor and focal changes in I_2BS expression.[140] The specificity of this tracer for the I_2BS site in rat brain remains to be investigated by MAO displacement studies. While relative expression levels of I_2BS and MAO differ from species to species, human brain uptake and in vivo binding characteristics of [^{11}C]BU99008 seem likely to match that in NHP brain. [^{11}C]BU99008 may thus prove useful for elucidating new functions of I_2BS in human neuroinflammation and neurodegenerative disorders,[140] although further preclinical evaluation seems warranted to address questions with respect to radioligand specificity for this target. In addition, selective radioligands could aid in the neurochemical search for the endogenous I_2BS ligand.

Cyclooxygenase

Cyclooxygenase is the key rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins, which are lipid mediators involved in several physiological and pathological processes including inflammation.[141] The 2 distinct COX isoforms, namely, COX-1 and COX-2, have differing tissue distribution and regulatory mechanisms, although both are blocked by aspirin and ibuprofen. The COX-1 is a constitutively expressed "housekeeping" enzyme present in most tissues, which is primarily responsible for homeostatic prostaglandin synthesis.[142] The COX-2 is mainly induced in response to inflammatory stimuli, although COX-2 is also constitutively expressed in hippocampal and cortical glutamatergic neurons, where it regulates synaptic activity and long-term synaptic plasticity.[143,144]

In the CNS, COX-1 immunoreactivity was shown to be enriched in midbrain, pons, and medulla.[145] The COX-1 has recently emerged as a prominent player in neuroinflammation, and COX-1–expressing activated microglia are found around Aβ plaques in human AD brain.[146] Moreover, a selective increase in COX-1 mRNA expression was reported in the hippocampus of aged rats, highlighting the broad relationship between neuroinflammation and aging.[147] The COX-1–deficient mice exhibit decreased inflammatory response, leukocyte infiltration, oxidative stress, and neuronal damage after central injection of the proinflammatory compounds LPS or Aβ1–42.[148,149] Consistent with these findings, treatment of 20-month-old triple transgenic AD mice with the COX-1 selective inhibitor SC-560, improved spatial learning and memory, reduced Aβ deposits and tau hyperphosphorylation, attenuated glial activation and brain expression of inflammatory markers, and switched the activated microglia to an M2 phenotype with enhanced phagocytic ability.[150] In a mouse model of chronic neuroinflammation due to IL-1β overexpression, COX-1 and prostaglandin E2 were upregulated, but this effect was completely abrogated upon genetic deletion or pharmacological inhibition of COX-1.[151] Thus, COX-1 activation is a mediator in acute and chronic neuroinflammatory conditions.

In contrast to COX-1, there are conflicting views of the role of COX-2 in neuroinflammation. The COX-2 inhibition or genetic deletion has been linked to detrimental effects, suggesting a neuroprotective role of COX-2. Thus, COX-2-null mice show increased glial activation and inflammatory markers after LPS injection compared to wild type.[152] The expression of COX-2 changes across disease stages in AD, with early upregulation and late downregulation, in a manner tracking the prostaglandin E2 levels in cerebrospinal fluid.[153,154]

The clear role of COX-1 in neuroinflammation has motivated the search for a selective COX-1 imaging probe. ^{11}C-labeled arylpropionic acids and their methyl esters, including [^{11}C]ibuprofen and [^{11}C]ketoprofen-methyl ester ([^{11}C]KTP-Me), have been prepared as potential PET tracers for COX-1.[155,156] Increased plasma protein binding associated with ibuprofen and relatively lower potency for COX-1 compared to KTP-Me (IC_{50}: 7.6 vs 0.047 μmol/L, respectively)

likely discouraged further pursuit of [^{11}C]ibuprofen for PET imaging studies.[157,158] [^{11}C]KTP-Me was the first successful PET radioligand for COX-1 in activated microglia.[156] Ex vivo autoradiographic analysis revealed a significant reduction in [^{11}C]KTP-Me accumulation only in the hippocampus, cerebral cortex, and cerebellum of COX-1–deficient mice, but not COX-2–deficient mice, when compared to respective wild-type controls,[156] providing evidence that [^{11}C]KTP-Me selectively binds to COX-1 in the brain. PET imaging with [^{11}C]KTP-Me in rats demonstrated good brain uptake and retention in microglia of inflammatory lesions formed after intrastriatal LPS injection. The first-in-human exploratory study with this tracer revealed favorable biodistribution.[159] [^{11}C]KTP-Me readily entered the human brain, attaining an SUV_{peak} of 1.5 in cortex at 2 minutes postinjection, followed by a slow washout to 40% of peak activity at 60 minutes.[159] Analysis of plasma extracts showed rapid metabolism of circulating [^{11}C]KTP-Me to [^{11}C]KTP, which may confound analysis of the brain PET signal.

Recent studies report the in vivo evaluation of novel and selective PET radioligands for COX-1 in rhesus monkeys.[160,161] A ^{11}C-labeled radiotracer was developed from PS13 (1,5-bis(4-methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H -1,2,4 trazole) that exhibited high affinity and 1000-fold selectivity for NHP and human COX-1 (IC_{50} ~1 nM) versus COX-2 (IC_{50} >1 μmol/L).[160] In vivo PET studies conducted in monkeys demonstrated high brain uptake after [^{11}C]PS13 injection (SUV_{peak} = 4.4), which was substantially displaced by pretreatment with COX-1 inhibitors (PS13 or KTP-Me). Most importantly, higher radioactivity uptake was found ipsilateral to intracerebral injections of an inflammogen (LPS) or excitotoxin (ibotenic acid) compared to the untreated contralateral side. A [^{18}F]fluoro-D_2-methoxy analog ([^{18}F]PS2), with a longer plasma half-life due to the deuterium isotope effect, showing high affinity for human COX-1 (IC_{50} = 4 nM) and high selectivity (>200-fold) over COX-2, was evaluated in vivo in monkeys.[161] The PET studies in rhesus monkey revealed rapid brain uptake following injection of [^{18}F]PS2 (SUV ~4.5 at 2 minutes). Moreover, the specific binding of [^{18}F]PS2 to COX-1 was reduced in a self-blocking experiment, supporting its use in further investigations.

A novel and selective ligand MC1 (6-methoxy-2-(4-(methylsulfonyl)phenyl)-N-(thiophen-2-ylmethyl)pyrimidin-4-amine) showed high affinity (IC_{50} = 3 nmol/L) and selectivity (>3000-fold over COX-1) for COX-2, and its PET analog [^{11}C]MC1 was evaluated in rhesus monkeys.[162,163] Dynamic PET imaging showed an SUV_{peak} (2.9) at 2 minutes postinjection of the radiotracer, which was blocked by pretreatment with nonradioactive MC1.[162] As a follow-up to this initial study, the same group evaluated brain uptake of [^{11}C]MC1 in monkeys with neuroinflammation due to intracerebral LPS injection[163]; this treatment increased the displaceable [^{11}C]MC1 uptake at the injection suite. Overall, [^{11}C]PS13, [^{18}F]PS2, and [^{11}C]MC1 are promising tracers for molecular imaging of COX-1 and COX-2 in neuroinflammation.

Arachidonic Acid

Arachidonic acid is a polyunsaturated omega-6 fatty acid derived from phospholipids by neuroreceptor-mediated activation of phospholipase A2 (PLA2) enzyme.[164] Upon mobilization into the intracellular space, arachidonic acid acts as a lipid second messenger for the regulation of signaling enzymes, such as other phospholipases and protein kinase C, while also serving as a precursor for the biosynthesis of prostaglandins and leukotrienes. Its liberation from phospholipids creates a sink in vivo for incorporation of [^{11}C]arachidonic acid into cell membranes such that the PET signal with [^{11}C]arachidonic acid indicates the activity of PLA2 in living brain.[165] The tracer uptake may occur in postsynaptic neuronal elements, and in astroglia and microglia, which likewise express PLA2. However, inflammatory signals arising from activated microglia can stimulate PLA2 via receptor-mediated effects of glutamate or cytokines such that [^{11}C]arachidonic acid can be an indirect index of microglial activation. Thus, increased cerebral [^{11}C]arachidonic acid uptake is taken to indicate a chronic inflammatory condition in patients with AD.[166]

Sphingosine-1-Phosphate Receptor 1

The S1P is a bioactive sphingolipid metabolite that regulates critical cellular processes including proliferation, survival, and migration.[167] It is derived from sphingosine upon adenosine triphosphate (ATP)-dependent phosphorylation by sphingosine kinase (predominantly sphingosine kinase 1). S1P is interconvertible with ceramide, a critical mediator of apoptosis, and the prevailing ratio between S1P and ceramide intracellular concentrations is postulated to determine cell fate, as the sphingolipid metabolites have opposing effects on cell survival.[167] Although there are proposed intracellular signaling pathways for S1P, it is extensively extruded via specific transporters from the intracellular to the extracellular environment, where it acts in an autocrine or paracrine manner on S1P receptors.[168,169] Data from in vitro studies and animal models suggest that S1P is important for several physiological CNS functions, including (1) migration of neuronal progenitor cells toward areas of damage; (2) migration of astrocytes and communication of astrocytes with other CNS cells; (3) the regulation of oligodendrocyte survival, function, and modulation of myelination following injury; (4) regulation of microglial number and activation; and (5) maintenance of BBB integrity.[170-175] Signals initiated by S1P are transduced by 5 G-protein–coupled receptors known as S1P1-5.[176] The understanding of S1P receptor function and biology has advanced rapidly, and these receptors have emerged as attractive therapeutic targets in chronic inflammatory pathologies, autoimmunity, and cancer. Herein, we particularly focus on the role of S1P1 receptors in the context of neuroinflammation and their potential as targets for PET radiotracer development.

The S1P1 receptors are widely distributed within the immune system and CNS. In the CNS, S1P1 is predominantly expressed in neurons and microglia and, to a relatively lesser

extent, in astrocytes and oligodendrocytes.[177] The SIP analog fingolimod (FTY720) is an immune-modifying drug derived from the natural product, myriocin.[178] Specifically, FTY720 acts as a functional S1P1 receptor antagonist with anti-inflammatory effects mediated through receptors expressed on lymphocytes, thereby preventing their transit from peripheral lymphoid organs into the CNS. The S1P1 receptor is highly expressed under neuroinflammatory conditions and particularly in MS lesions.[177] In 2010, the US Food and Drug Administration approved FTY720 as the first oral disease-modifying drug to treat relapses of MS.[179] In addition to blocking lymphocyte migration, FTY720 readily crosses the BBB and may attenuate neuroinflammation by regulating the activation and neuroprotective effects of microglia mainly via S1P1 blockade.[180-182] In vitro studies indicated that FTY720 downregulates production of proinflammatory molecules by microglia while increasing neurotrophic factor production, resulting in an overall neuroprotective phenotype.[182] FTY720 also inhibited secretory vesicle mobility and exocytic release by astroglia, thus attenuating the release of proinflammatory mediators.[183] In support of this finding, in vitro treatment of a human astrocyte cell line with FTY720 suppressed S1P-induced production of proinflammatory cytokines.[184]

The EAE is a well-established preclinical model of MS pathology.[185] Specific deletion of the S1P1 receptor in astrocytes resulted in decreased EAE pathology and a loss of FTY720 efficacy, indicating that the primary target of FTY720 during EAE was S1P1 receptors expressed specifically on astrocytes.[186] A potential therapeutic role of FTY720 in AD is also highlighted in a study revealing FTY720 treatment to decrease Aβ plaque density, activated microglia expression, and immunoreactivity of the astrocyte marker GFAP in an AD mouse model.[187] Overall, the discovery of FTY720 has advanced the understanding of physiological actions of S1P receptors and served as a pharmacological tool to identify their role in neuroinflammatory processes and diseases. Moreover, approval of FTY720 for MS treatment has accelerated drug discovery in this field and promoted the development of selective S1P1 agonists (ie, AUY954, CS-0777, KRP-203, SEW2871, ponesimod, and MT-1303) that present leads for developing radiotracers for PET imaging of the S1P1 receptor.[188]

To date, no compound has been translated for in vivo imaging of S1P1 receptors in humans, although several radiotracers have been developed and evaluated both in vitro and in vivo using murine models of MS. The S1P1 receptor antagonist [^{18}F](*R*)-1-[[3-(6-fluorohexyl)-phenyl]amino-4-oxobutyl]phosphonic acid showed good in vitro potency and serum stability, but PET imaging in wild-type mice revealed increased radioactivity uptake in bones due to rapid in vivo defluorination.[189] A subsequent in vivo biodistribution study was conducted to evaluate 2 fluorinated structural analogs of FTY720 as candidate PET radiotracers.[190] Quantitative PET time–activity curves indicated a rapid clearance from blood within 3 minutes postinjection, with the declining blood concentration coincident with an intense uptake of both tracers into the liver, reflecting predominant hepatic elimination. The PET imaging results also showed positive uptake in S1P1 receptor-rich tissues, without much evidence of defluorination in vivo. However, pharmacological specificity was not formally established for these tracers.

