



**HAL**  
open science

## **PET imaging of neuroinflammation: any credible alternatives to TSPO yet?**

Fabien Chauveau, Alexandra Winkeler, Sylvie Chalon, Hervé Boutin, Guillaume Becker

### **► To cite this version:**

Fabien Chauveau, Alexandra Winkeler, Sylvie Chalon, Hervé Boutin, Guillaume Becker. PET imaging of neuroinflammation: any credible alternatives to TSPO yet?. *Molecular Psychiatry*, 2024, <10.1038/s41380-024-02656-9>. <inserm-04648432>

**HAL Id: inserm-04648432**

**<https://inserm.hal.science/inserm-04648432v1>**

Submitted on 15 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

# PET imaging of neuroinflammation: Any credible alternatives to TSPO yet?

Fabien Chauveau<sup>1</sup>, Alexandra Winkeler<sup>2</sup>, Sylvie Chalon<sup>3</sup>, Hervé Boutin<sup>3\*</sup>, Guillaume Becker<sup>1#</sup>

<sup>1</sup>Université Claude Bernard Lyon 1, Centre de Recherche en Neurosciences de Lyon, Inserm U1028, CNRS UMR5292, BIORAN, Groupement Hospitalier Est – CERMEP, 59 boulevard Pinel, 69677 Bron Cedex, FRANCE

<sup>2</sup>Université Paris-Saclay, Inserm, CNRS, CEA, BioMaps, Service Hospitalier Frédéric Joliot, 4 place du général Leclerc, 91401 Orsay, FRANCE

<sup>3</sup>UMR 1253 iBrain, Université de Tours - INSERM, Bâtiment Planiol, UFR de Médecine, 10 Boulevard Tonnellé, 37032 Tours Cedex 01, FRANCE

\*Corresponding author: UMR 1253 iBrain, Université de Tours - INSERM, Bâtiment Planiol, UFR de Médecine, 10 Boulevard Tonnellé, 37032 Tours Cedex 01, France. ([herve.boutin@inserm.fr](mailto:herve.boutin@inserm.fr))

# Present address: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex

## Abstract

Over the last decades, the role of neuroinflammation in neuropsychiatric conditions has attracted an exponentially growing interest. A key driver for this trend was the ability to image brain inflammation *in vivo* using PET radioligands targeting the Translocator Protein 18 kDa (TSPO), which is known to be expressed in activated microglia and astrocytes upon inflammatory events as well as constitutively in endothelial cells. TSPO is a mitochondrial protein that is expressed mostly by microglial cells upon activation but is also expressed by astrocytes in some conditions and constitutively by endothelial cells. Therefore, our current understanding of neuroinflammation dynamics is hampered by the lack of alternative targets available for PET imaging. We performed a systematic search and review on radiotracers developed for neuroinflammation PET imaging apart from TSPO. The following targets of interest were identified through literature screening (including previous narrative reviews): P2Y<sub>12</sub>R, P2X<sub>7</sub>R, CSF1R, COX (microglial targets), MAO-B, I2BS (astrocytic targets), CB<sub>2</sub>R & S1PRs (not specific of a single cell type). We determined the level of development and provided a scoping review for each target. Strikingly, astrocytic biomarker MAO-B has progressed in clinical investigations the furthest, while few radiotracers (notably targeting S1PRs, CSF1R) are being implemented in clinical investigations. Other targets such as CB<sub>2</sub>R and P2X<sub>7</sub>R have proven disappointing in clinical studies (e.g. poor signal, lack of changes in disease conditions, etc.). While astrocytic targets are promising, development of new biomarkers and tracers specific for microglial activation has proven challenging.

**Keywords:** biomarkers, neuroinflammation, positron emission tomography

## General introduction

Over the last 3 decades, the role of neuroinflammation in neurodegenerative and neuropsychiatric conditions has attracted an exponentially growing interest as illustrated by the number of publications found on PubMed with “neuroinflammation AND (neurodeg\* OR neuropsych\*)” returning 270 publications over the first 48 years (1945-1993), 645 over the next 9 years (1994-2003) and 17604 between 2004 and 2024. For a long time, the brain was thought to be isolated from the immune system, notably because of the blood-brain-barrier. However, the concept of neuroinflammation dates as far back as 1847, with Jones<sup>1</sup> suggesting the concomitance of “inflammation of the brain” and *delirium tremens*, and later, demonstration in the early 20<sup>th</sup> century of macrophage-like or phagocytic cells in the brain by Nissl (1889), Robertson (1900) and Del Rio Ortega in the 1920-30 (for review see<sup>2</sup>). Thereafter, it seems that neuroinflammation (NI) did not attract much attention for several decades until new studies showed that inflammatory stimulus and acute injuries triggered the activation of glial cells and production of cytokines in the late 1990 to early 2000 (for review see<sup>3</sup>). Consequently, there was also a renewed interest in studying NI and its roles in health and diseases as it encompasses several processes with therapeutic potential. From this, the need to be able to investigate NI *in vivo* in human subjects emerged. The discovery in the 1980 of the peripheral benzodiazepine receptor (PBR)<sup>4, 5</sup>, later renamed translocator protein 18kDa<sup>6</sup>, that is expressed in activated microglia and astrocytes, and of the associated ligand PK11195 that could be radiolabelled with [<sup>11</sup>C] led to a long series of positron emission tomography (PET) investigations in a broad range of neurological conditions<sup>7-11</sup>. However, the main limitation of this radiotracer is the relatively high non-specific binding leading to a low sensitivity, leading to inconclusive findings in some clinical conditions<sup>11</sup>. Consequently, second generation TSPO radioligands were developed to overcome this limitation<sup>9</sup>; however one must note that findings from [<sup>11</sup>C]PK11195 PET studies have never been contradicted by more recent ones performed with new TSPO tracers. Moreover, with one of those radiotracers, [<sup>11</sup>C]PBR28, a single nucleotide polymorphism rs6971 of the TSPO gene was discovered<sup>12</sup>, and while this issue affects more or less severely all 2<sup>nd</sup> generation TSPO radiotracers<sup>13</sup>, it does not affect the binding of [<sup>11</sup>C]PK11195. Since then 3<sup>rd</sup> and 4<sup>th</sup> generation radiotracers that are also not affected by this polymorphism have been developed<sup>14, 15</sup> (for review see<sup>11</sup>). Although numerous studies provided solid evidence that TSPO is up regulated in several conditions, the sole use of TSPO as biomarker for NI does not allow the full characterisation of the various processes involved and regulating neuroinflammatory processes. Indeed, while the over-expression of TSPO in pro-inflammatory microglia seems well-established, it remains uncertain whether there is a comparable over-expression of TSPO in the anti-inflammatory phenotype of microglial cells. Moreover, various

studies have shown that there is a constitutive expression of TSPO in endothelial cells and that astrocytes can also express TSPO<sup>16-19</sup>.

TSPO PET studies in neuropsychiatric conditions have mostly focused on schizophrenia and depression. Most of the studies in depression are in agreement in consistently showing increased TSPO levels<sup>20-22</sup> (for review see<sup>23</sup>). In schizophrenia, the results are not as clear, whereas the first PET studies showed increases in TSPO<sup>24-26</sup> or positive correlation between symptoms and TSPO levels<sup>27</sup>, later studies showed no difference between patients and controls<sup>28-33</sup> or even significant decreases in schizophrenic patients<sup>34</sup>. Altogether, most of the recent studies with new TSPO tracers are supporting no changes or a decrease in TSPO tracer uptake rather than an increase (for review see<sup>11, 23, 35, 36</sup>); these studies warrant further investigations in schizophrenic patients, especially using new biomarkers and tracers for NI and taking into account other factors such as peripheral inflammation<sup>37</sup> or medication status<sup>29, 33</sup>.

Overall, the above studies indicate that there is a need for biomarkers and associated radiotracers that are more specific of cell types and neuroinflammatory phenotypes than TSPO. Numerous targets have been considered for this aim and research is ongoing to develop the tools that will unravel more precisely the roles of NI in neurological and neuropsychiatric conditions. In this review, we aim to provide an update on the current radiotracer development for targets specific for microglia (P2Y12, P2X7, CSF1R and COX) or astroglial (MAO-B and I2BS) and those unspecific of a particular cell type (S1PRs and CB2).

## Methods

The targets of interest were identified through literature screening (including previous narrative reviews<sup>38, 39</sup>). Although some other biomarkers could have been included, we felt that they did not specifically target NI associated with neurodegenerative or neuropsychiatric diseases. Namely, aquaporin-4 (AQ-4), which can be imaged with radiotracer [<sup>11</sup>C]TGN-020<sup>40</sup>, C-X-C chemokine receptor type 4 (CXCR4), imaged with [<sup>68</sup>Ga]<sup>41, 42</sup> and Tissue transglutaminase (TG2)<sup>43, 44</sup>, are all exclusively used in cancer imaging, including brain tumours. Numerous radiotracers have also been employed to image adenosine receptors 1 (A1)<sup>45</sup> and 2 (A2)<sup>46</sup>, but the *in vivo* binding is mostly interpreted as originating from neurons rather than glial cells, with the exception however of one preclinical study using [<sup>18</sup>F]CPFPX to image A1 receptors after stroke<sup>47</sup> and one clinical study using [<sup>11</sup>C]TMSX to image A2 receptors in multiple sclerosis<sup>48</sup>.

The basic search was done in PubMed with the following search string: (brain OR cereb\* OR neuro\*) AND (inflammation OR neuroinflammation) AND (microglia\* OR astrocyte OR astroglial) AND (PET OR positron OR SPECT) AND <target>. In some cases, the search was refined to exclude

irrelevant references, such as the one unrelated to imaging or the specified target. Although preprints are now listed on PubMed, we have decided to not cite preprints in this review as they have not gone through peer review.

## Part 1: Microglia-specific biomarkers

Microglial activation encompasses different phenotypes (resting, anti- and pro-inflammatory) which can dynamically evolve depending on the pathology and environmental stress. TSPO has been useful in demonstrating the implications of microglia in pathological conditions, but it does not provide refined information about those different phenotypes. Moreover, as mentioned earlier, TSPO is also expressed by other cell types in the brain. Hence, many other biomarkers have been envisaged and identified that would provide microglial specificity as well as, for some, phenotypic specificity. These and their associated radiotracers are described below. The respective advances in term of development from radiochemistry to preclinical and clinical use for each microglial target are shown Figure 1 and for each tracer per target in Figures S1 to S4. All the results shown in Figures 1 to 3 are also available as an interactive Figure here: <https://infogram.com/radar-spider-1h984wv3llqnd2p>).

### 1.1 Purinergic receptor type-2X7 (P2X7R)

P2X7 receptors (P2X7R) are ATP-gated cation channels whose crystal structure has recently been resolved<sup>49, 50</sup>. Although they are expressed by different cell types, in particular the peripheral immune cells<sup>51, 52</sup> and glial cells, i.e. oligodendrocytes and astrocytes<sup>53, 54</sup>, they are mainly expressed by microglia<sup>55</sup>, while their expression by neurons is still controversial<sup>56</sup>. P2X7R are also expressed by tumour cells<sup>57</sup>.

The involvement of P2X7R in several CNS disorders was initially highlighted by Burnstock<sup>58</sup>. It was proposed that ATP released by injured neurons induce the microglial activation through P2X7R stimulation<sup>59</sup>. These receptors appear therefore as key actors in a number of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) (see for reviews<sup>60, 61</sup>), and also in mood disorders (see for review<sup>62</sup>).

Regarding their involvement in a wide panel of pathophysiological processes, the exploration of P2X7R by PET imaging would be of great importance, and first attempts to develop suitable tracers for this aim occurred almost a decade ago, with different [<sup>11</sup>C]-labelled antagonists, A-740003<sup>63</sup> and GSK1482160<sup>64</sup>. Since then, new radioligands from different chemical families labelled either with [<sup>11</sup>C] or [<sup>18</sup>F] have been developed (Figure S1), and their main characteristics have recently been reviewed<sup>65</sup>.

These ligands belong mainly to four different chemical families. Some of them have not yet been studied in humans, such as [<sup>11</sup>C]GSK1482160 and its fluorinated derivative [<sup>18</sup>F]4A<sup>66, 67</sup>. Other tracers seemed promising at the stage of preclinical evaluation in animal models but gave rather

disappointing results in clinical applications. This was the case with [<sup>11</sup>C]SMW139 in relapsing remitting multiple sclerosis<sup>68</sup>. Several [<sup>11</sup>C]-labelled and [<sup>18</sup>F]-labelled JNJ derivatives, although having better affinity and suitable properties for human use<sup>69,70</sup>, were not able to discriminate healthy controls from patients, as assessed for [<sup>11</sup>C]JNJ-54173717 in PD<sup>71</sup>. The fluorinated derivative [<sup>18</sup>F]JNJ-64413739 with promising properties *in vivo* in healthy humans<sup>72,73</sup> has not yet been used in pathological conditions, but recent findings obtained with its tritiated analogue on human brain sections did not show any correlation between the tracer binding and the duration of epilepsy in treatment-resistant patients<sup>74</sup>.

To date, the development of PET tracers suitable for *in vivo* P2X7R imaging needs further investigations among them a potential effect of the polymorphism of the gene encoding the receptor on the binding in physiological and pathological conditions. The evaluation of receptor occupancy using PET tracers could also be investigated. Although it remains relevant to search for alternative or complementary targets to TSPO as a biomarker of NI<sup>75</sup>, it seems that, at least in several pathological conditions, the exploration of P2X7R offers little added value when compared to TSPO<sup>74,76</sup>.

## 1.2 Purinergic receptor type-2Y12 (P2Y12R)

A few years ago, work by Butovsky *et al.* and Keren-Shaul *et al.* resulted in the definition of unique homeostatic and disease-associated microglia signatures in adult mice<sup>77,78</sup>. Besides others, expression of the purinergic G protein-coupled P2Y12 receptor was shown to be specific for homeostatic microglia as compared to peripheral myeloid cells or further CNS cells like astrocytes, neurons and oligodendrocytes<sup>77</sup>. Furthermore, *P2ry12* gene was confirmed to be expressed by human microglia<sup>79-82</sup>. Such disease-associated signatures have been identified in models of AD, amyotrophic lateral sclerosis and MS and have been termed microglial neurodegenerative phenotype (MGnD)<sup>83</sup> or disease-associated microglia (DAM)<sup>78</sup>.

Increasing evidence indicates that P2Y12R expression changes according to disease development, such that down-regulation of the *P2ry12* gene was found in the MGnD signature. Interestingly, in a mouse with experimental autoimmune encephalomyelitis (EAE), immunofluorescence data showed that P2Y12R expression was lost at the onset and on the peak of the disease whereas it re-appeared in the recovery phase<sup>83</sup>. Other preclinical studies utilizing models of MS also demonstrated changes in P2Y12R expression depending on the disease state<sup>84,85</sup>. In addition to models of MS, an Alzheimer's tauopathy mouse model study showed that *P2ry12* was repressed before accumulation of intraneuronal tau deposits. The reduction in P2Y12R in association with tau pathologies was also observed in human AD entorhinal cortices<sup>86</sup>.

This accumulating evidence suggests that the P2Y12R is an interesting and promising target for PET imaging to evaluate microglial homeostasis vs a disease-associated microglial phenotype, but

one should keep in mind that it would act as a biomarker being down-regulated in the early phase of ND events and up-regulated during regenerative processes.

The P2Y<sub>12</sub> receptor belongs to the P2Y family of G protein-coupled receptors including eight subtypes: P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>, P2Y<sub>13</sub>, and P2Y<sub>14</sub>, which are activated by extracellular nucleotides. They are further subdivided into P2Y<sub>1</sub>-like, G<sub>q</sub> protein-coupled receptors (P2Y<sub>1,2,4,6,11</sub>) and P2Y<sub>12</sub>-like receptors (P2Y<sub>12,13,14</sub>) which are coupled to G<sub>i</sub> protein, and thus leading to reduced intracellular cAMP production upon activation. Besides its expression on microglia, P2Y<sub>12</sub> receptor is highly expressed on platelets and is activated by adenosine diphosphate (ADP). Due to its important role in thrombosis, potent and selective synthetic agonists and antagonists for P2Y<sub>12</sub> receptor have been identified<sup>51, 87</sup>.

The group of Janssen *et al.* (2015) published one of the first reports on radioligands for the P2Y<sub>12</sub> receptor, describing the synthesis of a carbon-11 labelled P2Y<sub>12</sub> receptor antagonist<sup>88</sup>. Later the same group used this compound for autoradiographic studies *i*) in a rat EAE model<sup>84</sup>, *ii*) in a mouse model of middle cerebral artery occlusion (MCAO), *iii*) in rat and mouse models of intracranially injected recombinant interleukin-4 (rIL4) and *iv*) on human stroke and control tissue<sup>89</sup>. Their results confirmed specific binding of the [<sup>11</sup>C]-P2Y<sub>12</sub>R antagonist in mouse brain sections, with less binding in the diseased (MCAO) compared to the control tissue, but a 1.4-fold increased binding in rIL4-injected vs vehicle-injected mouse brains<sup>89</sup>. The work by Villa *et al.*<sup>89</sup> is of further interest as the authors were specifically looking for molecular PET targets of the microglial anti-inflammatory activation state. However, the investigated radioligand did not cross the blood-brain-barrier (BBB). Recently, radiosynthesis of the P2Y<sub>12</sub>R antagonist [<sup>11</sup>C]AZD1283 has been described by two groups<sup>86, 90</sup>. Although Maeda and colleagues could show disease-related changes of P2Y<sub>12</sub>R expression in mouse models of Alzheimer's and tau pathologies using [<sup>11</sup>C]AZD1283 autoradiography, no significant brain uptake was found *in vivo*<sup>86</sup>. The same is true for Jackson and colleagues who investigated metabolism and *in vivo* uptake of [<sup>11</sup>C]AZD1283 in mice and non-human primates (NHPs)<sup>90</sup>. While results indicated good metabolic stability of the compound, early and high liver uptake as well as negligible brain uptake in both species question the use of this radioligand for *in vivo* imaging of the CNS. Likewise, two high-affinity thienopyrimidine-based P2Y<sub>12</sub>R antagonists, which have been radiolabelled and evaluated by *in vivo* imaging, *in vitro* autoradiography and metabolite analysis demonstrated only negligible brain uptake in rats, whereas blocking of efflux transporters with tariquidar led to a strong increase in brain SUVs<sup>91</sup>. Except in the case of improved characteristics for humans, the fact that these molecules are substrates for efflux transporters is likely to affect their further use. In summary, although the P2Y<sub>12</sub>R is an interesting target for PET imaging to assess microglial homeostasis, radioligands developed so far (Figure S2) did not show suitable properties for *in vivo* brain imaging.

### 1.3 Colony-Stimulating Factor-1 Receptor (CSF1R)

The Colony-Stimulating Factor-1 Receptor (CSF1R) is a receptor tyrosine kinase that has two known endogenous ligands, the colony-stimulating factor-1 and interleukin-34, which regulate the proliferation, differentiation, chemotaxis, migration, activation/polarisation and survival of macrophages in the periphery and of microglia in the brain. As such, CSF1R is a crucial regulator of inflammation and NI<sup>92</sup>. These functions, the specific microglial expression of CSF1R in the brain as well as its up regulation in activated microglia in many neurological diseases<sup>93</sup> made CSF1R a potential biomarker and hence an attractive imaging target of NI (Figure S3).

The first publication<sup>94</sup> to report the radiolabelling of a CSF1R inhibitor investigated fluorinated derivatives of the CSF1R inhibitor GW2580 and showed that one of the compounds (#10 in this publication<sup>94</sup>, later renamed [<sup>18</sup>F]FOMPyD<sup>95</sup>) retained the specificity and selectivity for CSF1R. However, this radiotracer suffered from cumbersome radiochemistry conditions, and inconclusive blocking studies<sup>95</sup>. In the meantime, another compound, [<sup>11</sup>C]AZ683 ([<sup>11</sup>C]-radiolabelled AZD6495 referred to in<sup>96</sup>), had been synthesised and tested in rats and primate, but showed a low brain uptake<sup>97</sup>. Soon after, Horti *et al.*<sup>98</sup> investigated the new CSF1R PET tracer, [<sup>11</sup>C]CPPC<sup>99</sup>, in several mouse NI models (LPS, EAE, AD) and in primates, as well as in post-mortem human brain tissue. This publication showed increased [<sup>11</sup>C]CPPC uptake - with partial blocking achieved *in vivo* using cold CPPC or other blocking CSF1R ligands - in all models of NI and in AD post-mortem brain tissue. Subsequently, using CSF1R KO mice or mice with depleted microglia, the authors showed little to no difference in [<sup>11</sup>C]CPPC uptake, which they attributed to a low level of expression of CSF1R in basal condition vs high non-specific binding. Finally, they examined [<sup>11</sup>C]CPPC uptake in LPS-treated baboons and showed a doubled volume of distribution of the tracer in various brain regions ( $V_T$ ) after the inflammatory challenge. Based on these observations, they concluded that [<sup>11</sup>C]CPPC was a suitable CSF1R radiotracer but that plasma analysis and full kinetic modelling with arterial input function was required to accurately measure CSF1R density *in vivo* with this tracer<sup>98</sup>. Following this characterisation study, the same group performed the first-in-man PET study<sup>100</sup>, although without blocking experiments. However, the results of Horti *et al.*<sup>98</sup> must be examined in the context of another study by Knight *et al.*<sup>101</sup> who investigated further the binding and specificity of [<sup>3</sup>H]CPPC for CSF1R *in vitro*. Worryingly, Knight *et al.*<sup>101</sup> demonstrated by autoradiography of brain sections after central administration of LPS or in a model of glioblastoma in rat that [<sup>3</sup>H]CPPC binding was low and did not colocalise with immunostaining for CSF1R, microglia Iba1 or [<sup>3</sup>H]PBR28 (TSPO) binding. Similarly, saturation experiments showed that [<sup>3</sup>H]CPPC binding did not saturate and was hardly displaceable suggesting that most of the binding is in fact off-target and/or non-specific binding, with a lack of increase in the ipsilateral (LPS) vs the contralateral side, whereas the TSPO tracer [<sup>3</sup>H]PBR28 demonstrated a

significant increase in displaceable and saturable binding in the ipsilateral side. Knight *et al.*<sup>101</sup> also questioned the specificity of [<sup>3</sup>H]CPPC for CSF1R using competition experiments with unlabelled CPPC and specific CSF1R inhibitors (PLX3397, PLX5622, and BLZ945), which all showed limited displacement, 30 days after LPS challenge, in brain but also in spleen. Finally, they found off-target binding with IC<sub>50</sub> in the nanomolar range for insulin receptor (70 nM), tyrosine protein kinase LCK (22 nM), and vascular endothelial growth factor receptor 2 (74 nM)<sup>101</sup>. Thus, CPPC might not be such a suitable CSF1R radiotracer.

Despite these observations, Rubin *et al.*<sup>102</sup> recently used [<sup>11</sup>C]CPPC in a clinical study in virally suppressed HIV (VS-PWH) patients (n=16) vs controls (n=15), but reported no difference. This suggested a poor sensitivity, which contrasts with multiple TSPO studies which invariably reported increases in TSPO binding in HIV patients or primates carrying the simian immunodeficiency virus<sup>103-108</sup>, except for one study in VS-PWH<sup>109</sup>.

Zhou *et al.*<sup>110</sup> developed the <sup>11</sup>C-radiolabelling of the CSF1R reference inhibitor GW2580 and compared it directly with [<sup>11</sup>C]CPPC in a model of acute inflammation induced by intra-striatal LPS injection and in a model of AD in mice. In line with the results of Knight *et al.*<sup>101</sup>, they found that [<sup>11</sup>C]GW2580 was a slightly better tracer than [<sup>11</sup>C]CPPC in terms of displaceable (specific) binding and consequently signal-to-noise ratio. More recently, Altomonte *et al.*<sup>111</sup> developed fluorinated analogues of [<sup>11</sup>C]CPPC, identifying Psa374 as the best candidate. As Psa374 can also be labelled with <sup>11</sup>C, they used [<sup>11</sup>C]Psa374 for PET imaging in naïve mice and rhesus monkeys. Interestingly, they showed a modest but significant affinity for mouse and monkey Pgp transporters which may preclude the use of [<sup>11</sup>C]CPPC and [<sup>11</sup>C]- or [<sup>18</sup>F]-Psa374 in PET imaging. Altogether, this evaluation of Psa374 highlighted significant issues with this tracer, notably the unexplained higher uptake in blocking conditions, the presence in significant proportion of radiometabolites in plasma and its low but significant affinity for Pgp transporters. These findings highlight the need for further investigations using Psa374 notably in preclinical models of neuroinflammation.

Van der Wildt *et al.*<sup>96, 112, 113</sup> also performed the radiolabelling of identified or chemically derived CSF1R inhibitors. Unfortunately, BLZ945<sup>96, 114</sup> was shown to be a Pgp substrate and displayed low selectivity towards CSF1R vs other kinases, while compound #5<sup>112, 115</sup> displayed low brain uptake, and hybrid molecules such as compound #4<sup>113</sup> had only a modest reduction of tracer uptake in blocking experiments. Recently, other groups have investigated new derivatives of existing CSF1R compounds such as a fluoro-ethyl derivative of CPPC (compound #1 in Lee *et al.*<sup>116</sup>) and fluorinated derivative (compound #4 in An *et al.*<sup>117</sup>) of a recently described CSF1R inhibitor<sup>118</sup>. Interestingly, compound #1 developed by Lee *et al.*<sup>116</sup> exhibited lower brain uptake than most other CSF1R candidates in control mice but tracer uptake doubled after systemic administration of LPS in mice.

Again, blocking experiments showed a significant but modest blocking, but it must be noted that the blocking agent was CPPC which, as mentioned above, may have high non-specific and some off-target binding. Clearly, this new compound #1 deserve further investigation and a full characterisation in other models and in NHPs. Conversely, the compounds developed by An *et al.*<sup>117</sup>, including their best candidate, compound #4, do not seem suitable PET tracers as the authors established that they were Pgp substrates.

Although [<sup>11</sup>C]CPPC is certainly the most tested radiotracer for CSF1R and has now reached clinical investigation, it is important to note that most [<sup>11</sup>C]CPPC studies have reported worryingly high level of non-specific binding and that some studies are even questioning its selectivity towards CSF1R vs other targets; altogether it is important that these points must be clarified before findings obtained with [<sup>11</sup>C]CPPC can be fully trusted.

#### 1.4 Cyclooxygenase

Cyclooxygenase (COX) enzymes are involved in the biosynthesis of prostaglandins from arachidonic acid. It is now admitted that the inducible isoform COX-2 but also the constitutive form COX-1 can be up-regulated in the brain consecutively to inflammation<sup>119</sup>. PET imaging of these enzymes emerged therefore as potential biomarkers of NI (Figure S4-A). As soon as 20 years ago, two fluor-18-labeled compounds derived from known COX-2 inhibitors belonging to the nonsteroidal anti-inflammatory drug (NSAID) category, celecoxib and rofecoxib, were developed for this aim, i.e., [<sup>18</sup>F]SC58125 for COX-2 and [<sup>18</sup>F]SC63217 for COX-1. However, although *in vitro* uptake of [<sup>18</sup>F]SC58125 corresponding to COX-2 expression by LPS+IFN $\gamma$ -stimulated macrophages was promising, both tracers had overall low and non-specific *in vivo* brain uptake in rat and NHP<sup>120</sup>.

Afterwards, several attempts to develop COX-2 PET tracers (Figure S4B) through direct labelling of these inhibitors also gave disappointing results such as a very high defluorination of [<sup>18</sup>F]celecoxib in rat<sup>121</sup>, and a non-specific binding of [<sup>11</sup>C]celecoxib in mouse brain<sup>122</sup>. Better results (heterogeneous uptake) were obtained in NHP, but requiring high injected dose rather incompatible with human use<sup>123</sup>. The evaluation of [<sup>11</sup>C]rofecoxib was also disappointing. Although an heterogeneous uptake, partially blocked by specific inhibitor, could be obtained in the rat brain, no modification was detected in 2 models of severe induced inflammation<sup>124</sup> or in a model of brain ischemia<sup>122</sup> in mice. The radiolabelling with fluor-18 of another COX-2 inhibitor, valdecoxib<sup>125</sup>, or its methoxy analogue [<sup>11</sup>C]MOV<sup>126</sup> resulted also in unsuitable probes because of rapid defluorination and/or insufficient brain-specific uptake. Similar unsatisfactory results were obtained with other tracer derivatives from NSAIDs such as indomethacin<sup>127</sup> and more recently Nimesulide<sup>128</sup>.

Besides attempts to develop NSAID derivative tracers, other chemical families were considered such as azaindazole<sup>129</sup> and pyrimidine from which the [<sup>11</sup>C]MC1 (<sup>11</sup>C-6-methoxy-2-(4-

(methylsulfonyl)phenyl)-N-(thiophen-2-ylmethyl)pyrimidin-4-amine) had a high sub-nanomolar affinity and selectivity for COX-2 (<100-fold towards COX-1)<sup>130</sup>. However, despite these promising properties, [<sup>11</sup>C]MC1 showed low specific to non-displaceable binding in monkey brain<sup>131</sup>. This tracer could however be eventually useful to evaluate COX-2 inhibitors occupancy and/or in peripheral disorders such as rheumatoid arthritis<sup>132</sup>. Another tracer with high COX-2 affinity and selectivity, [<sup>11</sup>C]TMI (<sup>11</sup>C-3-(4-methylsulfonylphenyl)-4-phenyl-5-trifluoromethyl isoxazole) seemed also promising in NHPs<sup>133</sup> but no human evaluation has been published to date. Thus, it appears that promising PET results with various COX-2 PET tracers<sup>134</sup> call for further investigations in neuroinflammatory conditions (Figure S4).

The current status on PET imaging tracers for COX-1 seems more advanced than for COX-2. As with COX-2 ligands, NSAIDs were at first the structural starting point to develop selective PET tracers. The [<sup>11</sup>C]ketoprofen methyl ester appeared promising, with a high *in vivo* selectivity for COX-1 vs COX-2 in mouse brain confirmed using KO animals<sup>135</sup>. The brain uptake of this tracer was clearly increased after acute NI induced by LPS or quinolinic acid in mouse models<sup>135</sup> and also in a transgenic mouse model of Alzheimer's disease<sup>136</sup>. In addition, an interesting complementarity between COX-1 and TSPO PET imaging was revealed in the LPS mouse model, demonstrating earlier uptake for [<sup>11</sup>C]ketoprofen than for [<sup>11</sup>C]PK-11195, which could correspond to the labelling of different cell populations or phenotypes. The same research group obtained similar promising results with the fluorinated analogue [<sup>18</sup>F]FKTP-Me<sup>137</sup> but no data in NHP or human are yet available.

Pike *et al.* developed the highly promising COX-1 PET tracer [<sup>11</sup>C]PS13 (11C-1,5-bis(4-methoxyphenyl)-3-(2,2,2-trifluoroethoxy)-1H-1,2,4-triazole), using a triazole heterocyclic scaffold, on the basis of its nanomolar affinity and selectivity (<1000-fold towards COX-2). [<sup>11</sup>C]PS13 showed a significant uptake in NHP brain which was blocked by COX-1 inhibitors<sup>131, 138, 139</sup>. These promising properties were then confirmed in healthy humans, in terms of stability, target quantification<sup>140</sup>, and even the ability to measure the NSAIDs site occupancy *in vivo*<sup>141</sup>. The possibility to use the fluorinated analogue [<sup>11</sup>C]PS13 is being investigated by the same research group<sup>142</sup>; both the carbon-11 and fluorine-18 labelled analogues of PS13 will require further investigation in neuroinflammatory conditions.

## 1.5 Conclusion on microglia-specific biomarkers

Outside proving that they are truly cell specific, and if possible selective of a pro- or anti-inflammatory phenotype, the main difficulty for new microglial biomarkers and radiotracers is to break through the current *status quo* of the well-established TSPO radiotracers used to image NI in clinical PET. As a result, and although full characterisation is needed to ascertain cell or target specificity, and despite promising results preclinically for some of those biomarkers/radiotracers, very few have yet reached clinical use (Figure 1, also available as an interactive [figure](#):

<https://infogram.com/radar-spider-1h984wv3llqnd2p>). Such example can be found with CSF1R tracers and the facts that some CSF1R radiotracer candidates are Pgp substrates or have suspected off-target binding have certainly limited the development of new tracers. For all the microglial targets, the current status of the research exemplifies that further investigations are needed to *i)* fully ascertain the selectivity of the tracers, *ii)* confirm that they exclusively reflect microglial activation (and/or macrophages infiltration) in a range of disease models and *iii)* characterise the phenotype of the activated microglia over-expressing these biomarkers in these models or clinical conditions.

## Part 2: Astrocyte-specific biomarkers

Astrocytes have been recognized for several decades now as having a critical role in CNS pathophysiological processes including psychiatric diseases. While the number of molecular markers continuously increased over time, including markers for the cytoskeleton (the most used is glial fibrillary acidic protein, GFAP), metabolism or transcription factors, we do not have as many potential radiolabelled probes for *in vivo* imaging. However, the available astrocyte-specific PET radiotracers have provided major knowledge, notably in Alzheimer's disease induced neurodegeneration and reactive astrogliosis dynamics. The nuclear imaging community worked on new PET radiopharmaceuticals directed towards other astrocytic targets that are now available for clinical studies (Figure 2). Adequate nomenclature for accurate phenotypic and genotypic description of astrocytes is available to help in-depth analysis of *in vivo* imaging thanks to translational *in vitro* comparison<sup>143</sup>.

### 2.1 Monoamine oxidase B (MAO-B)

Monoamine oxidase (MAO) enzymes catalyse the oxidative deamination of monoamines at the outer membrane of mitochondria. Two isoforms, MAO-A and MAO-B are present in the human brain. The midbrain serotonergic neurons of the raphe nucleus and astrocytes contain predominantly MAO-B, whereas catecholaminergic neurons contain mainly MAO-A<sup>144</sup>. The relatively specific astrocytic expression of MAO-B (apart from central serotonergic neurons) and the observed enhanced MAO-B activity in reactive astrocytes led to the use of MAO-B activity as proxy of brain inflammation, and particularly astrogliosis. Therefore, radiolabelled MAO-B inhibitors may serve as imaging biomarkers of astrogliosis. Historical collaboration between research groups at the Brookhaven National Laboratory, New York, USA, and at the University of Uppsala, Sweden have provided the first MAO-B PET radiotracer described in their seminal paper on the L-deprenyl MAO-B inhibitor radiolabelled with <sup>11</sup>C<sup>145</sup>. Despite the high specificity of [<sup>11</sup>C]L-deprenyl for the MAO-B isoform, the reference L-deprenyl molecule is a suicide inactivator which involves the irreversible covalent binding of the radiotracer to the flavin cofactor of MAO-B<sup>146</sup>. This feature makes [<sup>11</sup>C]-L-

deprenyl a suboptimal PET tracer regarding pharmacokinetic and modelling. Hence, these groups developed a deuterium-substituted L-deprenyl ( $[^{11}\text{C}]$ L-deprenyl-D2) to reduce the rate of radiotracer trapping in the human brain and to improve sensitivity<sup>147</sup>.  $[^{11}\text{C}]$ L-deprenyl-D2 rapidly became the reference PET radiotracer being used to study astrogliosis in different pathologies such as epilepsy<sup>148-150</sup>, traumatic brain injury<sup>151</sup> or amyotrophic lateral sclerosis<sup>152-155</sup>. In humans, age is a factor contributing to the variability among subjects given the established increased brain expression of MAO-B in normal aging<sup>146, 156, 157</sup>. In animals, anaesthesia should be carefully chosen considering the inhibitory effect of sevoflurane on radioligand binding to MAO-B<sup>158</sup>.