TZ3321, a novel S1P1 receptor antagonist with high affinity in vitro (K_i = 0.2 nM), was first introduced by Merck Serono.[191] The potential utility of this ligand for PET imaging was explored in a biodistribution study conducted in C57BL/6 mice, which showed excellent brain uptake of (SUV = 7.1 at 60 minutes postinjection).[192] The biodistribution results were encouraging for the use of [^{11}C]TZ3321 as a PET tracer for S1P1 receptors. Recently, [^{11}C]TZ3321 PET studies in the EAE rat model revealed 20% to 30% higher uptake in the lumbar spinal cord of symptomatic EAE rats versus sham controls.[193] Increased tracer uptake was correlated with increased S1P1 receptor expression in the EAE spinal cord, glial cell activation, and infiltrating IL-17–producing T cells as confirmed by immunohistochemical localization of GFAP, IBA1, and IL-17 in EAE rat spinal cord sections. Second-generation S1P1-specific ^{18}F-labeled radiotracers were developed that demonstrated specificity for S1P1 receptors, crossed the BBB, and showed elevated binding in the murine EAE model of MS by PET.[194] In rodent biodistribution studies, the S1P1 receptor ligands [^{18}F]TZ35110 (IC_{50} = 2.6 nmol/L), [^{18}F]TZ43113 (IC_{50} = 9.8 nM) and [^{18}F]TZ35104 (IC_{50} = 6.7 nmol/L) exhibited acceptable brain uptake with peak uptake occurring 1 hour postinjection.[194] The PET studies of [^{18}F]TZ43113 in the female Lewis rat EAE model showed 31% higher uptake in the lumbar spinal cord. Furthermore, PET imaging of [^{18}F]TZ35104 showed excellent uptake and washout in NHP brain.[194] The same research group then proceeded to optimize the kinetics of TZ35104, the lead structure identified from their previous study, by incorporating polyethylene glycol chains into the structures.[195] Small animal PET imaging studies with polyethylene glycol derivatives of TZ35104,such as [^{18}F]TZ4877 and [^{18}F]TZ4881, revealed 10% higher spinal cord uptake in the rat EAE model.[195] Further in vivo validation in different animal models of neuroinflammation as well as tracer metabolism studies are warranted for translating a suitable S1P1 receptor ligand as a PET radiotracer for clinical use in MS and other neuroinflammation conditions.

Cannabinoid-2 Receptor

The type 2 cannabinoid receptors (CB2) are G-protein (Gi/o) coupled and implicated in immunomodulation and endogenous response to injury.[196] Rat microglial cells normally express CB2 receptors, and neuroinflammatory treatments provoke upregulation of CB2 mRNA and protein, which are mainly colocalized with activated microglial cells in cerebellum, cortex, and brain stem,[197] although low levels of CB2 mRNA and protein have been detected in Purkinje cells and other neuron types of healthy brain.[198] Although the neuronal function is unclear, the CB2 receptors play an important role in microglial migration in response to inflammatory stimuli.[199]

Immunohistochemical studies revealed increased CB2 receptor protein expression in microglia clustering at Aβ plaques in AD brain tissues, compared to those of healthy individuals.[200] Thus, CB2 presents an important target for PET imaging of the microglial dynamics of neuroinflammation.

Recent studies report the in vivo evaluation of potent ^{11}C- and ^{18}F-labeled CB2 receptor agonists. Among the candidates, [^{11}C]NE40 demonstrated specific CB2 receptor binding in the spleen and blood of normal rats and high brain uptake in rhesus monkey.[201] Moreover, [^{11}C]NE40 showed specific and reversible binding to CB2 receptors in a rat model with local overexpression of human CB2 receptors. [^{11}C]NE40 uptake in the right striatum (hCB2R vector) was 2.5 times higher compared to that in the left striatum (control vector). However, results from the first-in-human PET study using [^{11}C]NE40 failed to reveal an upregulation in patients with AD.[202] In a dual tracer study to image Aβ plaques and CB2 receptors using [^{11}C]PIB and [^{11}C]NE40, respectively, in patients with AD, there was no correlation between regional Aβ binding and homologous CB2 receptor availability, suggesting insufficient affinity and/or selectivity of the PET radiotracer for CB2 receptors in human brain. The same research group then developed [^{11}C]MA2 and [^{18}F]MA3, which are radiolabeled analogs of a highly potent N-arylamide oxadiazole CB2 agonist ($EC_{50} = 0.02$ nmol/L).[203] MA2 and MA3 display high potency for hCB2 receptors (EC_{50}: 3 and 0.1 nmol/L, respectively), and high binding affinity in vitro for hCB2 (87 and 0.8 nmol/L, respectively). Biodistribution studies employing these radiotracers in mice demonstrated high brain uptake and efficient clearance from blood and all major organs by the hepatobiliary pathway. Taken together, [^{11}C]MA2 and [^{18}F]MA3 represent potential probes for PET in vivo imaging of brain CB2 receptors in neuroinflammation.

CX3CR1

CX3CR1, a Gαi-coupled 7-transmembrane chemokine receptor, is expressed in lymphocytes and microglia.[204] It has the distinction that its only known natural ligand arises from proteolysis of a transmembrane protein; the active CX3CR1 ligand, which is known as fractalkine, is shed through activity of a disintegrin/metaproteases such as ADAM17/TACE.[204] The chemokine fractalkine has the properties of a chemoattractant and adhesion molecule, being chemotactic for T cells and monocytes in inflammatory conditions such as osteoarthritis, and likewise involved in synaptic pruning and migration of microglia.[205,206] The broad-spectrum viral macrophage inflammatory protein-II linked to nanoparticles in conjunction with ^{64}Cu has served for PET imaging of macrophages in an inflammatory atherosclerosis model.[207] Although no small molecular weight chemokine ligand has been identified for brain imaging studies, the CX3R1 antagonist AZD8797 has been characterized as a selective and orally active agent, with disease-modifying effects in a chronic-relapsing rat model for MS.[208,209] With adequate derivatization and optimization, this work might serve to identify lead compounds for development of a brain-penetrating PET tracer for the CX3R1.

Purinergic Receptors—P2X$_7$ and P2Y$_{12}$

Purinergic signaling is mediated by the P2 family of receptors that plays a crucial role in physiology of the nervous system.[210] P2 receptors are broadly categorized into 2 types: (1) P2X ionotropic receptors, which are ATP-gated ion channels and (2) P2Y metabotropic receptors, which are G-protein–coupled receptors such as the inhibitory adenosine receptors blocked by caffeine.

The $P2X_7$ receptor, a subtype of P2X ionotropic receptors, comprises 2 transmembrane domains, an extracellular loop (with the ATP-binding site), and intracellular N- and C-terminal domains.[211] In brain, the $P2X_7$ is predominantly expressed in activated microglia and to a lesser extent in neurons and astrocytes, being most abundant in spinal cord, cerebellum, hypothalamus, and substantia nigra.[212-214] Under normal physiological conditions, $P2X_7$ is considered a "silent receptor," but it is functionally upregulated during pathological states. Following an insult, ATP is released into the extracellular space at higher concentrations, where it initiates inflammatory signaling cascades, principally via $P2X_7$.[215] Overexpression of $P2X_7$ receptors in rat primary hippocampal cultures was shown to drive the activation and proliferation of microglia in the absence of pathological insults.[216] Persistent elevations in extracellular ATP and proinflammatory cytokines will promote neuroinflammation and neurodegeneration. The expression and functioning of $P2X_7$ receptors is significantly upregulated in postmortem brain of patients with AD as well as various neurodegenerative disease animal models.[217] Given this background, pharmacological suppression of the $P2X_7$ pathway may offer a novel therapeutic approach for ameliorating neuroinflammatory processes.

A-740003, a selective $P2X_7$ receptor antagonist (IC_{50}: $rP2X_7R = 18$ nmol/L; $hP2X_7R = 40$ nmol/L) was pursued in the first efforts to develop $P2X_7$ receptor imaging by PET.[211] Biodistribution studies in healthy rats revealed only traces of [^{11}C]A-740003 uptake, either due to poor BBB permeability or due to low receptor expression levels in healthy brain. The same research group then developed an $P2X_7$ receptor allosteric antagonist, [^{11}C]SMW139.[218] The PET imaging studies conducted with [^{11}C]SMW139 in rats overexpressing the $hP2X_7$ receptor in the right striatum demonstrated 1.5-fold higher radioactivity uptake in the right striatum compared to the contralateral striatum, and this effect was blocked by pretreatment with JNJ-4796556, a potent P2X7 receptor antagonist.

JNJ-54173717, which has nanomolar affinity for rat $P2X_7$ R and $hP2X_7$ R,[219,220] was radiolabeled with ^{11}C and evaluated in vivo in rats and NHPs.[221] Biodistribution studies conducted in normal rats demonstrated that [^{11}C]JNJ-54173717 readily crossed the BBB and was cleared from plasma mainly via the hepatobiliary pathway.[221] To conduct further in vivo evaluation and validation of this PET tracer, a humanized animal model was developed in which $hP2X_7$ R was overexpressed in the rat striatum after stereotaxic injection of viral vectors.[221] The PET studies in these rats showed binding of [^{11}C]JNJ-

54173717 in the striatum expressing hP2X$_7$ R, with rapid washout from the noninjected control striatum and other brain regions. Furthermore, in the NHP brain, pretreatment with cold JNJ-54173717 and with the P2X$_7$ R antagonist JNJ-42253432 reduced uptake of [^{11}C]JNJ-54173717, suggesting P2X$_7$R-selective binding of this radiotracer.[221] This radioligand appears to be a promising candidate for further evaluation of P2X$_7$ R expression levels in neurodegenerative disorders.

The potent P2X$_7$ antagonist GSK1482160 (IC_{50}hP2X$_7$R = 3 nM) was labeled with carbon-11 to investigate its fitness as a radiotracer for PET imaging.[222] Biodistribution studies with [^{11}C]GSK1482160 revealed increased radiotracer accumulation in brain of LPS-treated mice compared to control animals[223]; this effect was blocked by pretreatment with an excess of authentic standard GSK1482160. The PET studies demonstrated slow in vivo kinetics and homogeneous distribution in the brain of healthy NHP, but responses to a neuroinflammatory challenge were not investigated.[224] In the EAE rat model, uptake of [^{11}C]GSK1482160 in lumbar spinal cord was the highest at the EAE-peak symptomatic stage, when radiotracer uptake correlated strongly with P2X$_7$-positive cell counts, activated microglia numbers, and disease severity.

The P2Y$_{12}$ receptor is an adenosine diphosphate-responsive G-protein–coupled receptor that is selectively overexpressed on activated microglia of the anti-inflammatory M2 phenotype.[225,226] There is sparse P2Y$_{12}$ mRNA expression throughout the normal adult rat brain, including the neocortex, hippocampus, cerebellum, and brain stem.[227] Immunohistochemical studies showed decreased P2Y$_{12}$ receptor expression in microglia near the Aβ plaques in postmortem brains from patients with AD and near the demyelinated lesions in patients with MS.[225] This decrease in P2Y$_{12}$ receptor expression in such neuroinflammatory diseases implies a shift in the balance away from anti-inflammatory to proinflammatory M1 microglial cells near the lesions.