*In vivo* PET imaging of astrogliosis has been studied mainly in the context of pathophysiological processes of Alzheimer's disease (AD), notably thanks to the work done at the Karolinska Institute<sup>159-161</sup>. An excellent review on this specific point has recently been published<sup>162</sup>. The complex relationship between fibrillar A $\beta$  deposition and astrogliosis was usually investigated thanks to the comparison of regional binding patterns of  $[^{11}\text{C}]$ PIB and  $[^{11}\text{C}]$ L-deprenyl-D2 respectively in cohorts of different AD stage patients. The increased MAO-B activity appears as an early event in AD, preceding A $\beta$  deposition, with main areas of increased  $[^{11}\text{C}]$ L-deprenyl-D2 binding being in the frontal, parietal, temporal and medial temporal lobes<sup>163-166</sup>. Complementary insight on the complex relationship between pathological hallmarks of AD and astrogliosis arose from studies in autosomal dominant Alzheimer's disease (ADAD), considered to some extent as a model for predicting the time course of neuropathological changes in the much more common and complex sporadic Alzheimer's disease<sup>167, 168</sup>. These studies confirmed an early increased  $[^{11}\text{C}]$ L-deprenyl-D2 binding (presymptomatic mutation carriers) followed by patterns of steady  $[^{11}\text{C}]$ L-deprenyl-D2 decrease concomitant with increasing  $[^{11}\text{C}]$ PiB retention. These results are in line with an increased MAO-B activity in the presence of soluble amyloid beta forms. The secondary  $[^{11}\text{C}]$ L-deprenyl-D2 binding decline has been shown to correspond to a decreasing glucose metabolism<sup>169</sup>. The decreasing tendency of MAO-B activity in the symptomatic stage of ADAD was interpreted as a sign of astrocyte cell loss or loss of function<sup>170</sup>. Nevertheless, further investigations are warranted given the differences in the pathological cascade between variants of AD recently described<sup>171</sup>. Autoradiography studies on post-mortem samples, which allow a sub-anatomical distribution assessment of MAO-B activity, depicted a laminar binding pattern in the frontal cortex of AD which was absent for the amyloid plaques but matched Tau deposition which spreads in all layers in the frontal cortex<sup>172-174</sup>. Overall, imaging MAO-B activity agrees with the view of a beneficial role for astrocytes in contributing to A $\beta$  clearance but also that excess A $\beta$  can lead to oxidative stress and damage, and therefore to reduced astrocyte functionality, leading to reactive changes and decreased neuronal support, and thereby contributing to neurodegeneration. Regarding the therapeutic potential of MAO-B inhibition in AD,  $[^{11}\text{C}]$ L-deprenyl-D2 has also been used in early

clinical investigations, for the evaluation of new selective and reversible MAO-B inhibitors<sup>175, 176</sup>. Despite the great success of [<sup>11</sup>C]L-deprenyl-D2, concerns subsist on the accuracy of binding levels quantification, notably regarding putative subtle changes related in neuropsychiatric diseases. More specifically, the poor binding reversibility of the tracer raises questions about the influence of blood flow on binding measures. Besides, the metabolic pathway generates the brain-penetrating radiometabolite [<sup>11</sup>C]metamphetamine which also contributes to the PET signal. These reasons prompted the radiochemistry community to search for new MAO-B PET ligands (Figure S5). Beyond the fluorinated deprenyl<sup>177, 178</sup> and the corresponding deuterium-substituted ([<sup>18</sup>F]fluorodeprenyl-D2) for which a clinical proof-of-concept has been recently established<sup>179, 180</sup>, many other potential new radiotracers for MAO-B activity were proposed in the last decade, with varying degrees of success<sup>181-184</sup>. The most successful developments relate to two new PET tracers for MAO-B activity, [<sup>18</sup>F]SMBT-1 on the one hand and [<sup>11</sup>C]SL25.1188 on the other. [<sup>18</sup>F]SMBT-1 is the most recent and demonstrated high selectivity and reversible binding<sup>185</sup>. The favourable features allowed a rapid first-in-human trial and convincing cross-sectional human PET studies across AD spectrum which overall confirmed previous knowledge, although the [<sup>18</sup>F]SMBT-1 would benefit from a full kinetic analysis with metabolite-corrected arterial input function to formally validate its use as a surrogate imaging biomarker<sup>186-188</sup>. However, the relationship between astrocytes and amyloid- $\beta$  load (and probably tau) may not be the same across different brain regions reflecting a complex regional relationship rather than a global response<sup>187, 189</sup>. The other alternative radiotracer is the [<sup>11</sup>C]SL25.1188. Although fluorinated analogues were recently evaluated, most of the results were obtained with the original carbonated compound which display good selectivity and affinity for the target<sup>190, 191</sup>. Radiochemical optimization was needed for first-in-human testing and a formal pharmacokinetic and modelling validation study revealed a similar binding pattern than [<sup>11</sup>C]L-deprenyl-D2 PET, which is also the case in NHPs, and [<sup>11</sup>C]SL25.1188 is a predictor of the known concentration of MAO-B *post-mortem* in human<sup>192, 193</sup>. Interestingly, a case-control study in patients suffering from major depressive disorder (MDD) demonstrated that [<sup>11</sup>C]SL25.1188 distribution volume was significantly increased in the prefrontal cortex of patients with major depressive episodes<sup>194</sup>. The same group also reported preliminary results in post-traumatic stress disorders (PTSD) with or without comorbid MDD<sup>195</sup>. These studies definitively confirmed the potential of radiolabelled specific MAO-B inhibitors as imaging biomarkers in biological psychiatry (Figure S5). These tools could also represent major assets in drug development, either to investigate potential pro-psychotic adverse events, or in the future for new anti-psychotic drugs<sup>196</sup>. In a recent study extending beyond strict neuro-psychiatric applications, researchers investigated patients who had undergone traumatic brain injuries. Utilizing [<sup>11</sup>C]SL25.1188 PET scans, the study unveiled a persistent presence of astrogliosis in cortical areas. Specifically, an

increase in [<sup>11</sup>C]SL25.1188 VT was found to correlate with enduring symptoms, prompting the authors to suggest that markers of astrogliosis could serve as potential tools for patient stratification and therapeutic targeting<sup>197</sup>.

Development of fluorinated MAO-B radiotracers with reversible binding properties is still ongoing with a recent description of the radiolabelling and translational *in vitro* validation of a chromone derivative<sup>198</sup>. This new radioligand named [<sup>18</sup>F]GEH200449 has been quantified very recently in NHPs using an arterial input function and Logan graphical analysis and established as a reversible radiotracer in blocking paradigm using rasagiline and deprenyl as well as in displacement paradigm using deprenyl<sup>199</sup>.

## 2.2 Imidazoline type-2 binding sites (I2BS)

Historically, a non-adrenergic imidazoline binding was identified in astrocytes, and increased in AD<sup>200</sup>. Indeed, radiopharmacological studies distinguished two subtypes of imidazoline binding sites (I2BS): type 1, labelled by clonidine and type 2, labelled by idazoxan. I2BS are located on the mitochondrial outer membranes and co-expressed with the monoamine oxidase B (MAO-B) in astrocytes. Its functional role remains uncertain. An endogenous ligand, agmatine, has been shown to provide neuroprotective effects<sup>201</sup>.

Pharmacological exploration of I2BS started in 2003 with the tritium labelling of BU224 and related compound 2BF1<sup>202</sup>, which then enabled the testing of multiple compounds by an *in vitro* binding assay<sup>203</sup> (Figure S6). This led to the identification and tritium-labelling of the lead compound BU99008 in 2012<sup>204</sup>. After successful radiolabelling and biodistribution studies in large animals (pig<sup>205</sup>, monkey<sup>206</sup>), the first-in-man studies, including competition scans to assess selectivity and specificity<sup>207</sup>, along with biodistribution and dosimetry<sup>208</sup>, were reported in 2018.

Increased [<sup>3</sup>H]BU99008 binding localized with the astrocyte's glial fibrillary acidic protein (GFAP), whose expression relates to I2BS density. Hence brain imaging with I2BS PET is thought to represent a valuable marker of astrogliosis *in vivo*. However, preclinical data (*in vivo* imaging / *ex vivo* validation) is strikingly lacking for this radiotracer. Autoradiography in human tissue supported different targets for [<sup>3</sup>H]BU99008 and [<sup>3</sup>H]Deprenyl but an interaction of [<sup>3</sup>H]BU99008 with MAO-B binding sites cannot be ruled out and could explain the apparent detection of multiple binding sites and different affinity status<sup>209</sup>. So far, clinical [<sup>11</sup>C]BU99008 PET studies were performed in neurodegenerative diseases. In AD, a reduced uptake of [<sup>11</sup>C]BU99008 was noted in regions with increased amyloid load like the temporal lobe, while an increased uptake of the tracer was evidenced in areas in which amyloid deposition occurs later, like the primary motor and sensory areas<sup>210</sup>. These observations support a bi-phasic hypothesis with high astrocyte reactivity initially triggered by early A $\beta$  deposition, followed by astrocyte dystrophy in the advanced stages of the disease<sup>210, 211</sup>. The same

pattern emerged from two pilot studies in PD, with widespread increases of binding in the early stage<sup>212</sup> and no difference or decreases of binding in advanced Parkinson, compared to controls<sup>212, 213</sup>. However, astrogliosis seemed to increase with aging<sup>213</sup>.

Few alternative radioligands are available. [<sup>11</sup>C]TEIMD, and [<sup>11</sup>C]metrazoline were radiolabelled and evaluated in mice<sup>214</sup>. [<sup>11</sup>C]FTIMD biodistribution was examined in normal rats<sup>215, 216</sup> and in monkeys<sup>217</sup>. A fluorinated radiotracer, [<sup>18</sup>F]BU99018 or FEBU, was evaluated in rodents and showed high specific-binding in the hypothalamus<sup>218</sup>. The same group later used [<sup>11</sup>C]BU99008 in a genetic rat model of obesity, and reported increased uptake in the hypothalamus<sup>219</sup>, using ultra-high specific activity<sup>215, 220</sup>.

### 2.3 Conclusion on Astrocytes-specific biomarkers

The main astrocytic PET radiotracers were developed with a very efficient method consisting in a nearly direct transfer after translational *in vitro* validation (Figure 2). Although these transfers were done twenty years ago when regulation were not as strict as there are now, one reason is the intrinsic quality of the vector molecules. New generation of MAO-B radiotracers follow the same process with rapid human transfer after just one or two radiochemistry and preclinical studies (Figure S5). Effective I2BS radiotracers are available, although the main one is carbonated ([<sup>11</sup>C]BU99008), which restrict its use to specific dedicated research centres (Figure S6). Given this successful development, it is surprising that astrocyte imaging remains under-used, compared to microglial TSPO imaging.

## Part 3: Biomarkers without cellular specificity

### 3.1 Cannabinoid type-2 receptor (CB<sub>2</sub>R)

Cannabinoid receptors (CBRs) are part of the endocannabinoid system and belong to the G protein-coupled receptor superfamily. There are two types of CBRs, CB<sub>1</sub> and CB<sub>2</sub>, with CB<sub>1</sub>R primarily found in the brain and expressed on neurons, but its expression can also occur in cells of the periphery. CB<sub>2</sub> receptors instead are primarily expressed in peripheral cells of the immune system, and in the brain, low expression can be found mainly in microglia under healthy conditions<sup>221, 222</sup>. In fact, over-expression of CB<sub>2</sub>R has been linked to NI and activation of microglia and brain macrophages<sup>222, 223</sup>, thus making it an interesting target for therapeutic intervention and PET radioligand development (Figure S7). However, it should be mentioned that neuronal CB<sub>2</sub>R expression has been detected in the brain<sup>224</sup>. In particular, Onaivi and colleagues have shown CB<sub>2</sub>R expression in the brain of rats and mice exposed to chronic mild stress or in model of drug abuse<sup>225, 226</sup>. Further findings support an impact of CB<sub>2</sub>R in anxiety, depression, schizophrenia, and additional neuropsychiatric disorders<sup>227, 228</sup>.

Due to the relatively low expression of CB<sub>2</sub> receptors in the brain under normal conditions, and in contrast the abundance of CB<sub>1</sub>, an important aspect for the development of radioligands targeting CB<sub>2</sub>R is their selectivity over CB<sub>1</sub>R. The first PET radioligand with high specificity, selectivity and first-in-human application is the 2-oxoquinoline derivative [<sup>11</sup>C]NE40. The properties of this radioligand, developed at KU Leuven, were intensively studied in several biodistribution and preclinical *in vivo* PET imaging studies in healthy, CB<sub>2</sub>R overexpressing and diseased rodents or NHPs before the first-in-human application<sup>229-232</sup>. Interestingly it was a group from the Hamamatsu University School of Medicine that showed increased binding of [<sup>11</sup>C]NE40 at early times points in a rat model of stroke as well as in a senescence-accelerated mouse model and observed that CB<sub>2</sub>R expression was linked to activated microglial cells<sup>233, 234</sup>. Surprisingly, later the Leuven group did not detect the anticipated up-regulation of CB<sub>2</sub>R in the brains of AD patients but rather a decrease of CB<sub>2</sub>R bioavailability compared to healthy controls<sup>235</sup>. Although [<sup>11</sup>C]NE40 showed a high-affinity (K<sub>i</sub>-CB<sub>2</sub>R = 9.6nM and a 100-fold selectivity over CB<sub>1</sub>R), these unexpected findings could be due to an insufficient selectivity over CB<sub>1</sub>R, which is highly expressed in the human brain, and thus an off-target binding effect, alternatively this could also be due to the loss of neuronal cells expressing CB<sub>2</sub>R rather than a change in microglial expression. Other oxoquinoline, oxadiazole or indole derivatives have also been studied with PET by the Leuven group<sup>236-239</sup>, however [<sup>11</sup>C]NE40 remains the only radioligand of the KU team with translation into the clinics.

Another PET radioligand with a first-in-human study comes from Horti and colleagues, who successfully radiolabelled the thiazole derivative A-836339 with carbon-11<sup>240</sup>. *In vivo* preclinical studies with this radioligand showed increased binding of [<sup>11</sup>C]A-836339 in mouse brains from LPS-treated animals as well as in two different mouse models of Alzheimer's disease<sup>240, 241</sup>. In contrast, Pottier *et al.* did not find increased binding of this radioligand in rat models of local CNS inflammation (induced by intrastriatal injection of LPS or AMPA) or after cerebral ischemia<sup>242</sup>. Interestingly, activation of CB<sub>2</sub>R by the selective CB<sub>2</sub> agonist JWH133, induced a decrease in binding of the TSPO PET radioligand [<sup>18</sup>F]DPA-714 in the ischemic model, linking its effect to NI. Despite the differences in the animal models, [<sup>11</sup>C]A-836339 (named [<sup>11</sup>C]MDTC in the publication) has recently been published in a first-in-human study resuming its safety and pharmacokinetic data<sup>243</sup>. Brain V<sub>T</sub> values were low in the relatively young, healthy volunteers, and radio-metabolite studies in mice indicated some radio-metabolites entering the brain. Thus, further investigation of the radioligand and its metabolites are needed for future clinical studies. Besides these two radiotracers, a huge variety of radioligands targeting CB<sub>2</sub>R have been developed so far<sup>244-256</sup>, however only a few have been investigated by *in vivo* PET and demonstrated significant CB<sub>2</sub>R binding in preclinical models of NI or CB<sub>2</sub>R overexpression. Of those [<sup>18</sup>F]LU14 and [<sup>18</sup>F]LU13, with nano- and sub-nanomolar binding affinity, demonstrated specific

and significant binding in a rat model with local CB<sub>2</sub>R overexpression<sup>257, 258</sup>. Others as [<sup>11</sup>C]RS-016, [<sup>11</sup>C]RSR-056, and [<sup>18</sup>F]29 were shown to have specific and increased binding in the brain of LPS-treated mice in comparison to controls or, as for [<sup>18</sup>F]RoSMA-18-d6, in animals with cerebral ischemia<sup>259-262</sup>.

### 3.2 Sphingosine 1 Phosphate Receptors (S1PRs)

Sphingosine 1 Phosphate Receptors (S1PRs) comprises five different receptor subtypes (S1P1–S1P5) belonging to a family of G-protein-coupled receptors. Their endogenous ligand is Sphingosine-1-phosphate (S1P), a signalling sphingolipid. S1PRs are ubiquitously expressed in cerebral grey matter (with S1P1 being the most abundant)<sup>263</sup> and are implicated in numerous physiological CNS functions<sup>264, 265</sup>.

Interest in targeting S1PRs was initiated by pharmacological developments for multiple sclerosis (MS). Indeed, fingolimod (FTY720, Gilenya®, Novartis), an efficient immunosuppressive therapy for MS, was shown to act as a non-specific agonist for S1PRs<sup>266</sup>, and was first iodinated by Novartis in 2011 to yield the radiotracer [<sup>123/124</sup>I]BZM055<sup>267</sup>. In the following years, independent groups reported the fluorine-18 labelling of other molecules (a 6-substituted indole-3-carboxylic acid amide compound<sup>268</sup>, the antagonist W146<sup>269</sup>, two new sphingosine analogues, and a siponimod derivative<sup>270</sup>). But none of these initial tracers were further developed (Figure S8).

Following the development of a binding assay for S1PR ligands using [<sup>32</sup>P]S1P<sup>271</sup>, Tu and co-workers started to label and evaluate several compounds with enhanced selectivity for S1P1 and S1P2 receptors, hereby reporting the first S1PRs distribution, under inflammatory challenges (EAE<sup>272</sup> and LPS<sup>273</sup>). Three different radiotracers were used in these seminal studies. Importantly, vascular diseases (stenosis, balloon injury, atherosclerosis) were also explored in these initial studies with [<sup>11</sup>C]TZ3321<sup>274-276</sup>.

The clinical translation of [<sup>11</sup>C]TZ3321, also named [<sup>11</sup>C]CS1P1, was carefully prepared through *i*) autoradiographic studies performed on human tissues with its tritiated derivative<sup>277, 278</sup>, *ii*) a dosimetry study in rodents<sup>279</sup> and *iii*) the implementation of radiosynthesis under Good Manufacturing Practices (GMP)<sup>280</sup>. The phase 1 evaluation (safety and dosimetry) of this tracer was finally reported in 2022<sup>281</sup>. The fluorine-18 labelling of the same molecule was recently implemented, giving rise to the radiotracer [<sup>18</sup>F]FS1P1, which shares the same structure as [<sup>11</sup>C]CS1P1<sup>282</sup>.

In parallel, a wealth of new tracers is continuously reported by the same research group (Pr Zhude Tu, St. Louis, USA), targeting S1P1<sup>283</sup> or S1P2<sup>284</sup>, and evaluated in rodents<sup>285, 286</sup> and in non-human primates (Figure 3)<sup>287, 288</sup>. Of note, a monoclonal antibody against S1P1 receptor was labelled with <sup>99m</sup>Tc for immunopET<sup>289</sup>.

While the potential of S1P1 imaging largely extends the field of brain inflammation, it is for now difficult to assess its expected impact as most of the work arises from a single research group, and few experimental models of brain injury were studied (Figure S8). A pilot autoradiography study exhibited enhanced specific binding of [<sup>3</sup>H]CS1P1 in type 2 schizophrenia subjects compared to type 1 (n=5 each), matching the known differential expression of S1P1 receptor<sup>278</sup>.

As for TPSO PET imaging, a probable difficulty will be to unambiguously interpret changes in radiotracer uptake in relation to the lack of cell specificity and ubiquitous expression of S1PRs.

### 3.3 Conclusion on non-specific biomarkers

These types of targets come with an inherent difficulty owing to their expression by a variety of cells in the CNS. Additional difficulty is the lack of valid antibody for CB2R, and the existence of multiple receptor subtypes for S1P. Despite this, CB2R has attracted considerable interest which however led to overall disappointing results up to now. Radiotracer development for S1PRs is more recent and mainly driven par a single research group. Results in clinical studies with [<sup>11</sup>C]CS1P1 are eagerly awaited to get a better idea of the potential of S1PR imaging (Figure 3, also available as an interactive [supplementary figure: https://infogram.com/radar-spider-1h984wv3llqnd2p](https://infogram.com/radar-spider-1h984wv3llqnd2p)).

## Conclusion

Biomarker identification and radiotracer development in psychiatric disorders is facing multiples challenges, notably owing to the heterogenous nature of these diseases with possible shared phenotypes or symptoms. *In vivo* PET imaging of brain inflammation is considered as a promising strategy to investigate potential targets of therapeutic interest. NI is a complex and dynamic process comprising of many mechanistic and histological features. The best-known related imaging biomarker is the 18kDa translocator protein (TSPO) which has been investigated for 40 years now. While acknowledging both the merits and limitations of TSPO for PET imaging of brain inflammation, this review highlights that the forefront alternatives lie in astrocyte-specific radiotracers, with many reaching clinical investigations. Due to their astrocytic specificity, these radiotracers serve to complement, rather than fully replace, TSPO PET imaging. Simultaneously, a growing number of potential microglial molecular markers have been studied resulting in the development of dozens of radiotracers. However, the molecular complexity as well as the dynamic aspect of NI, for instance, but not limited to, the evolution between pro- and anti- inflammatory status of microglia, are part of the obstacles that have limited the development of such radiotracers and their transfer to clinical imaging.

It is worth mentioning that while our present review focused on potential NI targets that are the subject of current radiotracer developments other biological targets are emerging but are not yet accompanied by dedicated radiotracers, but their development will be of considerable interest in the

future. For instance, we can cite the Calcitonin Receptor-Like Receptor, and the receptor activity-modifying protein (Ramp1) as well as the enzyme Arginase 1 (Arg1). However, some of these potential targets have limited scope for clinical application due to, for example, species-specific expression. Finally, the established immunoPET (*i.e.* the use of positron emitters-radiolabelled antibodies, and by extension nanobodies or antibody's fragments) suggests that we will be able to visualize a large diversity of targets thanks to biochemical engineering, knowing that a significant hurdle persists in finding ways for them to cross the BBB. Current research explores the active transfer using bifunctional antibodies, pre-targeting in conjunction with *in vivo* click-chemistry or strategies to bypass the BBB<sup>290</sup>. However, as of now, none of these technologies has advanced to the clinical stage.

To conclude, researchers and clinicians interested in neuroinflammatory correlates of psychiatric diseases have at their disposal PET radiotracers for clinical molecular imaging in patients that are rather complementary to the well-known TSPO imaging. These tools are currently mainly directed toward astrocytes and conducting clinical investigations alongside TSPO imaging would be of significant interest for the understanding of psychiatric diseases and for therapeutic strategies evaluation, while microglia-specific radiotracers are continuously being developed.

## Conflict of interest

The authors have no conflict of interest to declare.

## Author Contributions

All authors contributed equally to the writing of this review. HB coordinated and collated the contributions of all authors into the final manuscript. GB finalised the figures of this review; FC made the online interactive one (<https://infogram.com/radar-spider-1h984wv3llqnd2p>).

## Acknowledgements

This work was performed within the frameworks of LABEX PRIMES (ANR-11-LABX-0063, University of Lyon), the “Investissements d’Avenir” program (ANR-11-IDEX-0007, University of Lyon), the research program PURImaging (ANR-21-CE18-0067-01, University of Paris-Saclay, SHFJ-CEA Orsay), and the LABEX IRON (ANR-11-LABX-18-01, University of Tours) of the French National Research Agency (ANR). GB was supported by the “Fondation ARC pour la recherche sur le cancer” through the “passerelle 2023” grant.

## References

1. Jones HB. Some instances of the contrast between delirium tremens and inflammation of the brain, as regards the quantity of phosphoric acid excreted by the kidneys. *Med Chir Trans* 1847; **30**: 21-38.
2. Gomez-Nicola D, Perry VH. Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. *Neuroscientist* 2015; **21**(2): 169-184.
3. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001; **2**(10): 734-744.
4. Benavides J, Quarteronet D, Imbault F, Malgouris C, Uzan A, Renault C *et al.* Labelling of "peripheral-type" benzodiazepine binding sites in the rat brain by using [3H]PK 11195, an isoquinoline carboxamide derivative: kinetic studies and autoradiographic localization. *J Neurochem* 1983; **41**(6): 1744-1750.
5. Le Fur G, Vaucher N, Perrier ML, Flamier A, Benavides J, Renault C *et al.* Differentiation between two ligands for peripheral benzodiazepine binding sites, [3H]RO5-4864 and [3H]PK 11195, by thermodynamic studies. *Life Sci* 1983; **33**: 449-457.
6. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P *et al.* Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* 2006; **27**(8): 402-409.
7. Banati RB. Visualising microglial activation in vivo. *Glia* 2002; **40**(2): 206-217.
8. Owen DR, Matthews PM. Imaging brain microglial activation using positron emission tomography and translocator protein-specific radioligands. *Int Rev Neurobiol* 2011; **101**: 19-39.
9. Chauveau F, Boutin H, Van Camp N, Dolle F, Tavitian B. Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. *Eur J Nucl Med Mol Imaging* 2008; **35**(12): 2304-2319.
10. Wimberley C, Buvat I, Boutin H. Imaging translocator protein expression with positron emission tomography. *Eur J Nucl Med Mol Imaging* 2021; **49**(1): 74-76.
11. Chauveau F, Becker G, Boutin H. Have (R)-[11C]PK11195 challengers fulfilled the promise? A scoping review of clinical TSPO PET studies. *Eur J Nucl Med Mol Imaging* 2021; **49**(1): 201-220.
12. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A *et al.* An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab* 2012; **32**(1): 1-5.
13. Owen DR, Gunn RN, Rabiner EA, Bennacef I, Fujita M, Kreisl WC *et al.* Mixed-affinity binding in humans with 18-kDa translocator protein ligands. *J Nucl Med* 2011; **52**(1): 24-32.

14. Tiwari AK, Ji B, Yui J, Fujinaga M, Yamasaki T, Xie L *et al.* [18F]FEBMP: Positron Emission Tomography Imaging of TSPO in a Model of Neuroinflammation in Rats, and in vitro Autoradiograms of the Human Brain. *Theranostics* 2015; **5**(9): 961-969.
15. MacAskill MG, Stadulyte A, Williams L, Morgan TEF, Sloan NL, Alcaide-Corral CJ *et al.* Quantification of Macrophage-Driven Inflammation During Myocardial Infarction with (18)F-LW223, a Novel TSPO Radiotracer with Binding Independent of the rs6971 Human Polymorphism. *J Nucl Med* 2021; **62**(4): 536-544.
16. Gui Y, Marks JD, Das S, Hyman BT, Serrano-Pozo A. Characterization of the 18 kDa translocator protein (TSPO) expression in post-mortem normal and Alzheimer's disease brains. *Brain Pathol* 2020; **30**(1): 151-164.
17. Rizzo G, Veronese M, Tonietto M, Bodini B, Stankoff B, Wimberley C *et al.* Generalization of endothelial modelling of TSPO PET imaging: Considerations on tracer affinities. *J Cereb Blood Flow Metab* 2019; **39**(5): 874-885.
18. Wimberley C, Lavisse S, Brulon V, Peyronneau MA, Leroy C, Bodini B *et al.* Impact of Endothelial 18-kDa Translocator Protein on the Quantification of (18)F-DPA-714. *J Nucl Med* 2018; **59**(2): 307-314.
19. Veronese M, Reis Marques T, Bloomfield PS, Rizzo G, Singh N, Jones D *et al.* Kinetic modelling of [(11)C]PBR28 for 18 kDa translocator protein PET data: A validation study of vascular modelling in the brain using XBD173 and tissue analysis. *J Cereb Blood Flow Metab* 2018; **38**(7): 1227-1242.
20. Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G *et al.* Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* 2015; **72**(3): 268-275.
21. Su L, Faluyi YO, Hong YT, Fryer TD, Mak E, Gabel S *et al.* Neuroinflammatory and morphological changes in late-life depression: the NIMROD study. *Br J Psychiatry* 2016; **209**(6): 525-526.
22. Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM *et al.* Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study. *Biol Psychiatry* 2018; **83**(1): 61-69.
23. Meyer JH, Cervenka S, Kim MJ, Kreisl WC, Henter ID, Innis RB. Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. *Lancet Psychiatry* 2020; **7**(12): 1064-1074.
24. van Berckel BN, Bossong MG, Boellaard R, Kloet R, Schuitemaker A, Caspers E *et al.* Microglia activation in recent-onset schizophrenia: a quantitative (R)-[11C]PK11195 positron emission tomography study. *Biol Psychiatry* 2008; **64**(9): 820-822.
25. Doorduyn J, de Vries EF, Willemsen AT, de Groot JC, Dierckx RA, Klein HC. Neuroinflammation in schizophrenia-related psychosis: a PET study. *J Nucl Med* 2009; **50**(11): 1801-1807.
26. Bloomfield PS, Selvaraj S, Veronese M, Rizzo G, Bertoldo A, Owen DR *et al.* Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study. *Am J Psychiatry* 2016; **173**(1): 44-52.

27. Takano A, Arakawa R, Ito H, Tateno A, Takahashi H, Matsumoto R *et al.* Peripheral benzodiazepine receptors in patients with chronic schizophrenia: a PET study with [<sup>11</sup>C]DAA1106. *The international journal of neuropsychopharmacology* 2010; **13**(7): 943-950.
28. Kenk M, Selvanathan T, Rao N, Suridjan I, Rusjan P, Remington G *et al.* Imaging neuroinflammation in gray and white matter in schizophrenia: an in-vivo PET study with [<sup>18</sup>F]-FEPPA. *Schizophr Bull* 2015; **41**(1): 85-93.
29. Holmes SE, Hinz R, Drake RJ, Gregory CJ, Conen S, Matthews JC *et al.* In vivo imaging of brain microglial activity in antipsychotic-free and medicated schizophrenia: a [(11)C](R)-PK11195 positron emission tomography study. *Mol Psychiatry* 2016; **21**(12): 1672-1679.
30. van der Doef TF, de Witte LD, Sutterland AL, Jobse E, Yaqub M, Boellaard R *et al.* In vivo (R)-[(11)C]PK11195 PET imaging of 18kDa translocator protein in recent onset psychosis. *NPJ Schizophr* 2016; **2**: 16031.
31. Di Biase MA, Zalesky A, O'Keefe G, Laskaris L, Baune BT, Weickert CS *et al.* PET imaging of putative microglial activation in individuals at ultra-high risk for psychosis, recently diagnosed and chronically ill with schizophrenia. *Transl Psychiatry* 2017; **7**(8): e1225.
32. Hafizi S, Tseng HH, Rao N, Selvanathan T, Kenk M, Bazinet RP *et al.* Imaging Microglial Activation in Untreated First-Episode Psychosis: A PET Study With [(18)F]FEPPA. *Am J Psychiatry* 2017; **174**(2): 118-124.
33. Conen S, Gregory CJ, Hinz R, Smallman R, Corsi-Zuelli F, Deakin B, Talbot PS. Neuroinflammation as measured by positron emission tomography in patients with recent onset and established schizophrenia: implications for immune pathogenesis. *Mol Psychiatry* 2021; **26**(9): 5398-5406.
34. Collste K, Plaven-Sigray P, Fatouros-Bergman H, Victorsson P, Schain M, Forsberg A *et al.* Lower levels of the glial cell marker TSPO in drug-naive first-episode psychosis patients as measured using PET and [(11)C]PBR28. *Mol Psychiatry* 2017; **22**(6): 850-856.
35. De Picker LJ, Haarman BCM. Applicability, potential and limitations of TSPO PET imaging as a clinical immunopsychiatry biomarker. *Eur J Nucl Med Mol Imaging* 2021; **49**(1): 164-173.
36. Iliopoulou SM, Tsartsalis S, Kaiser S, Millet P, Tournier BB. Dopamine and Neuroinflammation in Schizophrenia - Interpreting the Findings from Translocator Protein (18kDa) PET Imaging. *Neuropsychiatr Dis Treat* 2021; **17**: 3345-3357.
37. Coughlin JM, Wang Y, Ambinder EB, Ward RE, Minn I, Vranesic M *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.
38. Narayanaswami V, Dahl K, Bernard-Gauthier V, Josephson L, Cumming P, Vasdev N. Emerging PET Radiotracers and Targets for Imaging of Neuroinflammation in Neurodegenerative Diseases: Outlook Beyond TSPO. *Mol Imaging* 2018; **17**: 1536012118792317.
39. Janssen B, Vugts DJ, Windhorst AD, Mach RH. PET Imaging of Microglial Activation-Beyond Targeting TSPO. *Molecules* 2018; **23**(3): 10.3390/molecules23030607.

40. Suzuki Y, Nakamura Y, Yamada K, Kurabe S, Okamoto K, Aoki H *et al.* Aquaporin Positron Emission Tomography Differentiates Between Grade III and IV Human Astrocytoma. *Neurosurgery* 2018; **82**(6): 842-846.
41. Chen Z, Yang A, Zhang J, Chen A, Zhang Y, Huang C *et al.* CXCR4-Directed PET/CT with [(68)Ga]Pentixafor in Central Nervous System Lymphoma: A Comparison with [(18)F]FDG PET/CT. *Mol Imaging Biol* 2022; **24**(3): 416-424.
42. Starzer AM, Berghoff AS, Traub-Weidinger T, Haug AR, Widhalm G, Hacker M *et al.* Assessment of Central Nervous System Lymphoma Based on CXCR4 Expression In Vivo Using 68Ga-Pentixafor PET/MRI. *Clin Nucl Med* 2021; **46**(1): 16-20.
43. van der Wildt B, Wilhelmus MM, Bijkerk J, Haveman LY, Kooijman EJ, Schuit RC *et al.* Development of carbon-11 labeled acryl amides for selective PET imaging of active tissue transglutaminase. *Nucl Med Biol* 2016; **43**(4): 232-242.
44. Ackermann U, Jager L, Rigopoulos A, Burvenich IJG, O'Keefe GJ, Scott AM. (18)F-labeling and initial in vivo evaluation of a Hitomi peptide for imaging tissue transglutaminase 2. *Nucl Med Biol* 2023; **116-117**: 108308.
45. Meyer PT, Elmenhorst D, Matusch A, Winz O, Zilles K, Bauer A. A1 adenosine receptor PET using [18F]CPFPX: displacement studies in humans. *Neuroimage* 2006; **32**(3): 1100-1105.
46. Barret O, Hannestad J, Vala C, Alagille D, Tavares A, Laruelle M *et al.* Characterization in humans of 18F-MNI-444, a PET radiotracer for brain adenosine 2A receptors. *J Nucl Med* 2015; **56**(4): 586-591.
47. Joya A, Ardaya M, Montilla A, Garbizu M, Plaza-Garcia S, Gomez-Vallejo V *et al.* In vivo multimodal imaging of adenosine A(1) receptors in neuroinflammation after experimental stroke. *Theranostics* 2021; **11**(1): 410-425.
48. Rissanen E, Virta JR, Paavilainen T, Tuisku J, Helin S, Luoto P *et al.* Adenosine A2A receptors in secondary progressive multiple sclerosis: a [(11)C]TMSX brain PET study. *J Cereb Blood Flow Metab* 2013; **33**(9): 1394-1401.
49. Karasawa A, Kawate T. Structural basis for subtype-specific inhibition of the P2X7 receptor. *Elife* 2016; **5**.
50. McCarthy AE, Yoshioka C, Mansoor SE. Full-Length P2X(7) Structures Reveal How Palmitoylation Prevents Channel Desensitization. *Cell* 2019; **179**(3): 659-670 e613.
51. Burnstock G, Boeynaems JM. Purinergic signalling and immune cells. *Purinergic Signal* 2014; **10**(4): 529-564.
52. Di Virgilio F, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 Receptor in Infection and Inflammation. *Immunity* 2017; **47**(1): 15-31.
53. Beltran-Lobo P, Reid MJ, Jimenez-Sanchez M, Verkhatsky A, Perez-Nievas BG, Noble W. Astrocyte adaptation in Alzheimer's disease: a focus on astrocytic P2X7R. *Essays Biochem* 2023; **67**(1): 119-130.