[^{11}C]**2** was developed and evaluated as a first potential PET tracer for the P2Y$_{12}$ receptor.[228] Specific binding of [^{11}C]**2** was evident to autoradiography in vitro of mouse brain in which neuroinflammation had been induced via intracerebroventricular injection of IL4, the increased [^{11}C]**2** binding was displaced by addition of ticagrelor, a P2Y$_{12}$ receptor antagonist, to the incubation medium. Ex vivo biodistribution study in rats revealed rapid plasma metabolism and extremely low brain uptake, thus disfavoring its use for PET imaging. Improving BBB permeability may be an important consideration in the development of improved tracers targeting P2Y$_{12}$. This effort seems justified, as promising initial results with [^{11}C]GSK1482160 open a path for developing tracers specific for the anti-inflammatory phenotype. Here it is important to recall that the existing TSPO PET literature is obscured by the lack of discrimination of M1 and M2 microglial subtypes. Overall, P2Y$_{12}$ is the only target yet identified that is exclusively expressed on M2-type microglia. Therefore, we can anticipate that clinical PET imaging of P2Y$_{12}$ receptors will facilitate identifying the specific microglial phenotype in different stages of neuroinflammation.

Receptor for Advanced Glycation End Products

The RAGE is a 45- to 55-kDa member of the immunoglobulin receptor superfamily with 3 extracellular domains, a transmembrane domain, and a highly charged intracellular domain that mediates interaction with oxidative stress-related signal transduction molecules (eg, mitogen-activated protein kinase and nuclear factor-κB).[229,230] In addition to advanced glycation end products, RAGE binds several other ligands, including Aβ, a central player in AD pathogenic mechanisms.[231-234] Two functional types of RAGE have been associated with neurological diseases: cell membrane-bound full length (flRAGE) and soluble (sRAGE).[235] In general, endogenous ligand binding to flRAGE initiates receptor-dependent signaling, resulting in inflammation and cellular stress. This process engages a positive feedback mechanism that can accelerate disease progression. In contrast, sRAGE lacks the cellular signaling domain but usually retains the ligand-binding domain whereby they can sequester endogenous ligand, thus preventing it from binding to flRAGE and activating damaging proinflammatory pathways.[235] Clearly, sRAGE constitutes a naturally occurring "governer" that prevents uncontrolled RAGE-mediated inflammation and cellular stress. The RAGE is constitutively expressed on microglia and neurons of the hippocampus, entorhinal cortex, and superior frontal gyrus.[236] Under normal physiological conditions, flRAGE is expressed at low levels, but its expression appears to increase with aging and in AD.[235-237] On the other hand, levels of sRAGE serve as a predictive marker for disease protection; compared to cognitively normal controls, sRAGE levels were lower in circulation of patients with AD and in individuals with early cognitive decline.[238,239] Circulating sRAGE is derived from enzymatic cleavage of flRAGE from the plasma membrane.[235] A decline in circulating sRAGE levels along with progressive cognitive decline in AD suggests that an imbalance between circulating sRAGE and endogenous RAGE ligands may influence or exacerbate pathological events in the brain. Whether monitoring the changes in sRAGE levels in the circulation can serve as an indicator for disease development and severity requires further investigation. Nevertheless, centrally acting RAGE antagonists or sRAGE mimetics may serve as novel therapeutic approaches for neuroinflammation.

[^{18}F]RAGER exhibited high affinity (K_d = 15 nmol/L) for RAGE in vitro in both AD and control tissues and was the first small-molecule radiotracer that was explored for in vivo PET imaging.[240] The PET studies conducted in rodents and NHP confirmed BBB permeability and increased uptake in the brain areas known to express RAGE. Autoradiographic images of [^{18}F]RAGER binding in postmortem brain revealed increased specific biding in frontal cortex gray matter from patients with AD compared to normal control donors. Moreover, [^{18}F]RAGER exhibited an in vitro B_{max}/K_D ratio of 2 in AD tissue, predicting a sufficient BP in vivo to justify using this scaffold for future applications in preclinical and clinical PET imaging.[240] As a step toward translation of [^{18}F]RAGER into clinical PET studies, a CNS receptor screening was performed

to identify any off-target binding.[241] Results of this screening showed that RAGER exhibited 93 nmol/L affinity toward the human melatonin MT1 receptor (in vitro). Nevertheless, competitive blocking studies with melatonin revealed that [^{18}F]RAGER binding was specific to RAGE, as melatonin did not displace [^{18}F]RAGER binding in human brain tissue autoradiography studies. Moreover, pretreatment with melatonin (10 μg/kg) failed to displace [^{18}F]RAGER binding in both rodent and NHP PET blocking studies. Further investigations are needed to confirm the selectivity of this tracer for either flRAGE or sRAGE subtypes, as this will enable monitoring alterations in the 2 RAGE subtypes in relation to disease progression and severity.

In order to explore the potential of RAGE as a new PET biomarker of AD diagnosis and monitoring, carbon-11-labeled RAGE inhibitor, [^{11}C]FPS-ZM1, was evaluated in autoradiography studies using brains from wild-type and AD mice.[242] Results from these studies highlighted high nonspecific binding in both wild-type and AD mice, thereby warranting further structural optimization. [^{18}F]FPS-ZM1 was developed to explore the link between diabetes and AD.[243] Biodistribution of [^{18}F]FPS-ZM1 in healthy C57BL/6J mice displayed high brain permeability followed by rapid clearance from brains and most other organs (except for liver, cecum, and gonad), indicating low nonspecific binding in brain and majority of the peripheral organs. Small animal PET imaging indicated that the uptake of [^{18}F]FPS-ZM1 in brain was higher in dementia models than in type 1 and type 2 diabetic models, suggesting that [^{18}F]FPS-ZM1 might be a promising biomarker for early detection of AD in patients with diabetes. Overall, a number of chemical entities that block RAGE activation by binding to the extracellular or intracellular domains have been reviewed elsewhere,[244] which may act as scaffolds for further chemical refinement and optimization of PET radiotracers that would elucidate alterations in RAGE expression and distribution in both healthy and pathological conditions.

Mer Tyrosine Kinase

The MerTK is a cell surface receptor tyrosine kinase that plays a critical role in the recognition, phagocytosis, and clearance of apoptotic cells in a variety of tissues.[245,246] In brain, MerTK is predominantly expressed in the microglia and astrocytes and is implicated in neuroinflammation and oncogenesis.[246,247] While MerTK protein levels are low to undetectable in normal brain, increased expression has been demonstrated in various neuroinflammatory disorders. For example, high levels of the soluble form of MerTK were found in a postmortem analysis of established MS lesions.[248] Also, polymorphisms in the *MerTK* gene are reportedly associated with MS susceptibility.[249] Although the precise role of MerTK in neuroinflammation remains unclear, PET imaging of this target should assist in devising new targeted therapeutic strategies for neuroinflammation and associated diseases.

[^{18}F]JHU16907, the first tracer developed for PET imaging of MerTK, was derived from a potent MerTK inhibitor 2-fluropyridine derivative, JHU16907 (IC_{50} = 2.5 nmol/L).[250] Robust brain uptake of [^{18}F]JHU16907 was found in control CD-1 mice, with peak at 5 minutes postinjection (SUV_{peak} 3% ID/g) cerebellum, hippocampus, and throughout cortex, followed by rapid washout. However, regional distribution of radioactivity showed relatively low heterogeneity, consistent with the globally low MerTK expression in healthy brain.[250] [^{18}F]JHU16907 was further evaluated in a biodistribution study conducted in an LPS rodent model of neuroinflammation. This study showed higher cerebral uptake of [^{18}F]JHU16907 in LPS-treated CD-1 mice than in control CD-1. Moreover, self-blocking studies (1 or 3 mg/kg JHU16907) significantly displaced [^{18}F]JHU16907 binding of LPS-treated mouse, confirming the specificity of this tracer for MerTK in vivo. There is considerable scope for imaging MerTK, thus warranting an extended search for improved MerTK tracers to enable eventual translational imaging research in human disease.

Triggering Receptor Expressed on Myeloid Cells-1

The TREM family of proteins comprises a group of cell surface innate immune receptors that are expressed on various myeloid cell populations throughout the body, including microglia in the brain.[251,252] The 2 subtypes of TREM, namely, TREM1 and TREM2, have distinct roles in immune function. Normal brain expresses very low to undetectable levels of TREM1. However, mice brain cell suspensions obtained 24 hours after intracerebral LPS injection revealed a significant increase in TREM1 expression and suppression in TREM2 expression as evidenced by gene expression analysis using qualitative polymerase chain reaction.[252] The differential expression of TREM1 and TREM2 in response to an acute inflammatory insult highlights the necessity to further understand how TREM expression is regulated in neurodegenerative diseases.

Initial efforts have been made toward developing brain-penetrating radiotracers for PET imaging of TREM1. A TREM1-specific PET tracer was recently developed by radiolabeling a selective anti-TREM1 antibody with copper-64 (^{64}Cu, $t_{½}$ = 12.7 hours).[253] Specificity of this PET radiotracer was verified in vitro by HEK293 cells with and without TREM1 transfection. Furthermore, in vivo PET imaging studies were conducted in different murine models of neuroinflammation, including LPS-induced systemic inflammation and ischemic stroke.[253,254] This thorough in vivo evaluation demonstrated higher binding of [^{64}Cu]TREM1-mAb in brain regions exhibiting inflammation in all mouse models investigated, compared to control mice. In vivo specificity of the PET radiotracer was further confirmed by studies employing TREM1 knockout mice. These results seem remarkable, given the caveat that the BBB is not usually permeable to large molecular weight tracers. Nonetheless, TREM1 knockout mice treated with LPS or following EAE induction exhibited negligible cerebral binding of [^{64}Cu]TREM1-mAb, thereby highlighting the specificity of the PET radiotracer. Overall, these results present a promising start for in vivo imaging of TREM1 and investigating its role in neuroinflammation, although there

likely remains a need for lower molecular weight ligands to achieve high specific binding of the PET ligand in vivo.

Conclusion

Neuroinflammation is a complex, orchestrated cellular response to harmful stimuli, which under acute conditions is aimed toward restoring tissue homeostasis. However, when inflammation is unresolved and becomes chronic, the otherwise salutogenic responses increase tissue damage and promote neurodegenerative disease initiation/progression. Emerging evidence suggests that chronic activation of microglia and astrocytes plays a more central role in neurodegenerative diseases than previously thought. Despite decades of research and the pharmaceutical industry's persistent efforts toward the discovery and development of treatment options for neurodegenerative diseases, translation of therapies to the clinic has faced considerable failure.[255] Taking as an example the case of AD, over 200 compounds have reached phase II clinical trials for AD since 2003, but no new drugs have been approved.[256,257] Nevertheless, important lessons have been learned from failures of anti-AD therapies in late-stage clinical trials, including dimebon (Medivation and Pfizer) and solanezumab (Eli Lilly). Therapy applied in late stages of AD seems unlikely to be ineffective, since ongoing chronic neuroinflammatory processes will have caused irreversible damage. Patients are generally recruited to trials only after their plaque burden and neurodegeneration has advanced and the disease is probably irreversible.[258] Moreover, detailed stratified analysis of clinical data reveals more promising results for treatment of patients with AD in earlier stages of the disease.[259] High heterogeneity in microglia and astrocyte reactions, as evidenced by molecular profiling in mouse models of neurodegenerative diseases, suggests that failure of neuroinflammation-targeted therapies might be due to their lack of immunoselectivity and inability to restore CNS cell function.[255] Clearly, the clinical development plan for neurodegenerative diseases is being revisited, and potential early diagnostic biomarkers and preventative strategies are being explored. To address the failures associated with AD drug development, the Committee for Medicinal Products for Human Use proposed changes in the diagnostic criteria, and more importantly encouraged the use of biomarkers in different stages of drug development.[260] In vivo imaging of biomarkers of neuroinflammation should assist in early detection of AD and other neurodegenerative diseases and help clinical researchers measure early responses to therapeutic interventions.[261] It is also noteworthy that neurodegenerative diseases involve multiple pathologies; therefore, a combination therapy appears to be a rational approach for treatment.[262] Given the significant role of neuroinflammation in the pathogenesis of neurodegenerative diseases, PET imaging biomarkers of neuroinflammation will not only assist in tracking disease progression but will also guide the design of combination therapies in an efficient and cost-effective way.