54. Illes P, Verkhratsky A, Burnstock G, Franke H. P2X receptors and their roles in astroglia in the central and peripheral nervous system. *Neuroscientist* 2012; **18**(5): 422-438.
55. Bhattacharya A, Biber K. The microglial ATP-gated ion channel P2X7 as a CNS drug target. *Glia* 2016; **64**(10): 1772-1787.
56. Illes P, Khan TM, Rubini P. Neuronal P2X7 Receptors Revisited: Do They Really Exist? *J Neurosci* 2017; **37**(30): 7049-7062.
57. Adinolfi E, Capece M, Amoroso F, De Marchi E, Franceschini A. Emerging roles of P2X receptors in cancer. *Current medicinal chemistry* 2015; **22**(7): 878-890.
58. Burnstock G. Purinergic signalling and disorders of the central nervous system. *Nat Rev Drug Discov* 2008; **7**(7): 575-590.
59. Monif M, Burnstock G, Williams DA. Microglia: proliferation and activation driven by the P2X7 receptor. *The international journal of biochemistry & cell biology* 2010; **42**(11): 1753-1756.
60. Oliveira-Giacomelli A, Petiz LL, Andrejew R, Turrini N, Silva JB, Sack U, Ulrich H. Role of P2X7 Receptors in Immune Responses During Neurodegeneration. *Front Cell Neurosci* 2021; **15**: 662935.
61. Territo PR, Zarrinmayeh H. P2X(7) Receptors in Neurodegeneration: Potential Therapeutic Applications From Basic to Clinical Approaches. *Front Cell Neurosci* 2021; **15**: 617036.
62. von Mucke-Heim IA, Deussing JM. The P2X7 receptor in mood disorders: Emerging target in immunopsychiatry, from bench to bedside. *Neuropharmacology* 2023; **224**: 109366.
63. Janssen B, Vugts DJ, Funke U, Spaans A, Schuit RC, Kooijman E *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.
64. 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.
65. Schmidt S, Isaak A, Junker A. Spotlight on P2X7 Receptor PET Imaging: A Bright Target or a Failing Star? *Int J Mol Sci* 2023; **24**(2).
66. Territo PR, Meyer JA, Peters JS, Riley AA, McCarthy BP, Gao M *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.
67. Huang G, Lu X, Qiu Y, Bi L, Ye P, Yang M *et al.* Hetero-aryl bromide precursor fluorine-18 radiosynthesis and preclinical evaluation of a novel positron emission tomography (PET) tracer [(18)F]GSK1482160. *Bioorg Med Chem* 2022; **73**: 116996.
68. Hagens MHJ, Golla SSV, Janssen B, Vugts DJ, Beaino W, Windhorst AD *et al.* The P2X7 receptor tracer [(11)C]SMW139 as an in vivo marker of neuroinflammation in multiple sclerosis: a first-in man study. *Eur J Nucl Med Mol Imaging* 2020; **47**(2): 379-389.

69. Ory D, Celen S, Gijbbers R, Van Den Haute C, Postnov A, Koole M *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.
70. Kolb HC, Barret O, Bhattacharya A, Chen G, Constantinescu C, Huang C *et al.* Preclinical Evaluation and Nonhuman Primate Receptor Occupancy Study of (18)F-JNJ-64413739, a PET Radioligand for P2X7 Receptors. *J Nucl Med* 2019; **60**(8): 1154-1159.
71. Van Weehaeghe D, Koole M, Schmidt ME, Deman S, Jacobs AH, Souche E *et al.* [(11)C]JNJ54173717, a novel P2X7 receptor radioligand as marker for neuroinflammation: human biodistribution, dosimetry, brain kinetic modelling and quantification of brain P2X7 receptors in patients with Parkinson's disease and healthy volunteers. *Eur J Nucl Med Mol Imaging* 2019; **46**(10): 2051-2064.
72. Koole M, Schmidt ME, Hijzen A, Ravenstijn P, Vandermeulen C, Van Weehaeghe D *et al.* (18)F-JNJ-64413739, a Novel PET Ligand for the P2X7 Ion Channel: Radiation Dosimetry, Kinetic Modeling, Test-Retest Variability, and Occupancy of the P2X7 Antagonist JNJ-54175446. *J Nucl Med* 2019; **60**(5): 683-690.
73. Mertens N, Schmidt ME, Hijzen A, Van Weehaeghe D, Ravenstijn P, Depre M *et al.* Minimally invasive quantification of cerebral P2X7R occupancy using dynamic [(18)F]JNJ-64413739 PET and MRA-driven image derived input function. *Sci Rep* 2021; **11**(1): 16172.
74. Mikkelsen JD, Aripaka SS, Kaad S, Pazarlar BA, Pinborg L, Finsen B *et al.* Characterization of the Novel P2X7 Receptor Radioligand [(3)H]JNJ-64413739 in Human Brain Tissue. *ACS Chem Neurosci* 2023; **14**(1): 111-118.
75. Notter T, Coughlin JM, Sawa A, Meyer U. Reconceptualization of translocator protein as a biomarker of neuroinflammation in psychiatry. *Mol Psychiatry* 2018; **23**(1): 36-47.
76. Van Weehaeghe D, Van Schoor E, De Vocht J, Koole M, Attili B, Celen S *et al.* TSPO Versus P2X7 as a Target for Neuroinflammation: An In Vitro and In Vivo Study. *J Nucl Med* 2020; **61**(4): 604-607.
77. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G *et al.* Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat Neurosci* 2014; **17**(1): 131-143.
78. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK *et al.* A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* 2017; **169**(7): 1276-1290 e1217.
79. Bennett ML, Bennett FC, Liddel SA, Ajami B, Zamanian JL, Fernhoff NB *et al.* New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci U S A* 2016; **113**(12): E1738-1746.
80. Zhu C, Kros JM, van der Weiden M, Zheng P, Cheng C, Mustafa DA. Expression site of P2RY12 in residential microglial cells in astrocytomas correlates with M1 and M2 marker expression and tumor grade. *Acta Neuropathol Commun* 2017; **5**(1): 4.

81. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci* 2018; **19**(10): 622-635.
82. Zrzavy T, Hametner S, Wimmer I, Butovsky O, Weiner HL, Lassmann H. Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* 2017; **140**(7): 1900-1913.
83. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R *et al.* The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* 2017; **47**(3): 566-581 e569.
84. Beaino W, Janssen B, Kooij G, van der Pol SMA, van Het Hof B, van Horssen J *et al.* Purinergic receptors P2Y12R and P2X7R: potential targets for PET imaging of microglia phenotypes in multiple sclerosis. *J Neuroinflammation* 2017; **14**(1): 259.
85. Klein B, Mrowetz H, Barker CM, Lange S, Rivera FJ, Aigner L. Age Influences Microglial Activation After Cuprizone-Induced Demyelination. *Front Aging Neurosci* 2018; **10**: 278.
86. Maeda J, Minamihisamatsu T, Shimojo M, Zhou X, Ono M, Matsuba Y *et al.* Distinct microglial response against Alzheimer's amyloid and tau pathologies characterized by P2Y12 receptor. *Brain Commun* 2021; **3**(1): fcab011.
87. Baqi Y, Muller CE. Antithrombotic P2Y(12) receptor antagonists: recent developments in drug discovery. *Drug Discov Today* 2019; **24**(1): 325-333.
88. Janssen AL, Vugts DJ, Funke U, Molenaar GT, Kruijer PS, Dolle F *et al.* Synthesis of the first carbon-11 labelled P2Y12 receptor antagonist for PET imaging of microglial activation in neuroinflammation. *Proceedings of the Journal of Labelled Compounds and Radiopharmaceuticals*; 2015, S44.
89. Villa A, Klein B, Janssen B, Pedragosa J, Pepe G, Zinnhardt B *et al.* Identification of new molecular targets for PET imaging of the microglial anti-inflammatory activation state. *Theranostics* 2018; **8**(19): 5400-5418.
90. Jackson IM, Buccino PJ, Azevedo EC, Carlson ML, Luo ASZ, Deal EM *et al.* Radiosynthesis and initial preclinical evaluation of [(11)C]AZD1283 as a potential P2Y12R PET radiotracer. *Nucl Med Biol* 2022; **114-115**: 143-150.
91. van der Wildt B, Janssen B, Pekosak A, Steen EJJ, Schuit RC, Kooijman EJM *et al.* Novel Thienopyrimidine-Based PET Tracers for P2Y(12) Receptor Imaging in the Brain. *ACS Chem Neurosci* 2021; **12**(23): 4465-4474.
92. Xiang C, Li H, Tang W. Targeting CSF-1R represents an effective strategy in modulating inflammatory diseases. *Pharmacol Res* 2023; **187**: 106566.
93. Chitu V, Biundo F, Stanley ER. Colony stimulating factors in the nervous system. *Semin Immunol* 2021; **54**: 101511.
94. Bernard-Gauthier V, Schirmmacher R. 5-(4-((4-[(18)F]Fluorobenzyl)oxy)-3-methoxybenzyl)pyrimidine-2,4-diamine: a selective dual inhibitor for potential PET imaging of Trk/CSF-1R. *Bioorg Med Chem Lett* 2014; **24**(20): 4784-4790.

95. Singleton TA, Bdair H, Bailey JJ, Choi S, Aliaga A, Rosa-Neto P *et al.* Efficient radiosynthesis and preclinical evaluation of [(18) F]FOMPpyD as a positron emission tomography tracer candidate for TrkB/C receptor imaging. *J Labelled Comp Radiopharm* 2020; **63**(3): 144-150.
96. van der Wildt B, Miao Z, Reyes ST, Park JH, Klockow JL, Zhao N *et al.* BLZ945 derivatives for PET imaging of colony stimulating factor-1 receptors in the brain. *Nucl Med Biol* 2021; **100-101**: 44-51.
97. Tanzey SS, Shao X, Stauff J, Arteaga J, Sherman P, Scott PJH, Mossine AV. Synthesis and Initial In Vivo Evaluation of [(11)C]AZ683-A Novel PET Radiotracer for Colony Stimulating Factor 1 Receptor (CSF1R). *Pharmaceuticals (Basel)* 2018; **11**(4).
98. Horti AG, Naik R, Foss CA, Minn I, Misheneva V, Du Y *et al.* PET imaging of microglia by targeting macrophage colony-stimulating factor 1 receptor (CSF1R). *Proc Natl Acad Sci U S A* 2019; **116**(5): 1686-1691.
99. Mathews WB, Wu Y, Horti AG, Naik R, Hall AW, Holt DP, Dannals RF. Radiosynthesis and validation of [5-cyano-N-(4-(4-[(11) C]methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl) furan-2-carboxamide] ([11) C]CPPC, a PET radiotracer for imaging CSF1R, a microglia-specific marker. *J Labelled Comp Radiopharm* 2019; **62**(13): 903-908.
100. Coughlin JM, Du Y, Lesniak WG, Harrington CK, Brosnan MK, O'Toole R *et al.* First-in-human use of (11)C-CPPC with positron emission tomography for imaging the macrophage colony-stimulating factor 1 receptor. *EJNMMI Res* 2022; **12**(1): 64.
101. Knight AC, Varlow C, Zi T, Liang SH, Josephson L, Schmidt K *et al.* In Vitro Evaluation of [(3)H]CPPC as a Tool Radioligand for CSF-1R. *ACS Chem Neurosci* 2021; **12**(6): 998-1006.
102. Rubin LH, Du Y, Sweeney SE, O'Toole R, Harrington CK, Jenkins K *et al.* Pilot imaging of the colony stimulating factor 1 receptor in the brains of virally-suppressed individuals with HIV. *AIDS* 2023; **37**(9): 1419-1424.
103. Venneti S, Wang G, Wiley CA. Activated macrophages in HIV encephalitis and a macaque model show increased [3H](R)-PK11195 binding in a PI3-kinase-dependent manner. *Neurosci Lett* 2007; **426**: 117-122.
104. Garvey LJ, Pavese N, Ramlackhansingh A, Thomson E, Allsop JM, Politis M *et al.* Acute HCV/HIV coinfection is associated with cognitive dysfunction and cerebral metabolite disturbance, but not increased microglial cell activation. *PLoS ONE* 2012; **7**: e38980.
105. Coughlin JM, Wang Y, Ma S, Yue C, Kim PK, Adams AV *et al.* Regional brain distribution of translocator protein using [(11)C]DPA-713 PET in individuals infected with HIV. *J Neurovirol* 2014; **20**(3): 219-232.
106. Garvey LJ, Pavese N, Politis M, Ramlackhansingh A, Brooks DJ, Taylor-Robinson SD, Winston A. Increased microglia activation in neurologically asymptomatic HIV-infected patients receiving effective ART. *AIDS* 2014; **28**(1): 67-72.
107. Vera JH, Guo Q, Cole JH, Boasso A, Greathead L, Kelleher P *et al.* Neuroinflammation in treated HIV-positive individuals: A TSPO PET study. *Neurology* 2016; **86**(15): 1425-1432.

108. Rubin LH, Sacktor N, Creighton J, Du Y, Endres CJ, Pomper MG, Coughlin JM. Microglial activation is inversely associated with cognition in individuals living with HIV on effective antiretroviral therapy. *AIDS* 2018; **32**(12): 1661-1667.
109. Boerwinkle AH, Strain JF, Burdo T, Doyle J, Christensen J, Su Y *et al.* Comparison of [11C]-PBR28 Binding Between Persons Living With HIV and HIV-Uninfected Individuals. *J Acquir Immune Defic Syndr* 2020; **85**(2): 244-251.
110. Zhou X, Ji B, Seki C, Nagai Y, Minamimoto T, Fujinaga M *et al.* PET imaging of colony-stimulating factor 1 receptor: A head-to-head comparison of a novel radioligand, (11)C-GW2580, and (11)C-CPPC, in mouse models of acute and chronic neuroinflammation and a rhesus monkey. *J Cereb Blood Flow Metab* 2021; **41**(9): 2410-2422.
111. Altomonte S, Yan X, Morse CL, Liow JS, Jenkins MD, Montero Santamaria JA *et al.* Discovery of a High-Affinity Fluoromethyl Analog of [(11)C]5-Cyano-N-(4-(4-methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide ([[(11)C]CPPC) and Their Comparison in Mouse and Monkey as Colony-Stimulating Factor 1 Receptor Positron Emission Tomography Radioligands. *ACS Pharmacol Transl Sci* 2023; **6**(4): 614-632.
112. van der Wildt B, Nezam M, Kooijman EJM, Reyes ST, Shen B, Windhorst AD, Chin FT. Evaluation of carbon-11 labeled 5-(1-methyl-1H-pyrazol-4-yl)-N-(2-methyl-5-(3-(trifluoromethyl)benzamido)phenyl)nicotinamide as PET tracer for imaging of CSF-1R expression in the brain. *Bioorg Med Chem* 2021; **42**: 116245.
113. van der Wildt B, Klockow JL, Miao Z, Reyes ST, Park JH, Shen B, Chin FT. Discovery of a CSF-1R inhibitor and PET tracer for imaging of microglia and macrophages in the brain. *Nucl Med Biol* 2022; **114-115**: 99-107.
114. Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF *et al.* CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med* 2013; **19**(10): 1264-1272.
115. Ramachandran SA, Jadhavar PS, Miglani SK, Singh MP, Kalane DP, Agarwal AK *et al.* Design, synthesis and optimization of bis-amide derivatives as CSF1R inhibitors. *Bioorg Med Chem Lett* 2017; **27**(10): 2153-2160.
116. Lee H, Park JH, Kim H, Woo SK, Choi JY, Lee KH, Choe YS. Synthesis and Evaluation of a (18)F-Labeled Ligand for PET Imaging of Colony-Stimulating Factor 1 Receptor. *Pharmaceuticals (Basel)* 2022; **15**(3).
117. An X, Wang J, Tong L, Zhang X, Fu H, Zhang J *et al.* (18)F-Labeled o-aminopyridyl alkynyl radioligands targeting colony-stimulating factor 1 receptor for neuroinflammation imaging. *Bioorg Med Chem* 2023; **83**: 117233.
118. Xie Z, Wu B, Liu Y, Ren W, Tong L, Xiang C *et al.* Novel Class of Colony-Stimulating Factor 1 Receptor Kinase Inhibitors Based on an o-Aminopyridyl Alkynyl Scaffold as Potential Treatment for Inflammatory Disorders. *J Med Chem* 2020; **63**(3): 1397-1414.
119. Ghazanfari N, van Waarde A, Dierckx R, Doorduyn J, de Vries EFJ. Is cyclooxygenase-1 involved in neuroinflammation? *J Neurosci Res* 2021; **99**(11): 2976-2998.