For 2 decades, TSPO PET imaging of neuroinflammation has held center stage in studies of neuroinflammatory diseases, despite several caveats related to quantitation, allelic dependence of binding, microglial phenotype, and incomplete cellular specificity for microglia. Nonetheless, TPSO PET imaging has, for example, highlighted the relationship between Aβ accumulation and microglial activation in AD,[263] and PET imaging of microglial activation, beyond TSPO, has recently been reviewed for CB2, COX-2, the $P2X_7$ receptor, and ROS.[264] Other PET targets discussed herein, such as GSK-3, MAO-B, COX, and S1P1, remain in a relatively preliminary stage of development and implementation as neuroimaging targets, but preclinical findings suggest that they offer immense potential for PET radiotracer development and clinical translation. In addition to the emerging neuroimmune targets discussed herein, ion channel linked receptors that include γ-aminobutyric acid—benzodiazepine receptor and nicotinic acetylcholine receptors (α_7 and $\alpha_4\beta_2$ subtypes)—also present promising potential for PET imaging, although further preclinical and clinical research is needed to establish the utility of these radiotracers for probing neuroinflammation and associated neurological disorders. We and others have previously reported selected PET radioligands developed for these targets.[265-268] Development of receptor subtype-specific radiotracers while maintaining or improving brain uptake remains a major challenge for PET radioligand development for these targets. Nevertheless, recent human PET imaging studies measuring α_7[269] and $\alpha_4\beta_2$[270] nicotinic acetylcholine receptor distribution in healthy volunteers using newly developed radioligands with superior in vivo characteristics support the translational potential of these targets for PET imaging of neurodegenerative diseases.

Alternate approaches are concurrently under development to identify clinically useful inflammatory cytokines in the plasma and establish the relationship between their peripheral and central distribution.[271] In this approach, proinflammatory biomarkers such as IL-1β, interferon-γ, IL-10, and IL-6 can be assessed in plasma and cerebrospinal fluid samples using Multiplex cytokine and proinflammatory biomarker analyses. Combining PET imaging results with proinflammatory biomarker analyses of plasma and cerebrospinal fluid samples from the same individuals in vivo may elucidate the molecular inflammatory signature in early-stage neurodegenerative disease and may assist in efficient translational application of relevant therapeutic interventions.[271] We contend that PET imaging of multiple biomarkers across the course of neurodegenerative disease will contribute toward better diagnosis and tracking of the sequence of pathophysiological events. One major remaining hurdle entails the distinction between the M1 and M2 microglial phenotypes. The $P2Y_{12}$ purinergic receptor is selectively expressed on M2 (neuroprotective) microglia, and PET imaging of this target may be especially beneficial in evaluating efficacy of potential therapies. For example, an anti-inflammatory therapy should ideally upregulate $P2Y_{12}$ expression, thus engaging a potentially neuroprotective mechanism. As discussed earlier, radiotracer development for PET brain imaging is a very challenging task. A fundamental criterion for a candidate radioligand is its ability

to cross the BBB. Some of the lead structures highlighted in this review displayed favorable in vitro binding properties, including good binding potential and selectivity. Nevertheless, upon transition to in vivo PET imaging, the radiotracer failed to display optimum BBB penetrability. Future studies are needed to acquire sufficient information to predict a radiotracer's brain penetrability, efflux protein interaction, and metabolism and to find a radiotracer with adequate specific binding and ideal metabolism that can overcome hurdles for human translation.

In conclusion, PET radiotracer development for neuroinflammation beyond TSPO, for imaging neurodegenerative diseases (and other neurological disorders), represents an active area of research with several emerging targets and tracers under various stages of preclinical and clinical research studies underway. Continuing development of PET radiotracers for imaging of neuroinflammation presents an attractive and non-invasive approach that can enable early diagnosis, track disease progression, and aid in the rational design and clinical assessment of patient responses to therapeutic intervention.

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References

1. Kumar V, Cotran RS, Robbins SL. *Robbins Basic Pathology*. 7th ed. New York, NY: Saunders; 2003.
2. Majno G, Joris I. *Cells, Tissues, and Disease: Principles of General Pathology*. 2nd ed. Oxford, England: Oxford University Press; 2004.
3. Medzhitov R. Origin and physiological roles of inflammation. *Nature*. 2008;454(7203):428–435. doi:10.1038/nature07201.
4. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. *J Neurochem*. 2016;139(suppl 2):136–153. doi:10.1111/jnc.13607.
5. Crutcher KA, Gendelman HE, Kipnis J, et al. Debate: "is increasing neuroinflammation beneficial for neural repair?". *J Neuroimmune Pharmacol*. 2006;1(3):195–211. doi:10.1007/s11481-006-9021-7.
6. Popovich PG, Longbrake EE. Can the immune system be harnessed to repair the CNS? *Nat Rev Neurosci*. 2008;9(6):481–493. doi:10.1038/nrn2398.
7. Schmid CD, Melchior B, Masek K, et al. Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. *J Neurochem*. 2009;109(suppl 1):117–125. doi:10.1111/j.1471-4159.2009.05984.x.
8. Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. *Exp Neurol*. 2007;208(1):1–25. doi:10.1016/j.expneurol.2007.07.004.
9. Skaper SD. The brain as a target for inflammatory processes and neuroprotective strategies. *Ann N Y Acad Sci*. 2007;1122:23–34. doi:10.1196/annals.1403.002.
10. Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog Neurobio.l* 2005;76(2):77–98. doi:10.1016/j.pneurobio.2005.06.004.
11. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. 2010;140(6):918–934. doi:10.1016/j.cell.2010.02.016.
12. Gonzalez H, Elgueta D, Montoya A, Pacheco R. Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J Neuroimmunol*. 2014;274(1-2):1–13. doi:10.1016/j.jneuroim.2014.07.012.
13. Molteni M, Rossetti C. Neurodegenerative diseases: the immunological perspective. *J Neuroimmunol*. 2017;313:109–115. doi:10.1016/j.jneuroim.2017.11.002.
14. Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science*. 2016;353(6301):777–783. doi:10.1126/science.aag2590.
15. Schain M, Kreisl WC. Neuroinflammation in neurodegenerative disorders – a review. *Curr Neurol Neurosci Rep*. 2017;17(3):25. doi:10.1007/s11910-017-0733-2.
16. Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease – a double-edged sword. *Neuron*. 2002;35(3):419–432.
17. Craft JM, Watterson DM, Van Eldik LJ. Neuroinflammation: a potential therapeutic target. *Expert Opin Ther Targets*. 2005;9(5):887–900. doi:10.1517/14728222.9.5.887.
18. McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology*. 1996;47(2):425–432.
19. Imbimbo BP, Solfrizzi V, Panza F. Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? *Front Aging Neurosci*. 2010;2:19. doi:10.3389/fnagi.2010.00019.
20. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging*. 2000;21(3):383–421.
21. Allaman I, Belanger M, Magistretti PJ. Astrocyte–neuron metabolic relationships: for better and for worse. *Trends Neurosci*. 2011;34(2):76–87. doi:10.1016/j.tins.2010.12.001.
22. Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci*. 2013;14(5):311–321. doi:10.1038/nrn3484.
23. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010;72:335–355. doi:10.1146/annurev-physiol-021909-135843.
24. Miller RH, Raff MC. Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. *J Neurosci*. 1984;4(2):585–592.

25. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci*. 2009;32(12):638–647. doi:10.1016/j.tins.2009.08.002.
26. Bush TG, Puvanachandra N, Horner CH, et al. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron*. 1999; 23(2):297–308.
27. Herrmann JE, Imura T, Song B, et al. STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. *J Neurosci*. 2008;28(28):7231–7243. doi:10.1523/JNEUROSCI.1709-08.2008.
28. Okada S, Nakamura M, Katoh H, et al. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med*. 2006;12(7):829–834. doi:10.1038/nm1425.
29. Wanner IB, Anderson MA, Song B, et al. Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. *J Neurosci*. 2013;33(31):12870–12886. doi:10.1523/JNEUROSCI.2121-13.2013.
30. Anderson MA, Burda JE, Ren Y, et al. Astrocyte scar formation aids central nervous system axon regeneration. *Nature*. 2016; 532(7598):195–200. doi:10.1038/nature17623.
31. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci*. 2015; 16(6):358–372. doi:10.1038/nrn3880.
32. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*. 2005;308(5726):1314–1318. doi:10.1126/science.1110647.
33. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev*. 2011;91(2):461–553. doi:10.1152/physrev.00011.2010.
34. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci*. 1996;19(8):312–318.
35. Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. *Nat Rev Neurol*. 2010;6(4):193–201. doi:10.1038/nrneurol.2010.17.
36. Davalos D, Grutzendler J, Yang G, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*. 2005;8(6):752–758. doi:10.1038/nn1472.
37. Hu X, Leak RK, Shi Y, et al. Microglial and macrophage polarization – new prospects for brain repair. *Nat Rev Neurol*. 2015; 11(1):56–64. doi:10.1038/nrneurol.2014.207.
38. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol*. 2009;27:119–145. doi:10.1146/annurev.immunol.021908.132528.
39. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. *Immunity*. 2005;23(4):344–346. doi:10.1016/j.immuni.2005.10.001.
40. Reus GZ, Fries GR, Stertz L, et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience*. 2015;300:141–154. doi:10.1016/j.neuroscience.2015.05.018.
41. Zamanian JL, Xu L, Foo LC, et al. Genomic analysis of reactive astrogliosis. *J Neurosci*. 2012;32(18):6391–6410. doi:10.1523/JNEUROSCI.6221-11.2012.
42. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017; 541(7638):481–487. doi:10.1038/nature21029.
43. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol*. 2010;119(1):7–35. doi:10.1007/s00401-009-0619-8.
44. Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S. Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res*. 1998;57(1):1–9.
45. Streit WJ, Braak H, Xue QS, Bechmann I. Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathol*. 2009;118(4):475–485. doi:10.1007/s00401-009-0556-6.
46. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J Neuroinflammation*. 2014;11:98. doi:10.1186/1742-2094-11-98.
47. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). *Neurochem Res*. 2000; 25(9-10):1439–1451.
48. Lucas CV, Bensch KG, Eng LF. In vitro polymerization of glial fibrillary acidic (GFA) protein extracted from multiple sclerosis (MS) brain. *Neurochem Res*. 1980;5(3):247–255.
49. Van de Bittner GC, Ricq EL, Hooker JM. A philosophy for CNS radiotracer design. *Acc Chem Res*. 2014;47(10):3127–3134. doi:10.1021/ar500233s.
50. Ametamey SM, Honer M, Schubiger PA. Molecular imaging with PET. *Chem Rev*. 2008;108(5):1501–1516. doi:10.1021/cr0782426.
51. Cumming P, Vasdev N. The assay of enzyme activity by positron emission tomography. In: Gründer G, ed. *Molecular Imaging in the Clinical Neurosciences Neuromethods*. New York, NY: Humana Press; 2012:111–135.
52. Halldin C, Gulyás B, Langer O, Farde L. Brain radioligands – state of the art and new trends. *Q J Nucl Med*. 2001;45(2):139–152.
53. Hicks JW, VanBrocklin HF, Wilson AA, Houle S, Vasdev N. Radiolabeled small molecule protein kinase inhibitors for imaging with PET or SPECT. *Molecules*. 2010;15(11):8260–8278. doi:10.3390/molecules15118260.
54. Holland JP, Cumming P, Vasdev N. PET radiopharmaceuticals for probing enzymes in the brain. *Am J Nucl Med Mol Imaging*. 2013;3(3):194–216.
55. Holland JP, Liang SH, Rotstein BH, et al. Alternative approaches for PET radiotracer development in Alzheimer's disease: imaging beyond plaque. *J Labelled Comp Radiopharm*. 2014;57(4):323–331. doi:10.1002/jlcr.3158.
56. Honer M, Gobbi L, Martarello L, Comley RA. Radioligand development for molecular imaging of the central nervous system with positron emission tomography. *Drug Discov Today*. 2014;19(12):1936–1944. doi:10.1016/j.drudis.2014.08.012.