120. McCarthy TJ, Sheriff AU, Graneto MJ, Talley JJ, Welch MJ. Radiosynthesis, in vitro validation, and in vivo evaluation of <sup>18</sup>F-labeled COX-1 and COX-2 inhibitors. *J Nucl Med* 2002; **43**(1): 117-124.
121. Prabhakaran J, Underwood MD, Parsey RV, Arango V, Majo VJ, Simpson NR *et al.* Synthesis and in vivo evaluation of [<sup>18</sup>F]-4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide as a PET imaging probe for COX-2 expression. *Bioorg Med Chem* 2007; **15**(4): 1802-1807.
122. Ji B, Kumata K, Onoe H, Kaneko H, Zhang MR, Seki C *et al.* Assessment of radioligands for PET imaging of cyclooxygenase-2 in an ischemic neuronal injury model. *Brain Res* 2013; **1533**: 152-162.
123. Kumar JSD, Bai B, Zanderigo F, DeLorenzo C, Prabhakaran J, Parsey RV, Mann JJ. In Vivo Brain Imaging, Biodistribution, and Radiation Dosimetry Estimation of [(11)C]Celecoxib, a COX-2 PET Ligand, in Nonhuman Primates. *Molecules* 2018; **23**(8).
124. de Vries EF, Doorduyn J, Dierckx RA, van Waarde A. Evaluation of [(11)C]rofecoxib as PET tracer for cyclooxygenase 2 overexpression in rat models of inflammation. *Nucl Med Biol* 2008; **35**(1): 35-42.
125. Toyokuni T, Kumar JS, Walsh JC, Shapiro A, Talley JJ, Phelps ME *et al.* Synthesis of 4-(5-[<sup>18</sup>F]fluoromethyl-3-phenylisoxazol-4-yl)benzenesulfonamide, a new [<sup>18</sup>F]fluorinated analogue of valdecoxib, as a potential radiotracer for imaging cyclooxygenase-2 with positron emission tomography. *Bioorg Med Chem Lett* 2005; **15**(21): 4699-4702.
126. Prabhakaran J, Underwood M, Zanderigo F, Simpson NR, Cooper AR, Matthew J *et al.* Radiosynthesis and in vivo evaluation of [(11)C]MOV as a PET imaging agent for COX-2. *Bioorg Med Chem Lett* 2018; **28**(14): 2432-2435.
127. Yamamoto Y, Toyohara J, Ishiwata K, Sano K, Yamamoto F, Mukai T, Maeda M. (1)(1)C-labeled analogs of indomethacin esters and amides for brain cyclooxygenase-2 imaging: radiosynthesis, in vitro evaluation and in vivo characteristics in mice. *Chem Pharm Bull (Tokyo)* 2011; **59**(8): 938-946.
128. Yamamoto Y, Tago T, Toyohara J, Saito Y, Yamamoto F. Radiosynthesis and in Vivo and ex Vivo Evaluation of Isomeric [(11)C]methoxy Analogs of Nimesulide as Brain Cyclooxygenase-2-Targeted Imaging Agents. *Biol Pharm Bull* 2022; **45**(1): 94-103.
129. Elie J, Vercoillie J, Arlicot N, Lemaire L, Bidault R, Bodard S *et al.* Design of selective COX-2 inhibitors in the (aza)indazole series. Chemistry, in vitro studies, radiochemistry and evaluations in rats of a [(18)F] PET tracer. *J Enzyme Inhib Med Chem* 2019; **34**(1): 1-7.
130. Cortes-Salva MY, Shrestha S, Singh P, Morse CL, Jenko KJ, Montero Santamaria JA *et al.* 2-(4-Methylsulfonylphenyl)pyrimidines as Prospective Radioligands for Imaging Cyclooxygenase-2 with PET-Synthesis, Triage, and Radiolabeling. *Molecules* 2018; **23**(11).
131. Kim MJ, Shrestha SS, Cortes M, Singh P, Morse C, Liow JS *et al.* Evaluation of Two Potent and Selective PET Radioligands to Image COX-1 and COX-2 in Rhesus Monkeys. *J Nucl Med* 2018; **59**(12): 1907-1912.

132. Shrestha S, Kim MJ, Eldridge M, Lehmann ML, Frankland M, Liow JS *et al.* PET measurement of cyclooxygenase-2 using a novel radioligand: upregulation in primate neuroinflammation and first-in-human study. *J Neuroinflammation* 2020; **17**(1): 140.
133. Kumar JSD, Zanderigo F, Prabhakaran J, Rubin-Falcone H, Parsey RV, Mann JJ. In vivo evaluation of [(11)C]TMI, a COX-2 selective PET tracer, in baboons. *Bioorg Med Chem Lett* 2018; **28**(23-24): 3592-3595.
134. Prabhakaran J, Molotkov A, Mintz A, Mann JJ. Progress in PET Imaging of Neuroinflammation Targeting COX-2 Enzyme. *Molecules* 2021; **26**(11).
135. Shukuri M, Takashima-Hirano M, Tokuda K, Takashima T, Matsumura K, Inoue O *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.
136. Shukuri M, Mawatari A, Ohno M, Suzuki M, Doi H, Watanabe Y, Onoe H. Detection of Cyclooxygenase-1 in Activated Microglia During Amyloid Plaque Progression: PET Studies in Alzheimer's Disease Model Mice. *J Nucl Med* 2016; **57**(2): 291-296.
137. Shukuri M, Mawatari A, Takatani S, Tahara T, Inoue M, Arakaki W *et al.* Synthesis and Preclinical Evaluation of (18)F-Labeled Ketoprofen Methyl Esters for Cyclooxygenase-1 Imaging in Neuroinflammation. *J Nucl Med* 2022; **63**(11): 1761-1767.
138. Singh P, Shrestha S, Cortes-Salva MY, Jenko KJ, Zoghbi SS, Morse CL *et al.* 3-Substituted 1,5-Diaryl-1 H-1,2,4-triazoles as Prospective PET Radioligands for Imaging Brain COX-1 in Monkey. Part 1: Synthesis and Pharmacology. *ACS Chem Neurosci* 2018; **9**(11): 2610-2619.
139. Shrestha S, Singh P, Cortes-Salva MY, Jenko KJ, Ikawa M, Kim MJ *et al.* 3-Substituted 1,5-Diaryl-1 H-1,2,4-triazoles as Prospective PET Radioligands for Imaging Brain COX-1 in Monkey. Part 2: Selection and Evaluation of [(11)C]PS13 for Quantitative Imaging. *ACS Chem Neurosci* 2018; **9**(11): 2620-2627.
140. Kim MJ, Lee JH, Juarez Anaya F, Hong J, Miller W, Telu S *et al.* First-in-human evaluation of [(11)C]PS13, a novel PET radioligand, to quantify cyclooxygenase-1 in the brain. *Eur J Nucl Med Mol Imaging* 2020; **47**(13): 3143-3151.
141. Kim MJ, Anaya FJ, Manly LS, Lee JH, Hong J, Shrestha S *et al.* Whole-Body PET Imaging in Humans Shows That (11)C-PS13 Is Selective for Cyclooxygenase-1 and Can Measure the In Vivo Potency of Nonsteroidal Antiinflammatory Drugs. *J Nucl Med* 2023; **64**(1): 159-164.
142. Taddei C, Morse CL, Kim MJ, Liow JS, Montero Santamaria J, Zhang A *et al.* Synthesis of [(18)F]PS13 and Evaluation as a PET Radioligand for Cyclooxygenase-1 in Monkey. *ACS Chem Neurosci* 2021; **12**(3): 517-530.
143. Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A *et al.* Reactive astrocyte nomenclature, definitions, and future directions. *Nat Neurosci* 2021; **24**(3): 312-325.
144. Westlund KN, Denney RM, Kochersperger LM, Rose RM, Abell CW. Distinct monoamine oxidase A and B populations in primate brain. *Science* 1985; **230**(4722): 181-183.

145. Fowler JS, MacGregor RR, Wolf AP, Arnett CD, Dewey SL, Schlyer D *et al.* Mapping human brain monoamine oxidase A and B with 11C-labeled suicide inactivators and PET. *Science* 1987; **235**(4787): 481-485.
146. 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.
147. Fowler JS, Wang GJ, Logan J, Xie S, Volkow ND, MacGregor RR *et al.* Selective reduction of radiotracer trapping by deuterium substitution: comparison of carbon-11-L-deprenyl and carbon-11-deprenyl-D2 for MAO B mapping. *J Nucl Med* 1995; **36**(7): 1255-1262.
148. Kumlien E, Bergstrom M, Lilja A, Andersson J, Szekeres V, Westerberg CE *et al.* Positron emission tomography with [11C]deuterium-deprenyl in temporal lobe epilepsy. *Epilepsia* 1995; **36**(7): 712-721.
149. Bergstrom M, Kumlien E, Lilja A, Tyrefors N, Westerberg G, Langstrom B. Temporal lobe epilepsy visualized with PET with 11C-L-deuterium-deprenyl--analysis of kinetic data. *Acta Neurol Scand* 1998; **98**(4): 224-231.
150. Kumlien E, Nilsson A, Hagberg G, Langstrom B, Bergstrom M. PET with 11C-deuterium-deprenyl and 18F-FDG in focal epilepsy. *Acta Neurol Scand* 2001; **103**(6): 360-366.
151. Fowler JS, Volkow ND, Cilento R, Wang GJ, Felder C, Logan J. Comparison of Brain Glucose Metabolism and Monoamine Oxidase B (MAO B) in Traumatic Brain Injury. *Clin Positron Imaging* 1999; **2**(2): 71-79.
152. Ekblom J, Jossan SS, Gillberg PG, Orelund L, Aquilonius SM. Monoamine oxidase-B in motor cortex: changes in amyotrophic lateral sclerosis. *Neuroscience* 1992; **49**(4): 763-769.
153. Aquilonius SM, Jossan SS, Ekblom JG, Askmark H, Gillberg PG. Increased binding of 3H-L-deprenyl in spinal cords from patients with amyotrophic lateral sclerosis as demonstrated by autoradiography. *J Neural Transm Gen Sect* 1992; **89**(1-2): 111-122.
154. Ekblom J, Jossan SS, Bergstrom M, Orelund L, Walum E, Aquilonius SM. Monoamine oxidase-B in astrocytes. *Glia* 1993; **8**(2): 122-132.
155. Johansson A, Engler H, Blomquist G, Scott B, Wall A, Aquilonius SM *et al.* Evidence for astrocytosis in ALS demonstrated by [11C](L)-deprenyl-D2 PET. *J Neurol Sci* 2007; **255**(1-2): 17-22.
156. Fowler JS, Volkow ND, Wang GJ, Logan J, Pappas N, Shea C, MacGregor R. Age-related increases in brain monoamine oxidase B in living healthy human subjects. *Neurobiol Aging* 1997; **18**(4): 431-435.
157. Arakawa R, Stenkrone P, Takano A, Nag S, Maior RS, Halldin C. Test-retest reproducibility of [(11)C]-L-deprenyl-D(2) binding to MAO-B in the human brain. *EJNMMI Res* 2017; **7**(1): 54.
158. Varnas K, Finnema SJ, Johnstrom P, Arakawa R, Halldin C, Eriksson LI, Farde L. Effects of sevoflurane anaesthesia on radioligand binding to monoamine oxidase-B in vivo. *Br J Anaesth* 2021; **126**(1): 238-244.

159. Gulyas B, Pavlova E, Kasa P, Gulya K, Bakota L, Varszegi S *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.
160. Kadir A, Marutle A, Gonzalez D, Scholl M, Almkvist O, Mousavi M *et al.* Positron emission tomography imaging and clinical progression in relation to molecular pathology in the first Pittsburgh Compound B positron emission tomography patient with Alzheimer's disease. *Brain* 2011; **134**(Pt 1): 301-317.
161. Farid K, Carter SF, Rodriguez-Vieitez E, Almkvist O, Andersen P, Wall A *et al.* Case Report of Complex Amyotrophic Lateral Sclerosis with Cognitive Impairment and Cortical Amyloid Deposition. *J Alzheimers Dis* 2015; **47**(3): 661-667.
162. Kumar A, Fontana IC, Nordberg A. Reactive astrogliosis: A friend or foe in the pathogenesis of Alzheimer's disease. *J Neurochem* 2023; **164**(3): 309-324.
163. Santillo AF, Gambini JP, Lannfelt L, Langstrom B, Ulla-Marja L, Kilander L, Engler H. In vivo imaging of astrogliosis in Alzheimer's disease: an (1)(1)C-L-deuteriodeprenyl and PIB PET study. *Eur J Nucl Med Mol Imaging* 2011; **38**(12): 2202-2208.
164. Rodriguez-Vieitez E, Ni R, Gulyas B, Toth M, Haggkvist J, Halldin C *et al.* Astrogliosis precedes amyloid plaque deposition in Alzheimer APP<sup>sw</sup> transgenic mouse brain: a correlative positron emission tomography and in vitro imaging study. *Eur J Nucl Med Mol Imaging* 2015; **42**(7): 1119-1132.
165. Carter SF, Scholl M, Almkvist O, Wall A, Engler H, Langstrom B, Nordberg A. Evidence for astrogliosis in prodromal Alzheimer disease provided by 11C-deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-FDG. *J Nucl Med* 2012; **53**(1): 37-46.
166. Choo IL, Carter SF, Scholl ML, Nordberg A. Astrogliosis measured by (1)(1)C-deprenyl PET correlates with decrease in gray matter density in the parahippocampus of prodromal Alzheimer's patients. *Eur J Nucl Med Mol Imaging* 2014; **41**(11): 2120-2126.
167. Scholl M, Carter SF, Westman E, Rodriguez-Vieitez E, Almkvist O, Thordardottir S *et al.* Early astrogliosis in autosomal dominant Alzheimer's disease measured in vivo by multi-tracer positron emission tomography. *Sci Rep* 2015; **5**: 16404.
168. Rodriguez-Vieitez E, Saint-Aubert L, Carter SF, Almkvist O, Farid K, Scholl M *et al.* Diverging longitudinal changes in astrogliosis and amyloid PET in autosomal dominant Alzheimer's disease. *Brain* 2016; **139**(Pt 3): 922-936.
169. Carter SF, Chiotis K, Nordberg A, Rodriguez-Vieitez E. Longitudinal association between astrocyte function and glucose metabolism in autosomal dominant Alzheimer's disease. *Eur J Nucl Med Mol Imaging* 2019; **46**(2): 348-356.
170. Vilaplana E, Rodriguez-Vieitez E, Ferreira D, Montal V, Almkvist O, Wall A *et al.* Cortical microstructural correlates of astrogliosis in autosomal-dominant Alzheimer disease. *Neurology* 2020; **94**(19): e2026-e2036.

171. Lemoine L, Gillberg PG, Bogdanovic N, Nennesmo I, Saint-Aubert L, Viitanen M *et al.* Amyloid, tau, and astrocyte pathology in autosomal-dominant Alzheimer's disease variants: AbetaPParc and PSEN1DE9. *Mol Psychiatry* 2021; **26**(10): 5609-5619.
172. Ni R, Rojdner J, Voytenko L, Dyrks T, Thiele A, Marutle A, Nordberg A. In vitro Characterization of the Regional Binding Distribution of Amyloid PET Tracer Florbetaben and the Glia Tracers Deprenyl and PK11195 in Autopsy Alzheimer's Brain Tissue. *J Alzheimers Dis* 2021; **80**(4): 1723-1737.
173. Marutle A, Gillberg PG, Bergfors A, Yu W, Ni R, Nennesmo I *et al.* (3)H-deprenyl and (3)H-PIB autoradiography show different laminar distributions of astroglia and fibrillar beta-amyloid in Alzheimer brain. *J Neuroinflammation* 2013; **10**: 90.
174. Lemoine L, Saint-Aubert L, Nennesmo I, Gillberg PG, Nordberg A. Cortical laminar tau deposits and activated astrocytes in Alzheimer's disease visualised by (3)H-THK5117 and (3)H-deprenyl autoradiography. *Sci Rep* 2017; **7**: 45496.
175. Hirvonen J, Kailajarvi M, Haltia T, Koskimies S, Nagren K, Virsu P *et al.* Assessment of MAO-B occupancy in the brain with PET and [11C]-L-deprenyl-D2: a dose-finding study with a novel MAO-B inhibitor, EVT 301. *Clin Pharmacol Ther* 2009; **85**(5): 506-512.
176. Sturm S, Forsberg A, Nave S, Stenkrona P, Seneca N, Varrone A *et al.* Positron emission tomography measurement of brain MAO-B inhibition in patients with Alzheimer's disease and elderly controls after oral administration of sembragiline. *Eur J Nucl Med Mol Imaging* 2017; **44**(3): 382-391.
177. Nag S, Lehmann L, Heinrich T, Thiele A, Kettschau G, Nakao R *et al.* Synthesis of three novel fluorine-18 labeled analogues of L-deprenyl for positron emission tomography (PET) studies of monoamine oxidase B (MAO-B). *J Med Chem* 2011; **54**(20): 7023-7029.
178. Nag S, Varrone A, Toth M, Thiele A, Kettschau G, Heinrich T *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.
179. Nag S, Fazio P, Lehmann L, Kettschau G, Heinrich T, Thiele A *et al.* In Vivo and In Vitro Characterization of a Novel MAO-B Inhibitor Radioligand, 18F-Labeled Deuterated Fluorodeprenyl. *J Nucl Med* 2016; **57**(2): 315-320.
180. Ballweg A, Klaus C, Vogler L, Katzdobler S, Wind K, Zatcepin A *et al.* [(18)F]F-DED PET imaging of reactive astrogliosis in neurodegenerative diseases: preclinical proof of concept and first-in-human data. *J Neuroinflammation* 2023; **20**(1): 68.
181. Vasdev N, Sadovski O, Moran MD, Parkes J, Meyer JH, Houle S, Wilson AA. Development of new radiopharmaceuticals for imaging monoamine oxidase B. *Nucl Med Biol* 2011; **38**(7): 933-943.
182. Dukic-Stefanovic S, Hang Lai T, Toussaint M, Clauss O, Jevtic, II, Penjisevic JZ *et al.* In vitro and in vivo evaluation of fluorinated indanone derivatives as potential positron emission tomography agents for the imaging of monoamine oxidase B in the brain. *Bioorg Med Chem Lett* 2021; **48**: 128254.