57. Piel M, Vernaleken I, Rosch F. Positron emission tomography in CNS drug discovery and drug monitoring. *J Med Chem*. 2014; 57(22):9232–9258. doi:10.1021/jm5001858.
58. Pike VW. PET radiotracers: crossing the blood–brain barrier and surviving metabolism. *Trends Pharmacol Sci*. 2009;30(8): 431–440. doi:10.1016/j.tips.2009.05.005.
59. Pike VW. Considerations in the development of reversibly binding PET radioligands for brain imaging. *Curr Med Chem*. 2016; 23(18):1818–1869.
60. Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol*. 1984; 15(3):217–227. doi:10.1002/ana.410150302.
61. Innis RB, Cunningham VJ, Delforge J, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab*. 2007;27(9):1533–1539. doi:10.1038/sj.jcbfm.9600493.
62. Patel S, Gibson R. In vivo site-directed radiotracers: a minireview. *Nucl Med Biol*. 2008;35(8):805–815. doi:10.1016/j.nucmedbio.2008.10.002.
63. Chesselet MF, Carmichael ST. Animal models of neurological disorders. *Neurotherapeutics*. 2012;9(2):241–244. doi:10.1007/s13311-012-0118-9.
64. Nazem A, Sankowski R, Bacher M, Al-Abed Y. Rodent models of neuroinflammation for Alzheimer's disease. *J Neuroinflammation*. 2015;12:74. doi:10.1186/s12974-015-0291-y.
65. Vingill S, Connor-Robson N, Wade-Martins R. Are rodent models of Parkinson's disease behaving as they should? *Behav Brain Res*. 2017. doi:10.1016/j.bbr.2017.10.021.
66. Le Fur G, Perrier ML, Vaucher N, 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):1839–1847.
67. Vivash L, O'Brien TJ. Imaging microglial activation with TSPO PET: lighting up neurologic diseases? *J Nucl Med*. 2016;57(2): 165–168. doi:10.2967/jnumed.114.141713.
68. Papadopoulos V, Baraldi M, Guilarte TR, et al. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci*. 2006;27(8):402–409. doi:10.1016/j.tips.2006.06.005.
69. Rupprecht R, Papadopoulos V, Rammes G, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2010;9(12): 971–988. doi:10.1038/nrd3295.
70. Wilms H, Claasen J, Röhl C, Sievers J, Deuschl G, Lucius R. Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: evidence from activated microglial cells in vitro. *Neurobiol Dis*. 2003;14(3):417–424.
71. Lavisse S, Guillermier M, Herard AS, et al. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. *J Neurosci*. 2012;32(32):10809–10818. doi:10.1523/JNEUROSCI.1487-12.2012.
72. Cumming P, Burgher B, Patkar O, et al. Sifting through the surfeit of neuroinflammation tracers. *J Cereb Blood Flow Metab*. 2018; 38(2):204–224. doi:10.1177/0271678X17748786.
73. Ikawa M, Lohith TG, Shrestha S, et al. 11C-ER176, a radioligand for 18-kDa translocator protein, has adequate sensitivity to robustly image all three affinity genotypes in human brain. *J Nucl Med*. 2017;58(2):320–325. doi:10.2967/jnumed.116.178996.
74. Dupont AC, 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). doi:10.3390/ijms18040785.
75. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multitasking kinase. *J Cell Sci*. 2003;116(Pt 7):1175–1186.
76. Woodgett JR. cDNA cloning and properties of glycogen synthase kinase-3. *Methods Enzymol*. 1991;200:564–577.
77. Leroy K, Brion JP. Developmental expression and localization of glycogen synthase kinase-3beta in rat brain. *J Chem Neuroanat*. 1999;16(4):279–293.
78. MacAulay K, Doble BW, Patel S, et al. Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab*. 2007;6(4):329–337. doi:10.1016/j.cmet.2007.08.013.
79. Bhat RV, Budd Haeberlein SL, Avila J. Glycogen synthase kinase 3: a drug target for CNS therapies. *J Neurochem*. 2004;89(6): 1313–1317. doi:10.1111/j.1471-4159.2004.02422.x.
80. Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. *Nat Rev Drug Discov*. 2004;3(6):479–487. doi:10.1038/nrd1415.
81. Maurer U, Preiss F, Brauns-Schubert P, Schlicher L, Charvet C. GSK-3 – at the crossroads of cell death and survival. *J Cell Sci*. 2014;127(Pt 7):1369–1378. doi:10.1242/jcs.138057.
82. Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. *Diabetes*. 2000; 49(2):263–271.
83. Robinson AP, Harp CT, Noronha A, Miller SD. The Experimental Autoimmune Encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handb Clin Neurol*. 2014;122:173–189. doi:10.1016/B978-0-444-52001-2.00008-X.
84. De Sarno P, Axtell RC, Raman C, Roth KA, Alessi DR, Jope RS. Lithium prevents and ameliorates experimental autoimmune encephalomyelitis. *J Immunol*. 2008;181(1):338–345.
85. Leroy K, Yilmaz Z, Brion JP. Increased level of active GSK-3beta in Alzheimer's disease and accumulation in argyrophilic grains and in neurones at different stages of neurofibrillary degeneration. *Neuropathol Appl Neurobiol*. 2007;33(1):43–55. doi:10.1111/j.1365-2990.2006.00795.x.
86. Llorens-Martín M, Jurado J, Hernández F, Avila J. GSK-3beta, a pivotal kinase in Alzheimer disease. *Front Mol Neurosci*. 2014;7: 46. doi:10.3389/fnmol.2014.00046.
87. Pandey MK, DeGrado TR. Glycogen synthase kinase-3 (GSK-3)-targeted therapy and imaging. *Theranostics*. 2016;6(4):571–593. doi:10.7150/thno.14334.
88. Bhat RV, Budd SL. GSK3beta signalling: casting a wide net in Alzheimer's disease. *Neurosignals*. 2002;11(5):251–261. doi:10.1159/000067423.

89. Takahashi M, Tomizawa K, Kato R, et al. Localization and developmental changes of tau protein kinase I/glycogen synthase kinase-3 beta in rat brain. *J Neurochem*. 1994;63(1): 245–255.
90. DaRocha-Souto B, Coma M, Perez-Nievas BG, et al. Activation of glycogen synthase kinase-3 beta mediates beta-amyloid induced neuritic damage in Alzheimer's disease. *Neurobiol Dis*. 2012;45(1):425–437. doi:10.1016/j.nbd.2011.09.002.
91. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem*. 2008;104(6):1433–1439. doi:10.1111/j.1471-4159.2007.05194.x.
92. Vasdev N, Garcia A, Stableford WT, et al. Synthesis and ex vivo evaluation of carbon-11 labelled N-(4-methoxybenzyl)-N′-(5-nitro-1,3-thiazol-2-yl)urea ([11C]AR-A014418): a radiolabelled glycogen synthase kinase-3beta specific inhibitor for PET studies. *Bioorg Med Chem Lett*. 2005;15(23):5270–5273. doi:10.1016/j.bmcl.2005.08.037.
93. Li L, Shao X, Cole EL, et al. Synthesis and initial in vivo studies with [(11)C]SB-216763: the first radiolabeled brain penetrative inhibitor of GSK-3. *ACS Med Chem Lett*. 2015;6(5):548–552. doi:10.1021/acsmedchemlett.5b00044.
94. Wang M, Gao M, Miller KD, Sledge GW, Hutchins GD, Zheng QH. The first synthesis of [(11)C]SB-216763, a new potential PET agent for imaging of glycogen synthase kinase-3 (GSK-3). *Bioorg Med Chem Lett*. 2011;21(1):245–249. doi:10.1016/j.bmcl.2010.11.026.
95. Cole EL, Shao X, Sherman P, et al. Synthesis and evaluation of [(11)C]PyrATP-1, a novel radiotracer for PET imaging of glycogen synthase kinase-3beta (GSK-3beta). *Nucl Med Biol*. 2014; 41(6):507–512. doi:10.1016/j.nucmedbio.2014.03.025.
96. Kumata K, Yui J, Xie L, et al. Radiosynthesis and preliminary PET evaluation of glycogen synthase kinase 3beta (GSK-3beta) inhibitors containing [(11)C]methylsulfanyl, [(11)C]methylsulfinyl or [(11)C]methylsulfonyl groups. *Bioorg Med Chem Lett*. 2015;25(16):3230–3233. doi:10.1016/j.bmcl.2015.05.085.
97. Hu K, Patnaik D, Collier TL, et al. Development of [(18)F]Maleimide-based glycogen synthase kinase-3beta ligands for positron emission tomography imaging. *ACS Med Chem Lett*. 2017; 8(3):287–292. doi:10.1021/acsmedchemlett.6b00405.
98. Liang SH, Chen JM, Normandin MD, et al. Discovery of a highly selective glycogen synthase kinase-3 inhibitor (PF-04802367) that modulates tau phosphorylation in the brain: translation for PET neuroimaging. *Angew Chem Int Ed Engl*. 2016;55(33):9601–9605. doi:10.1002/anie.201603797.
99. Cumming P. *Imaging Dopamine*. Cambridge, England: Cambridge University Press; 2009.
100. Grimsby J, Chen K, Wang LJ, Lan NC, Shih JC. Human monoamine oxidase A and B genes exhibit identical exon–intron organization. *Proc Natl Acad Sci U S A*. 1991;88(9):3637–3641.
101. Grimsby J, Lan NC, Neve R, Chen K, Shih JC. Tissue distribution of human monoamine oxidase A and B mRNA. *J Neurochem*. 1990;55(4):1166–1169.
102. Vincent SR. Histochemical localization of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine oxidation in the mouse brain. *Neuroscience*. 1989;28(1):189–199.
103. Ekblom J, Jossan SS, Oreland L, Walum E, Aquilonius SM. Reactive gliosis and monoamine oxidase B. *J Neural Transm Suppl*. 1994;41:253–258.
104. Smith MA, Rottkamp CA, Nunomura A, et al. Oxidative stress in Alzheimer's disease. *Biochim Biophys Acta*. 2000;1502(1): 139–144.
105. Carter SF, Scholl M, Almkvist O, et al. Evidence for astrocytosis in prodromal Alzheimer disease provided by ^{11}C-deuterium-L-deprenyl: a multitracer PET paradigm combining ^{11}C-Pittsburgh compound B and ^{18}F-FDG. *J Nucl Med*. 2012;53(1):37–46. doi:10.2967/jnumed.110.087031.
106. Gulyas B, Pavlova E, Kasa P, et al. Activated MAO-B in the brain of Alzheimer patients, demonstrated by [11C]-L-deprenyl using whole hemisphere autoradiography. *Neurochem Int*. 2011; 58(1):60–68. doi:10.1016/j.neuint.2010.10.013.
107. Shoulson I. DATATOP: a decade of neuroprotective inquiry. Parkinson study group. deprenyl and tocopherol antioxidative therapy of parkinsonism. *Ann Neurol*. 1998;44(3 suppl 1): S160–S166.
108. Fowler JS, Logan J, Volkow ND, Wang GJ. Translational neuroimaging: positron emission tomography studies of monoamine oxidase. *Mol Imaging Biol*. 2005;7(6):377–387. doi:10.1007/s11307-005-0016-1.
109. Fowler JS, Logan J, Volkow ND, Wang GJ, MacGregor RR, Ding YS. Monoamine oxidase: radiotracer development and human studies. *Methods*. 2002;27(3):263–277.
110. Nag S, Varrone A, Toth M, et al. In vivo evaluation in cynomolgus monkey brain and metabolism of [(1)(8)F]fluorodeprenyl: a new MAO-B pet radioligand. *Synapse*. 2012;66(4): 323–330. doi:10.1002/syn.21514.
111. Nag S, Fazio P, Lehmann L, et al. In vivo and in vitro characterization of a novel MAO-B inhibitor radioligand, ^{18}F-labeled deuterated fluorodeprenyl. *J Nucl Med*. 2016;57(2):315–320. doi:10.2967/jnumed.115.161083.
112. Nag S, Lehmann L, Kettschau G, et al. Development of a novel fluorine-18 labeled deuterated fluororasagiline ([(18)F]fluororasagiline-D2) radioligand for PET studies of monoamino oxidase B (MAO-B). *Bioorg Med Chem*. 2013;21(21):6634–6641. doi:10.1016/j.bmc.2013.08.019.
113. Brooks AF, Shao X, Quesada CA, Sherman P, Scott PJ, Kilbourn MR. In vivo metabolic trapping radiotracers for imaging monoamine oxidase-A and -B enzymatic activity. *ACS Chem Neurosci*. 2015;6(12):1965–1971. doi:10.1021/acschemneuro.5b00223.
114. Vasdev N, Sadovski O, Moran MD, et al. Development of new radiopharmaceuticals for imaging monoamine oxidase B. *Nucl Med Biol*. 2011;38(7):933–943. doi:10.1016/j.nucmedbio.2011.03.003.
115. Bramoullé Y PF, Saba W, Valette H, et al. Radiosynthesis of (S)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)benzo[d]isoxazol-3-yl] oxazolidin-2-[11C]one ([11C]SL25.1188), a novel radioligand for imaging monoamine oxidase-B with PET. *J Label Compd Radiopharm*. 2008;51(3):153–158.
116. Vasdev N, Sadovski O, Garcia A, et al. Radiosynthesis of [11C]SL25.1188 via [11C]CO_2 fixation for imaging monoamine oxidase B. *J Label Compd Radiopharm*. 2011;54(10):678–680.

117. Saba W, Valette H, Peyronneau MA, et al. [(11)C]SL25.1188, a new reversible radioligand to study the monoamine oxidase type B with PET: preclinical characterisation in nonhuman primate. *Synapse*. 2010;64(1):61–69. doi:10.1002/syn.20703.
118. Rusjan PM, Wilson AA, Miler L, et al. Kinetic modeling of the monoamine oxidase B radioligand [(1)(1)C]SL25.1188 in human brain with high-resolution positron emission tomography. *J Cereb Blood Flow Metab*. 2014;34(5):883–889. doi:10.1038/jcbfm.2014.34.
119. Hicks JW, Sadovski O, Parkes J, et al. Radiosynthesis and ex vivo evaluation of [(18)F]-(S)-3-(6-(3-fluoropropoxy)benzo[d]isoxazol-3-yl)-5-(methoxymethyl)oxazoli din-2-one for imaging MAO-B with PET. *Bioorg Med Chem Lett*. 2015;25(2):288–291. doi:10.1016/j.bmcl.2014.11.048.
120. Saint-Aubert L, Lemoine L, Chiotis K, Leuzy A, Rodriguez-Vieitez E, Nordberg A. Tau PET imaging: present and future directions. *Mol Neurodegener*. 2017;12(1):19. doi:10.1186/s13024-017-0162-3.
121. Vermeiren C, Motte P, Viot D, et al. The tau positron-emission tomography tracer AV-1451 binds with similar affinities to tau fibrils and monoamine oxidases. *Mov Disord*. 2018;33(2):273–281. doi:10.1002/mds.27271.
122. Ng KP, Pascoal TA, Mathotaarachchi S, et al. Monoamine oxidase B inhibitor, selegiline, reduces (18)F-THK5351 uptake in the human brain. *Alzheimers Res Ther*. 2017;9(1):25. doi:10.1186/s13195-017-0253-y.
123. Hsieh HL, Yang CM. Role of redox signaling in neuroinflammation and neurodegenerative diseases. *Biomed Res Int*. 2013;2013:484613. doi:10.1155/2013/484613.
124. Lee IT, Yang CM. Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. *Biochem Pharmacol*. 2012;84(5):581–590. doi:10.1016/j.bcp.2012.05.005.
125. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44–84. doi:10.1016/j.biocel.2006.07.001.
126. Rojo AI, McBean G, Cindric M, et al. Redox control of microglial function: molecular mechanisms and functional significance. *Antioxid Redox Signal*. 2014;21(12):1766–1801. doi:10.1089/ars.2013.5745.
127. Shi Q, Gibson GE. Oxidative stress and transcriptional regulation in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2007;21(4):276–291. doi:10.1097/WAD.0b013e31815721c3.
128. Jenner P, Olanow CW. The pathogenesis of cell death in Parkinson's disease. *Neurology*. 2006;66(10 suppl 4):S24–S36.
129. Carroll VN, Truillet C, Shen B, et al. [(11)C]Ascorbic and [(11)C]dehydroascorbic acid, an endogenous redox pair for sensing reactive oxygen species using positron emission tomography. *Chem Commun (Camb)*. 2016;52:4888–4890. doi:10.1039/c6cc00895j.
130. Abe K, Takai N, Fukumoto K, et al. In vivo imaging of reactive oxygen species in mouse brain by using [^{3} H]hydromethidine as a potential radical trapping radiotracer. *J Cereb Blood Flow Metab*. 2014;34(12):1907–1913. doi:10.1038/jcbfm.2014.160.
131. Wilson AA, Sadovski O, Nobrega JN, et al. Evaluation of a novel radiotracer for positron emission tomography imaging of reactive oxygen species in the central nervous system. *Nucl Med Biol*. 2017;53:14–20. doi:10.1016/j.nucmedbio.2017.05.011.
132. Hou C, Hsieh CJ, Li S, et al. Development of a positron emission tomography radiotracer for imaging elevated levels of superoxide in neuroinflammation. *ACS Chem Neurosci*. 2018;9(3):578–586. doi:10.1021/acschemneuro.7b00385.
133. Doxey JC, Roach AG, Smith CF. Studies on RX 781094: a selective, potent and specific antagonist of alpha 2-adrenoceptors. *Br J Pharmacol*. 1983;78(3):489–505.
134. Michel MC, Ernsberger P. Keeping an eye on the I site: imidazoline-preferring receptors. *Trends Pharmacol Sci*. 1992;13(10):369–370.
135. Olmos G, Alemany R, Escriba PV, García-Sevilla JA. The effects of chronic imidazoline drug treatment on glial fibrillary acidic protein concentrations in rat brain. *Br J Pharmacol*. 1994;111(4):997–1002.
136. Garcia-Sevilla JA, Escriba PV, Guimon J. Imidazoline receptors and human brain disorders. *Ann N Y Acad Sci*. 1999;881:392–409.
137. Martin-Gomez JI, Ruiz J, Callado LF, et al. Increased density of I2-imidazoline receptors in human glioblastomas. *Neuroreport*. 1996;7(8):1393–1396.
138. Parker CA, Nabulsi N, Holden D, et al. Evaluation of 11C-BU99008, a PET ligand for the imidazoline2 binding sites in rhesus brain. *J Nucl Med*. 2014;55(5):838–844. doi:10.2967/jnumed.113.131854.
139. Gargalidis-Moudanos C, Pizzinat N, Javoy-Agid F, Remaury A, Parini A. I2-imidazoline binding sites and monoamine oxidase activity in human postmortem brain from patients with Parkinson's disease. *Neurochem Int*. 1997;30(1):31–36.
140. Kawamura K, Shimoda Y, Yui J, et al. A useful PET probe [(11)C]BU99008 with ultra-high specific radioactivity for small animal PET imaging of I2-imidazoline receptors in the hypothalamus. *Nucl Med Biol*. 2017;45:1–7. doi:10.1016/j.nucmedbio.2016.10.005.
141. Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol Sci*. 2009;30(4):174–181. doi:10.1016/j.tips.2009.01.002.
142. Phillis JW, Horrocks LA, Farooqui AA. Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. *Brain Res Rev*. 2006;52(2):201–243. doi:10.1016/j.brainresrev.2006.02.002.
143. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron*. 1993;11(2):371–386.
144. Yang H, Chen C. Cyclooxygenase-2 in synaptic signaling. *Curr Pharm Des*. 2008;14(14):1443–1451.
145. Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. *J Comp Neurol*. 1995;355(2):296–315. doi:10.1002/cne.903550208.
146. Yermakova AV, Rollins J, Callahan LM, Rogers J, O'Banion MK. Cyclooxygenase-1 in human Alzheimer and control brain:

quantitative analysis of expression by microglia and CA3 hippocampal neurons. *J Neuropathol Exp Neurol*. 1999;58(11): 1135–1146.

147. Aid S, Bosetti F. Gene expression of cyclooxygenase-1 and Ca(2+)-independent phospholipase A(2) is altered in rat hippocampus during normal aging. *Brain Res Bull*. 2007;73(1-3): 108–113. doi:10.1016/j.brainresbull.2007.02.015.
148. Choi SH, Bosetti F. Cyclooxygenase-1 null mice show reduced neuroinflammation in response to beta-amyloid. *Aging (Albany NY)*. 2009;1(2):234–244. doi:10.18632/aging.100021.
149. Choi SH, Langenbach R, Bosetti F. Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. *FASEB J*. 2008;22(5):1491–1501. doi:10.1096/fj.07-9411com.
150. Choi SH, Aid S, Caracciolo L, et al. Cyclooxygenase-1 inhibition reduces amyloid pathology and improves memory deficits in a mouse model of Alzheimer's disease. *J Neurochem*. 2013; 124(1):59–68. doi:10.1111/jnc.12059.
151. Matousek SB, Hein AM, Shaftel SS, Olschowka JA, Kyrkanides S, Kerry O'Banion M. Cyclooxygenase-1 mediates prostaglandin E(2) elevation and contextual memory impairment in a model of sustained hippocampal interleukin-1beta expression. *J Neurochem*. 2010;114(1):247–258. doi:10.1111/j.1471-4159.2010.06759.x.
152. Aid S, Langenbach R, Bosetti F. Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2. *J Neuroinflammation*. 2008;5:17. doi:10.1186/1742-2094-5-17.
153. Combrinck M, Williams J, De Berardinis MA, et al. Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2006;77(1): 85–88. doi:10.1136/jnnp.2005.063131.
154. Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. *J Neuropathol Exp Neurol*. 2004; 63(9):901–910.
155. Kikuchi T, Okada M, Nengaki N, et al. Efficient synthesis and chiral separation of ^{11}C-labeled ibuprofen assisted by DMSO for imaging of in vivo behavior of the individual isomers by positron emission tomography. *Bioorg Med Chem*. 2011;19(10): 3265–3273. doi:10.1016/j.bmc.2011.03.041.
156. Shukuri M, Takashima-Hirano M, Tokuda K, et al. In vivo expression of cyclooxygenase-1 in activated microglia and macrophages during neuroinflammation visualized by PET with 11C-ketoprofen methyl ester. *J Nucl Med*. 2011;52(7): 1094–1101. doi:10.2967/jnumed.110.084046.
157. Paliwal JK, Smith DE, Cox SR, Berardi RR, Dunn-Kucharski VA, Elta GH. Stereoselective, competitive, and nonlinear plasma protein binding of ibuprofen enantiomers as determined in vivo in healthy subjects. *J Pharmacokinet Biopharm*. 1993; 21(2):145–161.
158. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc Natl Acad Sci U S A*. 1999;96(13):7563–7568.
159. Ohnishi A, Senda M, Yamane 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):594–599. doi:10.1016/j.nucmedbio.2014.04.008.
160. Shrestha S, Singh P, Eldridge M, et al. A novel PET radioligand, [11C]PS13, successfully images COX-1, a potential biomarker for neuroinflammation. *J Nucl Med*. 2016;57(suppl 2):115.
161. Singh P, Cortes M, Morse C, et al. [18F]PS-2 as a candidate radioligand for imaging COX-1 expression in brain: radiosynthesis and monkey PET imaging. *J Nucl Med*. 2015;56(suppl 2): 1091.
162. Cortes M, Singh P, Morse C, et al. Synthesis of PET radioligands as potential probes for imaging COX-2 in neuroinflammation. *J Nucl Med*. 2015;56(suppl 3):1092.
163. Kim M, Shrestha S, Eldridge M, et al. Novel PET radioligands show that, in rhesus monkeys, COX-1 is constitutively expressed and COX-2 is induced by inflammation. *J Nucl Med*. 2017; 58(suppl 1):203.
164. Balsinde J, Winstead MV, Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. *FEBS Lett*. 2002; 531(1):2–6.
165. Basselin M, Ramadan E, Rapoport SI. Imaging brain signal transduction and metabolism via arachidonic and docosahexaenoic acid in animals and humans. *Brain Res Bull*. 2012;87(2-3): 154–171. doi:10.1016/j.brainresbull.2011.12.001.
166. Esposito G, Giovacchini G, Liow JS, et al. Imaging neuroinflammation in Alzheimer's disease with radiolabeled arachidonic acid and PET. *J Nucl Med*. 2008;49(9):1414–1421. doi:10.2967/jnumed.107.049619.
167. Takabe K, Paugh SW, Milstien S, Spiegel S. "Inside-out" signaling of sphingosine-1-phosphate: therapeutic targets. *Pharmacol Rev*. 2008;60(2):181–195. doi:10.1124/pr.107.07113.
168. Brinkmann V. Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacol Ther*. 2007;115(1):84–105. doi:10.1016/j.pharmthera.2007.04.006.
169. Hla T, Brinkmann V. Sphingosine 1-phosphate (S1P): physiology and the effects of S1P receptor modulation. *Neurology*. 2011;76(8 suppl 3):S3–S8. doi:10.1212/WNL.0b013e31820d5ec1.
170. Haghikia A, Gold R. Sphingosine-1-phosphate and its receptors as a possible therapeutic target in autoimmune diseases of the nervous system. *J Neuroimmunol*. 2010;218(1-2):1–2. doi:10.1016/j.jneuroim.2009.09.018.
171. Miron VE, Schubart A, Antel JP. Central nervous system-directed effects of FTY720 (fingolimod). *J Neurol Sci*. 2008; 274(1-2):13–17. doi:10.1016/j.jns.2008.06.031.
172. Nayak D, Huo Y, Kwang WX, et al. Sphingosine kinase 1 regulates the expression of proinflammatory cytokines and nitric oxide in activated microglia. *Neuroscience*. 2010;166(1): 132–144. doi:10.1016/j.neuroscience.2009.12.020.
173. Pebay A, Toutant M, Premont J, et al. Sphingosine-1-phosphate induces proliferation of astrocytes: regulation by intracellular signalling cascades. *Eur J Neurosci*. 2001;13(12):2067–2076.
174. Rouach N, Pebay A, Meme W, et al. S1P inhibits gap junctions in astrocytes: involvement of G and Rho GTPase/ROCK. *Eur J*