183. Nag S, Lehmann L, Ketschau G, Heinrich T, Thiele A, Varrone A *et al.* Synthesis and evaluation of [(1)(8)F]fluororasagiline, a novel positron emission tomography (PET) radioligand for monoamine oxidase B (MAO-B). *Bioorg Med Chem* 2012; **20**(9): 3065-3071.
184. Nag S, Lehmann L, Ketschau G, Toth M, Heinrich T, Thiele A *et al.* Development of a novel fluorine-18 labeled deuterated fluororasagiline ([18F]fluororasagiline-D2) radioligand for PET studies of monoamine oxidase B (MAO-B). *Bioorg Med Chem* 2013; **21**(21): 6634-6641.
185. Harada R, Hayakawa Y, Ezura M, Lerdsirisuk P, Du Y, Ishikawa Y *et al.* (18)F-SMBT-1: A Selective and Reversible PET Tracer for Monoamine Oxidase-B Imaging. *J Nucl Med* 2021; **62**(2): 253-258.
186. Villemagne VL, Harada R, Dore V, Furumoto S, Mulligan R, Kudo Y *et al.* First-in-Humans Evaluation of (18)F-SMBT-1, a Novel (18)F-Labeled Monoamine Oxidase-B PET Tracer for Imaging Reactive Astrogliosis. *J Nucl Med* 2022; **63**(10): 1551-1559.
187. Villemagne VL, Harada R, Dore V, Furumoto S, Mulligan R, Kudo Y *et al.* Assessing Reactive Astrogliosis with (18)F-SMBT-1 Across the Alzheimer Disease Spectrum. *J Nucl Med* 2022; **63**(10): 1560-1569.
188. Olsen M, Aguilar X, Sehlin D, Fang XT, Antoni G, Erlandsson A, Syvanen S. Astroglial Responses to Amyloid-Beta Progression in a Mouse Model of Alzheimer's Disease. *Mol Imaging Biol* 2018; **20**(4): 605-614.
189. Chatterjee P, Dore V, Pedrini S, Krishnadas N, Thota R, Bourgeat P *et al.* Plasma Glial Fibrillary Acidic Protein Is Associated with 18F-SMBT-1 PET: Two Putative Astrocyte Reactivity Biomarkers for Alzheimer's Disease. *J Alzheimers Dis* 2023; **92**(2): 615-628.
190. Dahl K, Bernard-Gauthier V, Nag S, Varnas K, Narayanaswami V, Mahdi Moein M *et al.* Synthesis and preclinical evaluation of [(18)F]FSL25.1188, a reversible PET radioligand for monoamine oxidase-B. *Bioorg Med Chem Lett* 2019; **29**(13): 1624-1627.
191. Hicks JW, Sadovski O, Parkes J, Houle S, Hay BA, Carter RL *et al.* Radiosynthesis and ex vivo evaluation of [(18)F]-(S)-3-(6-(3-fluoropropoxy)benzo[d]isoxazol-3-yl)-5-(methoxymethyl)oxazolidin-2-one for imaging MAO-B with PET. *Bioorg Med Chem Lett* 2015; **25**(2): 288-291.
192. Saba W, Valette H, Peyronneau MA, Bramouille Y, Coulon C, Curet O *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.
193. Rusjan PM, Wilson AA, Miler L, Fan I, Mizrahi R, Houle S *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.
194. Moriguchi S, Wilson AA, Miler L, Rusjan PM, Vasdev N, Kish SJ *et al.* Monoamine Oxidase B Total Distribution Volume in the Prefrontal Cortex of Major Depressive Disorder: An [11C]SL25.1188 Positron Emission Tomography Study. *JAMA Psychiatry* 2019; **76**(6): 634-641.

195. Gill T, Watling SE, Richardson JD, McCluskey T, Tong J, Meyer JH *et al.* Imaging of astrocytes in posttraumatic stress disorder: A PET study with the monoamine oxidase B radioligand [(11)C]SL25.1188. *Eur Neuropsychopharmacol* 2022; **54**: 54-61.
196. Varnas K, Cselenyi Z, Arakawa R, Nag S, Stepanov V, Moein MM *et al.* The pro-psychotic metabotropic glutamate receptor compounds fenobam and AZD9272 share binding sites with monoamine oxidase-B inhibitors in humans. *Neuropharmacology* 2020; **162**: 107809.
197. Koshimori Y, Cusimano MD, Vieira EL, Rusjan PM, Kish SJ, Vasdev N *et al.* Astroglial marker 11C-SL25.1188 PET in traumatic brain injury with persistent symptoms. *Brain* 2023; **146**(11): 4469-4475.
198. Nag S, Jia Z, Svedberg M, Jackson A, Ahmad R, Luthra S *et al.* Synthesis and Autoradiography of Novel F-18 Labeled Reversible Radioligands for Detection of Monoamine Oxidase B. *ACS Chem Neurosci* 2020; **11**(24): 4398-4404.
199. Varnas K, Nag S, Halldin C, Farde L. PET Evaluation of the Novel F-18 Labeled Reversible Radioligand [(18)F]GEH200449 for Detection of Monoamine Oxidase-B in the Non-Human Primate Brain. *ACS Chem Neurosci* 2023; **14**(17): 3206-3211.
200. Ruiz J, Martin I, Callado LF, Meana JJ, Barturen F, Garcia-Sevilla JA. Non-adrenoceptor [3H]idazoxan binding sites (I2-imidazoline sites) are increased in postmortem brain from patients with Alzheimer's disease. *Neurosci Lett* 1993; **160**(1): 109-112.
201. Qiu WW, Zheng RY. Neuroprotective effects of receptor imidazoline 2 and its endogenous ligand agmatine. *Neurosci Bull* 2006; **22**(3): 187-191.
202. Hudson AL, Tyacke RJ, Lales MD, Davies N, Finn DP, Marti O *et al.* Novel ligands for the investigation of imidazoline receptors and their binding proteins. *Ann N Y Acad Sci* 2003; **1009**: 302-308.
203. Saczewski F, Tabin P, Tyacke RJ, Maconie A, Saczewski J, Kornicka A *et al.* 2-(4,5-Dihydroimidazol-2-yl)benzimidazoles as highly selective imidazoline I2/adrenergic alpha2 receptor ligands. *Bioorg Med Chem* 2006; **14**(19): 6679-6685.
204. Tyacke RJ, Fisher A, Robinson ES, Grundt P, Turner EM, Husbands SM *et al.* Evaluation and initial in vitro and ex vivo characterization of the potential positron emission tomography ligand, BU99008 (2-(4,5-dihydro-1H-imidazol-2-yl)-1-methyl-1H-indole), for the imidazoline(2) binding site. *Synapse* 2012; **66**(6): 542-551.
205. Kealey S, Turner EM, Husbands SM, Salinas CA, Jakobsen S, Tyacke RJ *et al.* Imaging imidazoline-I2 binding sites in porcine brain using 11C-BU99008. *J Nucl Med* 2013; **54**(1): 139-144.
206. Parker CA, Nabulsi N, Holden D, Lin SF, Cass T, Labaree 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.
207. Tyacke RJ, Myers JFM, Venkataraman A, Mick I, Turton S, Passchier J *et al.* Evaluation of (11)C-BU99008, a PET Ligand for the Imidazoline(2) Binding Site in Human Brain. *J Nucl Med* 2018; **59**(10): 1597-1602.

208. Venkataraman AV, Keat N, Myers JF, Turton S, Mick I, Gunn RN *et al.* First evaluation of PET-based human biodistribution and radiation dosimetry of (11)C-BU99008, a tracer for imaging the imidazoline(2) binding site. *EJNMMI Res* 2018; **8**(1): 71.
209. Kumar A, Koistinen NA, Malarte ML, Nennesmo I, Ingelsson M, Ghetti B *et al.* Astroglial tracer BU99008 detects multiple binding sites in Alzheimer's disease brain. *Mol Psychiatry* 2021; **26**(10): 5833-5847.
210. Livingston NR, Calsolaro V, Hinz R, Nowell J, Raza S, Gentleman S *et al.* Relationship between astrocyte reactivity, using novel (11)C-BU99008 PET, and glucose metabolism, grey matter volume and amyloid load in cognitively impaired individuals. *Mol Psychiatry* 2022; **27**(4): 2019-2029.
211. Calsolaro V, Matthews PM, Donat CK, Livingston NR, Femminella GD, Guedes SS *et al.* Astrocyte reactivity with late-onset cognitive impairment assessed in vivo using (11)C-BU99008 PET and its relationship with amyloid load. *Mol Psychiatry* 2021; **26**(10): 5848-5855.
212. Wilson H, Dervenoulas G, Pagano G, Tyacke RJ, Polychronis S, Myers J *et al.* Imidazoline 2 binding sites reflecting astroglia pathology in Parkinson's disease: an in vivo 11C-BU99008 PET study. *Brain* 2019; **142**(10): 3116-3128.
213. Mohamed MA, Zeng Z, Gennaro M, Lao-Kaim NP, Myers JFM, Calsolaro V *et al.* Astroglialosis in aging and Parkinson's disease dementia: a new clinical study with (11)C-BU99008 PET. *Brain Commun* 2022; **4**(5): fcac199.
214. Kawamura K, Yui J, Konno F, Yamasaki T, Hatori A, Wakizaka H *et al.* Synthesis and evaluation of PET probes for the imaging of I2 imidazoline receptors in peripheral tissues. *Nucl Med Biol* 2012; **39**(1): 89-99.
215. Kawamura K, Kimura Y, Yui J, Wakizaka H, Yamasaki T, Hatori A *et al.* PET study using [11C]FTIMD with ultra-high specific activity to evaluate I2-imidazoline receptors binding in rat brains. *Nucl Med Biol* 2012; **39**(2): 199-206.
216. Kawamura K, Naganawa M, Konno F, Yui J, Wakizaka H, Yamasaki T *et al.* Imaging of I2-imidazoline receptors by small-animal PET using 2-(3-fluoro-[4-11C]tolyl)-4,5-dihydro-1H-imidazole ([11C]FTIMD). *Nucl Med Biol* 2010; **37**(5): 625-635.
217. Kawamura K, Maeda J, Hatori A, Okauchi T, Nagai Y, Higuchi M *et al.* In vivo and in vitro imaging of I(2) imidazoline receptors in the monkey brain. *Synapse* 2011; **65**(5): 452-455.
218. Kawamura K, Shimoda Y, Kumata K, Fujinaga M, Yui J, Yamasaki T *et al.* In vivo evaluation of a new (1)(8)F-labeled PET ligand, [(1)(8)F]FEBU, for the imaging of I(2)-imidazoline receptors. *Nucl Med Biol* 2015; **42**(4): 406-412.
219. Kawamura K, Yamasaki T, Zhang Y, Wakizaka H, Hatori A, Xie L *et al.* Change in the Binding of [(11)C]BU99008 to Imidazoline I2 Receptor Using Brain PET in Zucker Rats. *Mol Imaging Biol* 2019; **21**(1): 105-112.
220. Kawamura K, Shimoda Y, Yui J, Zhang Y, Yamasaki T, Wakizaka H *et al.* A useful PET probe [(11)C]BU99008 with ultra-high specific radioactivity for small animal PET imaging of I(2)-imidazoline receptors in the hypothalamus. *Nucl Med Biol* 2017; **45**: 1-7.

221. Brunt TM, Bossong MG. The neuropharmacology of cannabinoid receptor ligands in central signaling pathways. *Eur J Neurosci* 2022; **55**(4): 909-921.
222. Komorowska-Muller JA, Schmole AC. CB2 Receptor in Microglia: The Guardian of Self-Control. *Int J Mol Sci* 2020; **22**(1).
223. Di Marzo V, Stella N, Zimmer A. Endocannabinoid signalling and the deteriorating brain. *Nat Rev Neurosci* 2015; **16**(1): 30-42.
224. Grabon W, Bodennec J, Rheims S, Belmeguenai A, Bezin L. Update on the controversial identity of cells expressing *cnr2* gene in the nervous system. *CNS Neurosci Ther* 2023; **29**(3): 760-770.
225. Onaivi ES. Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology* 2006; **54**(4): 231-246.
226. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al.* Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 2005; **310**(5746): 329-332.
227. Kibret BG, Ishiguro H, Horiuchi Y, Onaivi ES. New Insights and Potential Therapeutic Targeting of CB2 Cannabinoid Receptors in CNS Disorders. *Int J Mol Sci* 2022; **23**(2).
228. García-Gutiérrez MS, Navarrete F, Gasparyan A, Manzanares J. Therapeutic potential of the cannabinoid receptor 2 in neuropsychiatry. *Exploration of Neuroprotective Therapy* 2021: 55-71.
229. Evens N, Muccioli GG, Houbrechts N, Lambert DM, Verbruggen AM, Van Laere K, Bormans GM. Synthesis and biological evaluation of carbon-11- and fluorine-18-labeled 2-oxoquinoline derivatives for type 2 cannabinoid receptor positron emission tomography imaging. *Nucl Med Biol* 2009; **36**(4): 455-465.
230. Evens N, Vandeputte C, Coolen C, Janssen P, Sciort R, Baekelandt V *et al.* Preclinical evaluation of [<sup>11</sup>C]NE40, a type 2 cannabinoid receptor PET tracer. *Nucl Med Biol* 2012; **39**(3): 389-399.
231. Vandeputte C, Casteels C, Struys T, Koole M, van Veghel D, Evens N *et al.* Small-animal PET imaging of the type 1 and type 2 cannabinoid receptors in a photothrombotic stroke model. *Eur J Nucl Med Mol Imaging* 2012; **39**(11): 1796-1806.
232. Ahmad R, Koole M, Evens N, Serdons K, Verbruggen A, Bormans G, Van Laere K. Whole-body biodistribution and radiation dosimetry of the cannabinoid type 2 receptor ligand [<sup>11</sup>C]-NE40 in healthy subjects. *Mol Imaging Biol* 2013; **15**(4): 384-390.
233. Hosoya T, Fukumoto D, Kakiuchi T, Nishiyama S, Yamamoto S, Ohba H *et al.* In vivo TSPO and cannabinoid receptor type 2 availability early in post-stroke neuroinflammation in rats: a positron emission tomography study. *J Neuroinflammation* 2017; **14**(1): 69.
234. Yamagishi S, Iga Y, Nakamura M, Takizawa C, Fukumoto D, Kakiuchi T *et al.* Upregulation of cannabinoid receptor type 2, but not TSPO, in senescence-accelerated neuroinflammation in mice: a positron emission tomography study. *J Neuroinflammation* 2019; **16**(1): 208.

235. Ahmad R, Postnov A, Bormans G, Versijpt J, Vandenbulcke M, Van Laere K. 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.
236. Evens N, Bosier B, Lavey BJ, Kozlowski JA, Vermaelen P, Baudemprez L *et al.* Labelling and biological evaluation of [(11C)methoxy-Sch225336: a radioligand for the cannabinoid-type 2 receptor. *Nucl Med Biol* 2008; **35**(7): 793-800.
237. Vandeputte C, Evens N, Toelen J, Deroose CM, Bosier B, Ibrahimi A *et al.* A PET brain reporter gene system based on type 2 cannabinoid receptors. *J Nucl Med* 2011; **52**(7): 1102-1109.
238. Ahamed M, van Veghel D, Ullmer C, Van Laere K, Verbruggen A, Bormans GM. Synthesis, Biodistribution and In vitro Evaluation of Brain Permeable High Affinity Type 2 Cannabinoid Receptor Agonists [(11C)MA2 and [(18F)MA3. *Front Neurosci* 2016; **10**: 431.
239. Evens N, Vandeputte C, Muccioli GG, Lambert DM, Baekelandt V, Verbruggen AM *et al.* Synthesis, in vitro and in vivo evaluation of fluorine-18 labelled FE-GW405833 as a PET tracer for type 2 cannabinoid receptor imaging. *Bioorg Med Chem* 2011; **19**(15): 4499-4505.
240. Horti AG, Gao Y, Ravert HT, Finley P, Valentine H, Wong DF *et al.* Synthesis and biodistribution of [11C]A-836339, a new potential radioligand for PET imaging of cannabinoid type 2 receptors (CB2). *Bioorg Med Chem* 2010; **18**(14): 5202-5207.
241. Savonenko AV, Melnikova T, Wang Y, Ravert H, Gao Y, Koppel J *et al.* Cannabinoid CB2 Receptors in a Mouse Model of Abeta Amyloidosis: Immunohistochemical Analysis and Suitability as a PET Biomarker of Neuroinflammation. *PLoS One* 2015; **10**(6): e0129618.
242. Pottier G, Gomez-Vallejo V, Padro D, Boisgard R, Dolle F, Llop J *et al.* PET imaging of cannabinoid type 2 receptors with [(11C)A-836339 did not evidence changes following neuroinflammation in rats. *J Cereb Blood Flow Metab* 2017; **37**(3): 1163-1178.
243. Du Y, Coughlin JM, Brosnan MK, Chen A, Shinehouse LK, Abdallah R *et al.* First-in-human imaging using [(11C)MDTC: a radiotracer targeting the cannabinoid receptor type 2. *Eur J Nucl Med Mol Imaging* 2023; **50**(8): 2386-2393.
244. Fujinaga M, Kumata K, Yanamoto K, Kawamura K, Yamasaki T, Yui J *et al.* Radiosynthesis of novel carbon-11-labeled triaryl ligands for cannabinoid-type 2 receptor. *Bioorg Med Chem Lett* 2010; **20**(5): 1565-1568.
245. Turkman N, Shavrin A, Paolillo V, Yeh HH, Flores L, Soghomonian S *et al.* Synthesis and preliminary evaluation of [18F]-labeled 2-oxoquinoline derivatives for PET imaging of cannabinoid CB2 receptor. *Nucl Med Biol* 2012; **39**(4): 593-600.
246. Mu L, Bieri D, Slavik R, Drandarov K, Muller A, Cermak S *et al.* Radiolabeling and in vitro /in vivo evaluation of N-(1-adamantyl)-8-methoxy-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxamide as a PET probe for imaging cannabinoid type 2 receptor. *J Neurochem* 2013; **126**(5): 616-624.
247. Lueg C, Schepmann D, Gunther R, Brust P, Wunsch B. Development of fluorinated CB(2) receptor agonists for PET studies. *Bioorg Med Chem* 2013; **21**(23): 7481-7498.