Neurosci. 2006;23(6):1453–1464. doi:10.1111/j.1460-9568.2006.04671.x.

175. Yamagata K, Tagami M, Torii Y, et al. Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. *Glia*. 2003;41(2):199–206. doi:10.1002/glia.10180.
176. Blaho VA, Hla T. An update on the biology of sphingosine 1-phosphate receptors. *J Lipid Res*. 2014;55(8):1596–1608. doi:10.1194/jlr.R046300.
177. Groves A, Kihara Y, Chun J. Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *J Neurol Sci*. 2013;328(1-2):9–18. doi:10.1016/j.jns.2013.02.011.
178. Brinkmann V, Billich A, Baumruker T, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov*. 2010;9(11):883–897. doi:10.1038/nrd3248.
179. Kihara Y, Mizuno H, Chun J. Lysophospholipid receptors in drug discovery. *Exp Cell Res*. 2015;333(2):171–177. doi:10.1016/j.yexcr.2014.11.020.
180. Jackson SJ, Giovannoni G, Baker D. Fingolimod modulates microglial activation to augment markers of remyelination. *J Neuroinflammation*. 2011;8:76. doi:10.1186/1742-2094-8-76.
181. Kolahdooz Z, Nasoohi S, Asle-Rousta M, Ahmadiani A, Dargahi L. Sphingosin-1-phosphate receptor 1: a potential target to inhibit neuroinflammation and restore the sphingosin-1-phosphate metabolism. *Can J Neurol Sci*. 2015;42(3):195–202. doi:10.1017/cjn.2015.19.
182. Noda H, Takeuchi H, Mizuno T, Suzumura A. Fingolimod phosphate promotes the neuroprotective effects of microglia. *J Neuroimmunol*. 2013;256(1-2):13–18. doi:10.1016/j.jneuroim.2012.12.005.
183. Trkov S, Stenovec M, Kreft M, et al. Fingolimod – a sphingosine-like molecule inhibits vesicle mobility and secretion in astrocytes. *Glia*. 2012;60(9):1406–1416. doi:10.1002/glia.22361.
184. Seki N, Maeda Y, Kataoka H, Sugahara K, Chiba K. Role of sphingosine 1-phosphate (S1P) receptor 1 in experimental autoimmune encephalomyelitis. *Pharmacol Pharm*. 2013;4(8):628–637.
185. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol*. 2011;164(4):1079–1106. doi:10.1111/j.1476-5381.2011.01302.x.
186. Choi JW, Gardell SE, Herr DR, et al. FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proc Natl Acad Sci U S A*. 2011;108(2):751–756. doi:10.1073/pnas.1014154108.
187. Rothhammer V, Kenison JE, Tjon E, et al. Sphingosine 1-phosphate receptor modulation suppresses pathogenic astrocyte activation and chronic progressive CNS inflammation. *Proc Natl Acad Sci U S A*. 2017;114(8):2012–2017. doi:10.1073/pnas.1615413114.
188. Park SJ, Im DS. Sphingosine 1-phosphate receptor modulators and drug discovery. *Biomol Ther (Seoul)*. 2017;25(1):80–90. doi:10.4062/biomolther.2016.160.
189. Prasad VP, Wagner S, Keul P, et al. Synthesis of fluorinated analogues of sphingosine-1-phosphate antagonists as potential radiotracers for molecular imaging using positron emission tomography. *Bioorg Med Chem*. 2014;22(19):5168–5181. doi:10.1016/j.bmc.2014.08.009.
190. Shaikh RS, Schilson SS, Wagner S, et al. Synthesis and evaluation of fluorinated fingolimod (FTY720) analogues for sphingosine-1-phosphate receptor molecular imaging by positron emission tomography. *J Med Chem*. 2015;58(8):3471–3484. doi:10.1021/jm502021d.
191. Quattropani AMC, Sauer W, Crosignani S, Bombrun A. Oxadiazole derivatives. WO 2010112461 A1, Appl no PCT/EP2010/054103. 2010.
192. Jin H, Yang H, Liu H, et al. A promising carbon-11-labeled sphingosine-1-phosphate receptor 1-specific PET tracer for imaging vascular injury. *J Nucl Cardiol*. 2017;24(2):558–570. doi:10.1007/s12350-015-0391-1.
193. Liu H, Jin H, Yue X, et al. PET imaging study of S1PR1 expression in a rat model of multiple sclerosis. *Mol Imaging Biol*. 2016;18(5):724–732. doi:10.1007/s11307-016-0944-y.
194. Rosenberg A, Liu H, Yue X, Jin H, Tu Z. Development and in vivo evaluation of three F-18 labeled S1P1 ligands as PET tracers for MS. *J Nucl Med*. 2016;57(suppl 2):1.
195. Liu H, Han J, Jin H, et al. Optimization of S1P1-specific PET radioligands for imaging neuroinflammation. *J Nucl Med*. 2017;58(suppl 1):10.
196. Benito C, Tolón RM, Pazos MR, Núñez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol*. 2008;153(2):277–285. doi:10.1038/sj.bjp.0707505.
197. Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA. Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int Immunopharmacol*. 2002;2(1):69–82.
198. Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol*. 2010;160(3):467–479. doi:10.1111/j.1476-5381.2010.00729.x.
199. Walter L, Franklin A, Witting A, et al. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J Neurosci*. 2003;23(4):1398–1405.
200. Benito C, Nunez E, Tolon RM, et al. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci*. 2003;23(35):11136–11141.
201. Evens N, Vandeputte C, Coolen C, et al. Preclinical evaluation of [11C]NE40, a type 2 cannabinoid receptor PET tracer. *Nucl Med Biol*. 2012;39(3):389–399. doi:10.1016/j.nucmedbio.2011.09.005.
202. Ahmad R, Postnov A, Bormans G, et al. Decreased in vivo availability of the cannabinoid type 2 receptor in Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2016;43(12):2219–2227. doi:10.1007/s00259-016-3457-7.
203. Ahmad R, Postnov A, Bormans G, Versijpt J, Vandenbulcke M, Van Laere K. Synthesis, biodistribution and in vitro evaluation of brain permeable high affinity type 2 cannabinoid receptor

agonists [(11)C]MA2 and [(18)F]MA3. *Front Neurosci*. 2016; 10:431. doi:10.3389/fnins.2016.00431.

204. Wolf Y, Yona S, Kim KW, Jung S. Microglia, seen from the CX3CR1 angle. *Front Cell Neurosci*. 2013;7:26. doi:10.3389/fncel.2013.00026.
205. Maciejewski-Lenoir D, Chen S, Feng L, Maki R, Bacon KB. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR-1-expressing microglia. *J Immunol*. 1999;163(3):1628–1635.
206. Wojdasiewicz P, Poniatowski LA, Kotela A, Deszczyński J, Kotela I, Szukiewicz D. The chemokine CX3CL1 (fractalkine) and its receptor CX3CR1: occurrence and potential role in osteoarthritis. *Arch Immunol Ther Exp (Warsz)*. 2014;62(5): 395–403. doi:10.1007/s00005-014-0275-0.
207. Luehmann HP, Detering L, Fors BP, et al. PET/CT imaging of chemokine receptors in inflammatory atherosclerosis using targeted nanoparticles. *J Nucl Med*. 2016;57(7):1124–1129. doi:10.2967/jnumed.115.166751.
208. Karlstrom S, Nordvall G, Sohn D, et al. Substituted 7-amino-5-thio-thiazolo[4,5-d]pyrimidines as potent and selective antagonists of the fractalkine receptor (CX3CR1). *J Med Chem*. 2013; 56(8):3177–3190. doi:10.1021/jm3012273.
209. Ridderstad Wollberg A, Ericsson-Dahlstrand A, Jureus A, et al. Pharmacological inhibition of the chemokine receptor CX3CR1 attenuates disease in a chronic-relapsing rat model for multiple sclerosis. *Proc Natl Acad Sci U S A*. 2014;111(14):5409–5414. doi:10.1073/pnas.1316510111.
210. Puchałowicz K, Tarnowski M, Baranowska-Bosiacka I, Chlubek D, Dziedziejko V. P2X and P2Y receptors-role in the pathophysiology of the nervous system. *Int J Mol Sci*. 2014;15(12): 23672–23704. doi:10.3390/ijms151223672.
211. Janssen B, Vugts DJ, Funke U, et al. Synthesis and initial preclinical evaluation of the P2X7 receptor antagonist [(1)(1)C]A-740003 as a novel tracer of neuroinflammation. *J Labelled Comp Radiopharm*. 2014;57(8):509–516. doi:10.1002/jlcr.3206.
212. Cotrina ML, Nedergaard M. Physiological and pathological functions of P2X7 receptor in the spinal cord. *Purinergic Signal*. 2009;5(2):223–232. doi:10.1007/s11302-009-9138-2.
213. Sanchez-Nogueiro J, Marin-Garcia P, Miras-Portugal MT. Characterization of a functional P2X(7)-like receptor in cerebellar granule neurons from P2X(7) knockout mice. *FEBS Lett*. 2005;579(17):3783–3788. doi:10.1016/j.febslet.2005.05.073.
214. Wang X, Arcuino G, Takano T, et al. P2X7 receptor inhibition improves recovery after spinal cord injury. *Nat Med*. 2004;10(8): 821–827. doi:10.1038/nm1082.
215. Wiley JS, Sluyter R, Gu BJ, Stokes L, Fuller SJ. The human P2X7 receptor and its role in innate immunity. *Tissue Antigens*. 2011;78(5):321–332. doi:10.1111/j.1399-0039.2011.01780.x.
216. Monif M, Burnstock G, Williams DA. Microglia: proliferation and activation driven by the P2X7 receptor. *Int J Biochem Cell Biol*. 2010;42(11):1753–1756. doi:10.1016/j.biocel.2010.06.021.
217. Takenouchi T, Sekiyama K, Sekigawa A, et al. P2X7 receptor signaling pathway as a therapeutic target for neurodegenerative diseases. *Arch Immunol Ther Exp (Warsz)*. 2010;58(2):91–96. doi:10.1007/s00005-010-0069-y.
218. Janssen B. *Novel PET Tracers Targeting Purinergic Receptors for in Vivo Imaging of Microglial Activation*. Amsterdam, Netherlands: Vrije Universiteit Amsterdam; 2017.
219. Rudolph DA, Alcazar J, Ameriks MK, et al. Novel methyl substituted 1-(5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)methanones are P2X7 antagonists. *Bioorg Med Chem Lett*. 2015;25(16):3157–3163. doi:10.1016/j.bmcl.2015.06.004.
220. Savall BM, Wu D, De Angelis M, et al. Synthesis, SAR, and pharmacological characterization of brain penetrant P2X7 receptor antagonists. *ACS Med Chem Lett*. 2015;6(6):671–676. doi:10.1021/acsmedchemlett.5b00089.
221. Ory D, Celen S, Gijsbers R, et al. Preclinical evaluation of a P2X7 receptor-selective radiotracer: PET studies in a rat model with local overexpression of the human P2X7 receptor and in nonhuman primates. *J Nucl Med*. 2016;57(9):1436–1441. doi:10.2967/jnumed.115.169995.
222. Gao M, Wang M, Green MA, Hutchins GD, Zheng QH. Synthesis of [(11)C]GSK1482160 as a new PET agent for targeting P2X(7) receptor. *Bioorg Med Chem Lett*. 2015;25(9): 1965–1970. doi:10.1016/j.bmcl.2015.03.021.
223. Territo PR, Meyer JA, Peters JS, et al. Characterization of (11)C-GSK1482160 for targeting the P2X7 receptor as a biomarker for neuroinflammation. *J Nucl Med*. 2017;58(3):458–465. doi:10.2967/jnumed.116.181354.
224. Han J, Liu H, Liu C, et al. Pharmacologic characterizations of a P2X7 receptor-specific radioligand, [11C]GSK1482160 for neuroinflammatory response. *Nucl Med Commun*. 2017;38: 372–382. doi:10.1097/MNM.0000000000000660.
225. Mildner A, Huang H, Radke J, Stenzel W, Priller J. P2Y12 receptor is expressed on human microglia under physiological conditions throughout development and is sensitive to neuroinflammatory diseases. *Glia*. 2017;65(2):375–387. doi:10.1002/glia.23097.
226. Moore CS, Ase AR, Kinsara A, et al. P2Y12 expression and function in alternatively activated human microglia. *Neurol Neuroimmunol Neuroinflamm*. 2015;2(2):e80. doi:10.1212/NXI.0000000000000080.
227. Sasaki Y, Hoshi M, Akazawa C, et al. Selective expression of Gi/o-coupled ATP receptor P2Y12 in microglia in rat brain. *Glia*. 2003;44(3):242–250. doi:10.1002/glia.10293.
228. Janssen B, Vugts DJ, Molenaar GT, et al. *Synthesis of the first carbon-11 labelled P2Y12 receptor antagonist for imaging the anti-inflammatory phenotype of activated microglia*. In: *18th European Symposium on Radiopharmacy and Radiopharmaceuticals*. Salzburg, Austria: EJNMMI Radiopharmacy and Chemistry; 2016;1:10.
229. Ahmed N. Advanced glycation endproducts – role in pathology of diabetic complications. *Diabetes Res Clin Pract*. 2005;67(1): 3–21. doi:10.1016/j.diabres.2004.09.004.
230. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM. Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem*. 1997;272(28):17810–17814.