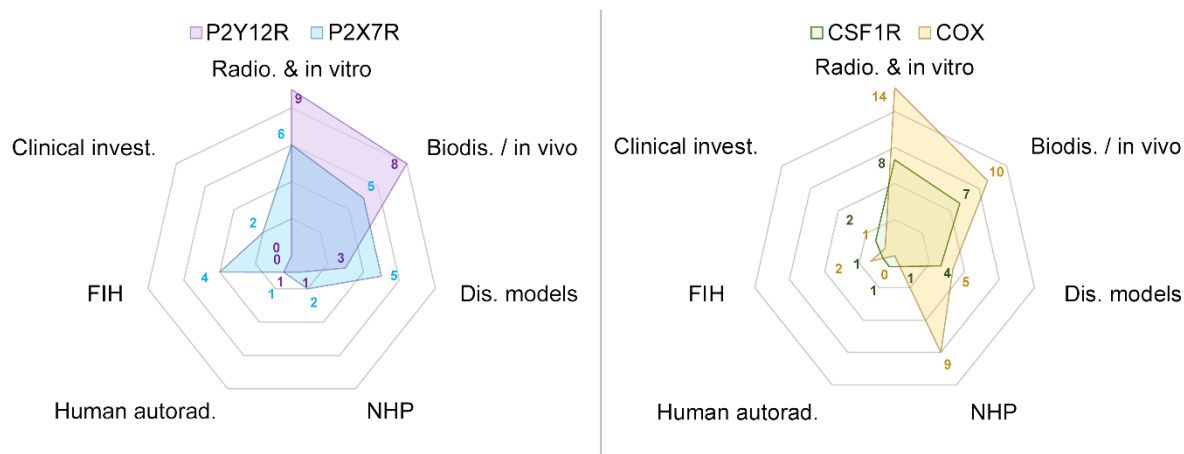
248. Slavik R, Bieri D, Cermak S, Muller A, Kramer SD, Weber M *et al.* Development and evaluation of novel PET tracers for imaging cannabinoid receptor type 2 in brain. *Chimia (Aarau)* 2014; **68**(4): 208-210.
249. Haider A, Muller Herde A, Slavik R, Weber M, Mugnaini C, Ligresti A *et al.* Synthesis and Biological Evaluation of Thiophene-Based Cannabinoid Receptor Type 2 Radiotracers for PET Imaging. *Front Neurosci* 2016; **10**: 350.
250. Slavik R, Muller Herde A, Haider A, Kramer SD, Weber M, Schibli R *et al.* Discovery of a fluorinated 4-oxo-quinoline derivative as a potential positron emission tomography radiotracer for imaging cannabinoid receptor type 2. *J Neurochem* 2016; **138**(6): 874-886.
251. Haider A, Spinelli F, Herde AM, Mu B, Keller C, Margelisch M *et al.* Evaluation of 4-oxo-quinoline-based CB2 PET radioligands in R6/2 chorea huntington mouse model and human ALS spinal cord tissue. *Eur J Med Chem* 2018; **145**: 746-759.
252. Haider A, Kretz J, Gobbi L, Ahmed H, Atz K, Burkler M *et al.* Structure-Activity Relationship Studies of Pyridine-Based Ligands and Identification of a Fluorinated Derivative for Positron Emission Tomography Imaging of Cannabinoid Type 2 Receptors. *J Med Chem* 2019; **62**(24): 11165-11181.
253. Kecheliev V, Spinelli F, Herde A, Haider A, Mu L, Klohs J *et al.* Evaluation of cannabinoid type 2 receptor expression and pyridine-based radiotracers in brains from a mouse model of Alzheimer's disease. *Front Aging Neurosci* 2022; **14**: 1018610.
254. Ueberham L, Gundel D, Kellert M, Deuther-Conrad W, Ludwig FA, Lonnecke P *et al.* Development of the High-Affinity Carborane-Based Cannabinoid Receptor Type 2 PET Ligand [(18)F]LUZ5-d(8). *J Med Chem* 2023; **66**(7): 5242-5260.
255. Kallinen A, Mardon K, Lane S, Montgomery AP, Bhalla R, Stimson DHR *et al.* Synthesis and Preclinical Evaluation of Fluorinated 5-Azaindoles as CB2 PET Radioligands. *ACS Chem Neurosci* 2023; **14**(16): 2902-2921.
256. Pascali G, Panetta D, De Simone M, Burchielli S, Lucchesi V, Sanguinetti E *et al.* Preliminary Investigation of a Novel 18F Radiopharmaceutical for Imaging CB2 Receptors in a SOD Mouse Model. *Australian Journal of Chemistry* 2021; **74**(6): 443-452.
257. Teodoro R, Gundel D, Deuther-Conrad W, Ueberham L, Toussaint M, Bormans G *et al.* Development of [(18)F]LU14 for PET Imaging of Cannabinoid Receptor Type 2 in the Brain. *Int J Mol Sci* 2021; **22**(15).
258. Gundel D, Deuther-Conrad W, Ueberham L, Kaur S, Otikova E, Teodoro R *et al.* Structure-Based Design, Optimization, and Development of [(18)F]LU13: A Novel Radioligand for Cannabinoid Receptor Type 2 Imaging in the Brain with PET. *J Med Chem* 2022; **65**(13): 9034-9049.
259. Slavik R, Herde AM, Bieri D, Weber M, Schibli R, Kramer SD *et al.* Synthesis, radiolabeling and evaluation of novel 4-oxo-quinoline derivatives as PET tracers for imaging cannabinoid type 2 receptor. *Eur J Med Chem* 2015; **92**: 554-564.

260. Slavik R, Grether U, Muller Herde A, Gobbi L, Fingerle J, Ullmer C *et al.* Discovery of a high affinity and selective pyridine analog as a potential positron emission tomography imaging agent for cannabinoid type 2 receptor. *J Med Chem* 2015; **58**(10): 4266-4277.
261. Moldovan RP, Teodoro R, Gao Y, Deuther-Conrad W, Kranz M, Wang Y *et al.* Development of a High-Affinity PET Radioligand for Imaging Cannabinoid Subtype 2 Receptor. *J Med Chem* 2016; **59**(17): 7840-7855.
262. Ni R, Muller Herde A, Haider A, Keller C, Louloudis G, Vaas M *et al.* In vivo Imaging of Cannabinoid Type 2 Receptors: Functional and Structural Alterations in Mouse Model of Cerebral Ischemia by PET and MRI. *Mol Imaging Biol* 2022; **24**(5): 700-709.
263. Bravo GA, Cedeno RR, Casadevall MP, Ramio-Torrenta L. Sphingosine-1-Phosphate (S1P) and S1P Signaling Pathway Modulators, from Current Insights to Future Perspectives. *Cells* 2022; **11**(13).
264. McGinley MP, Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis and other conditions. *Lancet* 2021; **398**(10306): 1184-1194.
265. Chen H, Wang J, Zhang C, Ding P, Tian S, Chen J *et al.* Sphingosine 1-phosphate receptor, a new therapeutic direction in different diseases. *Biomed Pharmacother* 2022; **153**: 113341.
266. Brinkmann V. FTY720 (fingolimod) in Multiple Sclerosis: therapeutic effects in the immune and the central nervous system. *Br J Pharmacol* 2009; **158**(5): 1173-1182.
267. Briard E, Orain D, Beerli C, Billich A, Streiff M, Bigaud M, Auberson YP. BZM055, an iodinated radiotracer candidate for PET and SPECT imaging of myelin and FTY720 brain distribution. *ChemMedChem* 2011; **6**(4): 667-677.
268. Rokka J, Federico C, Jurttila J, Snellman A, Haaparanta M, Rinne JO, Solin O. 19F/18F exchange synthesis for a novel [18F]S1P3-radiopharmaceutical. *J Labelled Comp Radiopharm* 2013; **56**(8): 385-391.
269. Prasad VP, Wagner S, Keul P, Hermann S, Levkau B, Schafers M, Haufe G. 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.
270. Briard E, Rudolph B, Desrayaud S, Krauser JA, Auberson YP. MS565: A SPECT Tracer for Evaluating the Brain Penetration of BAF312 (Siponimod). *ChemMedChem* 2015; **10**(6): 1008-1018.
271. Rosenberg AJ, Liu H, Tu Z. A practical process for the preparation of [(32)P]S1P and binding assay for S1P receptor ligands. *Appl Radiat Isot* 2015; **102**: 5-9.
272. Liu H, Jin H, Yue X, Luo Z, Liu C, Rosenberg AJ, Tu Z. PET Imaging Study of S1PR1 Expression in a Rat Model of Multiple Sclerosis. *Mol Imaging Biol* 2016; **18**(5): 724-732.
273. Rosenberg AJ, Liu H, Jin H, Yue X, Riley S, Brown SJ, Tu Z. Design, Synthesis, and In Vitro and In Vivo Evaluation of an (18)F-Labeled Sphingosine 1-Phosphate Receptor 1 (S1P1) PET Tracer. *J Med Chem* 2016; **59**(13): 6201-6220.

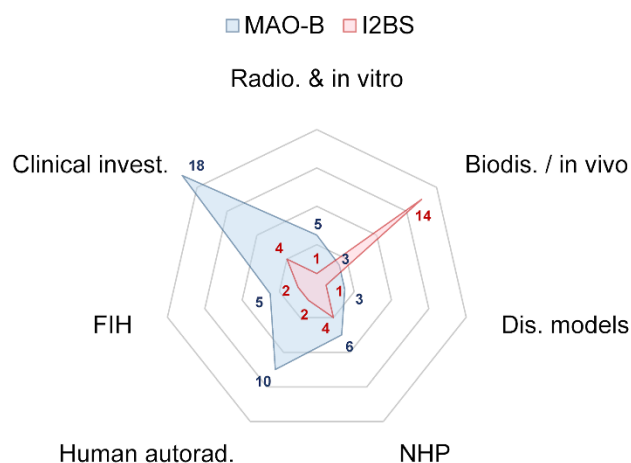
274. Jin H, Yang H, Liu H, Zhang Y, Zhang X, Rosenberg AJ *et al.* A promising carbon-11-labeled sphingosine-1-phosphate receptor 1-specific PET tracer for imaging vascular injury. *J Nucl Cardiol* 2017; **24**(2): 558-570.
275. Liu H, Jin H, Yue X, Han J, Baum P, Abendschein DR, Tu Z. PET Study of Sphingosine-1-Phosphate Receptor 1 Expression in Response to Vascular Inflammation in a Rat Model of Carotid Injury. *Mol Imaging* 2017; **16**: 1536012116689770.
276. Liu H, Jin H, Han J, Yue X, Yang H, Zayed MA *et al.* Upregulated Sphingosine 1-Phosphate Receptor 1 Expression in Human and Murine Atherosclerotic Plaques. *Mol Imaging Biol* 2018; **20**(3): 448-456.
277. Jiang H, Joshi S, Liu H, Mansor S, Qiu L, Zhao H *et al.* In Vitro and In Vivo Investigation of S1PR1 Expression in the Central Nervous System Using [(3)H]CS1P1 and [(11)C]CS1P1. *ACS Chem Neurosci* 2021; **12**(19): 3733-3744.
278. Chand GB, Jiang H, Miller JP, Rhodes CH, Tu Z, Wong DF. Differential Sphingosine-1-Phosphate Receptor-1 Protein Expression in the Dorsolateral Prefrontal Cortex Between Schizophrenia Type 1 and Type 2. *Front Psychiatry* 2022; **13**: 827981.
279. Liu H, Laforest R, Gu J, Luo Z, Jones LA, Gropler RJ *et al.* Acute Rodent Tolerability, Toxicity, and Radiation Dosimetry Estimates of the S1P1-Specific Radioligand [(11)C]CS1P1. *Mol Imaging Biol* 2020; **22**(2): 285-292.
280. Luo Z, Gu J, Dennett RC, Gaehle GG, Perlmutter JS, Chen DL *et al.* Automated production of a sphingosine-1 phosphate receptor 1 (S1P1) PET radiopharmaceutical [(11)C]CS1P1 for human use. *Appl Radiat Isot* 2019; **152**: 30-36.
281. Brier MR, Hamdi M, Rajamanikam J, Zhao H, Mansor S, Jones LA *et al.* Phase 1 Evaluation of (11)C-CS1P1 to Assess Safety and Dosimetry in Human Participants. *J Nucl Med* 2022; **63**(11): 1775-1782.
282. Qiu L, Jiang H, Yu Y, Gu J, Wang J, Zhao H *et al.* Radiosynthesis and evaluation of a fluorine-18 radiotracer [(18)F]FS1P1 for imaging sphingosine-1-phosphate receptor 1. *Org Biomol Chem* 2022; **20**(5): 1041-1052.
283. Liu H, Luo Z, Gu J, Jiang H, Joshi S, Shoghi KI *et al.* In vivo Characterization of Four (18)F-Labeled S1PR1 Tracers for Neuroinflammation. *Mol Imaging Biol* 2020; **22**(5): 1362-1369.
284. Luo Z, Liu H, Yu Y, Gropler RJ, Klein RS, Tu Z. Synthesis and evaluation of highly selective quinazoline-2,4-dione ligands for sphingosine-1-phosphate receptor 2. *RSC Med Chem* 2022; **13**(2): 202-207.
285. Luo Z, Rosenberg AJ, Liu H, Han J, Tu Z. Syntheses and in vitro evaluation of new S1PR1 compounds and initial evaluation of a lead F-18 radiotracer in rodents. *Eur J Med Chem* 2018; **150**: 796-808.
286. Jiang H, Gu J, Zhao H, Joshi S, Perlmutter JS, Gropler RJ *et al.* PET Study of Sphingosine-1-phosphate Receptor 1 Expression in Response to *S. aureus* Infection. *Mol Imaging* 2021; **2021**: 9982020.

287. Luo Z, Han J, Liu H, Rosenberg AJ, Chen DL, Gropler RJ *et al.* Syntheses and in vitro biological evaluation of S1PR1 ligands and PET studies of four F-18 labeled radiotracers in the brain of nonhuman primates. *Org Biomol Chem* 2018; **16**(47): 9171-9184.
288. Qiu L, Jiang H, Zhou C, Wang J, Yu Y, Zhao H *et al.* Discovery of a Promising Fluorine-18 Positron Emission Tomography Radiotracer for Imaging Sphingosine-1-Phosphate Receptor 1 in the Brain. *J Med Chem* 2023; **66**(7): 4671-4688.
289. Ye M, Gai Y, Ji H, Jiang Y, Qiao P, Wang W *et al.* A Novel Radioimmune (99m)Tc-Labeled Tracer for Imaging Sphingosine 1-Phosphate Receptor 1 in Tumor Xenografts: An In Vitro and In Vivo Study. *Front Immunol* 2021; **12**: 660842.
290. Sehlin D, Syvanen S, faculty M. Engineered antibodies: new possibilities for brain PET? *Eur J Nucl Med Mol Imaging* 2019; **46**(13): 2848-2858.

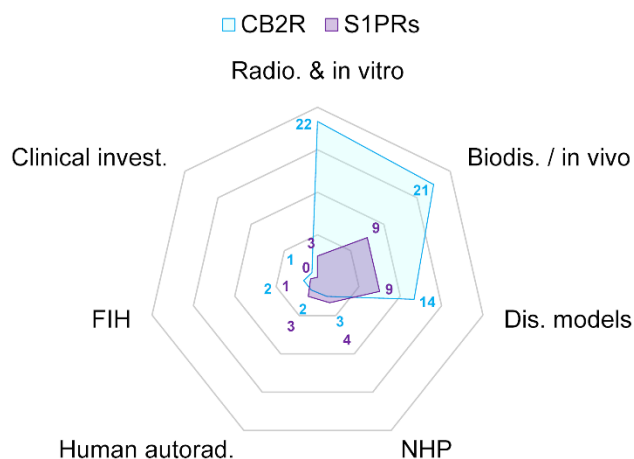
## Figure legends



**Figure 1:** Development stages of radiotracers for the currently explored microglial targets (purinergic receptors P2Y12R and P2X7R on the left, Colony-Stimulating Factor-1 Receptor (CSF1R) and cyclooxygenase (COX) on the right) based on the numbers of published articles in each of the category, listed as follow: **Radio. & in vitro**, Radiochemistry and *in vitro* studies; **Biodis. / in vivo**, Biodistribution and/or preclinical imaging in naive animals; **Dis. models**, Preclinical imaging in disease models; **NHP**, Non-Human Primate & large animal studies; **Human autorad.**, Translational autoradiography (human tissue); **FIH**, First-in-human & modelling; **Clinical invest.**, Clinical studies.



**Figure 2:** Development stages of radiotracers for the currently explored astrocytic targets based on the numbers of published articles in each of the category, listed as follow: **Radio. & in vitro**, Radiochemistry and *in vitro* studies; **Biodis. / in vivo**, Biodistribution and/or preclinical imaging in naive animals; **Dis. models**, Preclinical imaging in disease models; **NHP**, Non-Human Primate & large animal studies; **Human autorad.**, Translational autoradiography (human tissue); **FIH**, First-in-human & modelling; **Clinical invest.**, Clinical studies.



**Figure 3:** development stages of radiotracers for CB2R and S1PRs targets based on the numbers of published articles in each of the category, listed as follow: **Radio. & in vitro**, Radiochemistry and *in vitro* studies; **Biodis. / in vivo**, Biodistribution and/or preclinical imaging in naive animals; **Dis. models**, Preclinical imaging in disease models; **NHP**, Non-Human Primate & large animal studies; **Human autorad.**, Translational autoradiography (human tissue); **FIH**, First-in-human & modelling; **Clinical invest.**, Clinical studies.