231. Hofmann MA, Drury S, Fu C, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell*. 1999;97(7):889–901.
232. Huttunen HJ, Kuja-Panula J, Sorci G, Agneletti AL, Donato R, Rauvala H. Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. *J Biol Chem*. 2000;275(51):40096–40105. doi:10.1074/jbc.M006993200.
233. Wautier JL, Zoukourian C, Chappey O, et al. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J Clin Invest*. 1996;97(1):238–243. doi:10.1172/JCI118397.
234. Yan SD, Chen X, Fu J, et al. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature*. 1996;382(6593):685–691. doi:10.1038/382685a0.
235. Lue LF, Walker DG, Jacobson S, Sabbagh M. Receptor for advanced glycation end products: its role in Alzheimer's disease and other neurological diseases. *Future Neurol*. 2009;4(2):167–177. doi:10.2217/14796708.4.2.167.
236. Lue LF, Walker DG, Brachova L, et al. Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer's disease: identification of a cellular activation mechanism. *Exp Neurol*. 2001;171(1):29–45. doi:10.1006/exnr.2001.7732.
237. Jeynes B, Provias J. Evidence for altered LRP/RAGE expression in Alzheimer lesion pathogenesis. *Curr Alzheimer Res*. 2008;5(5):432–437.
238. Emanuele E, D'Angelo A, Tomaino C, et al. Circulating levels of soluble receptor for advanced glycation end products in Alzheimer disease and vascular dementia. *Arch Neurol*. 2005;62(11):1734–1736. doi:10.1001/archneur.62.11.1734.
239. Ghidoni R, Benussi L, Glionna M, et al. Decreased plasma levels of soluble receptor for advanced glycation end products in mild cognitive impairment. *J Neural Transm (Vienna)*. 2008;115(7):1047–1050. doi:10.1007/s00702-008-0069-9.
240. Cary BP, Brooks AF, Fawaz MV, et al. Synthesis and evaluation of [(18)F]RAGER: a first generation small-molecule PET radioligand targeting the receptor for advanced glycation endproducts. *ACS Chem Neurosci*. 2016;7:391–398. doi:10.1021/acschemneuro.5b00319.
241. Drake L, Brooks A, Scott P. Imaging the receptor for advanced glycation endproducts with [18F]RAGER. *J Nucl Med*. 2017;58(suppl 1):551.
242. Savickas V, Bongarzone S, Luzi F, Singh N, Gee TD. Development and evaluation of a novel positron emission tomography radiotracer for imaging the receptor for advanced glycation endproducts in Alzheimer's disease. *Alzheimer's Dementia*. 2017;13(7):P1536–P1537.
243. Kong YHF, Guan Y, et al. RAGE-specific probe 18F -FPS-ZM1 may be a promising biomarker for early detection of diabetes with Alzheimer's disease. *J Nucl Med*. 2016;57(Suppl 2):1049.
244. Bongarzone S, Savickas V, Luzi F, Gee AD. Targeting the receptor for advanced glycation endproducts (RAGE): a medicinal chemistry perspective. *J Med Chem*. 2017;60(17):7213–7232. doi:10.1021/acs.jmedchem.7b00058.
245. Lemke G, Rothlin CV. Immunobiology of the TAM receptors. *Nat Rev Immunol*. 2008;8(5):327–336. doi:10.1038/nri2303.
246. Pierce AM, Keating AK. TAM receptor tyrosine kinases: expression, disease and oncogenesis in the central nervous system. *Brain Res*. 2014;1542:206–220. doi:10.1016/j.brainres.2013.10.049.
247. Cahoy JD, Emery B, Kaushal A, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci*. 2008;28(1):264–278. doi:10.1523/JNEUROSCI.4178-07.2008.
248. Weinger JG, Omari KM, Marsden K, Raine CS, Shafit-Zagardo B. Up-regulation of soluble Axl and Mer receptor tyrosine kinases negatively correlates with Gas6 in established multiple sclerosis lesions. *Am J Pathol*. 2009;175(1):283–293. doi:10.2353/ajpath.2009.080807.
249. Ma GZ, Stankovich J, Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), Kilpatrick TJ, Binder MD, Field J. Polymorphisms in the receptor tyrosine kinase MERTK gene are associated with multiple sclerosis susceptibility. *PLoS One*. 2011;6(2):e16964. doi:10.1371/journal.pone.0016964.
250. Horti A, Dannals R, Pomper M. [18F]JHU16907 for PET imaging of MER tyrosine kinase (MERTK). *J Nucl Med*.2017;58(suppl 1):209.
251. Colonna M. TREMs in the immune system and beyond. *Nat Rev Immunol*. 2003;3(6):445–453. doi:10.1038/nri1106.
252. Owens R, Grabert K, Davies CL, et al. Divergent neuroinflammatory regulation of microglial TREM expression and involvement of NF-kappaB. *Front Cell Neurosci*. 2017;11:56. doi:10.3389/fncel.2017.00056.
253. James ML, Andreasson KI. Labeled probe and methods of use. WO2017083682 A1, Appl no PCT/US2016/061577. 2017.
254. Johnson EMA, Chaney A, et al. Development and evaluation of the first TREM1-specific PET tracer for imaging maladaptive inflammation in a mouse model of lipopolysaccharide-induced sepsis. *Mol Imaging Biol*. 2017;19(Suppl 1):5518–5758.
255. Masgrau R, Guaza C, Ransohoff RM, Galea E. Should we stop saying 'glia' and 'neuroinflammation'? *Trends Mol Med*. 2017;23(6):486–500. doi:10.1016/j.molmed.2017.04.005.
256. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res Ther*. 2014;6(4):37. doi:10.1186/alzrt269.
257. Godyn J, Jonczyk J, Panek D, Malawska B. Therapeutic strategies for Alzheimer's disease in clinical trials. *Pharmacol Rep*. 2016;68(1):127–138. doi:10.1016/j.pharep.2015.07.006.
258. Abbott A. Is 'friendly fire' in the brain provoking Alzheimer's disease? *Nature*. 2018;556(7702):426–428. doi:10.1038/d41586-018-04930-7.
259. Sheinerman KS, Umansky SR. Early detection of neurodegenerative diseases: circulating brain-enriched microRNA. *Cell Cycle*. 2013;12(1):1–2. doi:10.4161/cc.23067.
260. CHMP. *Concept Paper on No Need for Revision of the Guideline on Medicinal Products for the Treatment of Alzheimer's Disease and Other Dementias*. Committee for Medicinal Products for Human Use. London, England: European Medicines Agency.

261. Albrecht DS, Granziera C, Hooker JM, Loggia ML. In vivo imaging of human neuroinflammation. *ACS Chem Neurosci*. 2016;7(4):470–483. doi:10.1021/acschemneuro.6b00056.
262. Valera E, Masliah E. Combination therapies: the next logical step for the treatment of synucleinopathies? *Mov Disord*. 2016;31(2):225–234. doi:10.1002/mds.26428.
263. Yokokura M, Mori N, Yagi S, 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):343–351. doi:10.1007/s00259-010-1612-0.
264. Janssen B, Vugts DJ, Windhorst AD, Mach RH. PET imaging of microglial activation-beyond targeting TSPO. *Molecules*. 2018; 23(3). doi:10.3390/molecules23030607.
265. Andersson JD, Halldin C. PET radioligands targeting the brain GABAA /benzodiazepine receptor complex. *J Labelled Comp Radiopharm*. 2013;56(3-4):196–206. doi:10.1002/jlcr.3008.
266. Chalon S, Vercouillie J, Guilloteau D, Suzenet F, Routier S. PET tracers for imaging brain alpha7 nicotinic receptors: an update. *Chem Commun (Camb)*. 2015;51(80):14826–14831. doi:10.1039/c5cc04536c.
267. Kassenbrock A, Vasdev N, Liang SH. Selected PET radioligands for ion channel linked neuroreceptor imaging: focus on GABA, NMDA and nACh receptors. *Curr Top Med Chem*. 2016;16(16): 1830–1842.
268. Lin SF, Bois F, Holden D, et al. The search for a subtype-selective PET imaging agent for the GABAA receptor complex: evaluation of the radiotracer [(11)C]ADO in nonhuman primates. *Mol Imaging*. 2017;16:1536012117731258. doi:10.1177/1536012117731258.
269. Coughlin JM, Du Y, Rosenthal HB, et al. The distribution of the alpha7 nicotinic acetylcholine receptor in healthy aging: an in vivo positron emission tomography study with [(18)F]ASEM. *Neuroimage*. 2018;165:118–124. doi:10.1016/j.neuroimage.2017.10.009.
270. Coughlin JM, Slania S, Du Y, et al. (18)F-XTRA PET for enhanced imaging of the extrathalamic alpha4beta2 nicotinic acetylcholine receptor [published online ahead of print March 1, 2018]. *J Nucl Med*. 2018. doi:10.2967/jnumed.117.205492.
271. Coughlin JM, Wang Y, Ambinder EB, et al. In vivo markers of inflammatory response in recent-onset schizophrenia: a combined study using [(11)C]DPA-713 PET and analysis of CSF and plasma. *Transl Psychiatry*. 2016;6:e777. doi:10.1038/tp.2016.40.