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

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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): P2Y₁₂R, P2X₇R, CSF1R, COX (microglial targets), MAO-B, I2BS (astrocytic targets), CB₂R & 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 S₁P₁Rs, CSF1R) are being implemented in clinical investigations. Other targets such as CB₂R and P2X₇R 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 Jones¹ suggesting the concomitance of “inflammation of the brain” and *delirium tremens*, and later, demonstration in the early 20th 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 see²). 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 see³). 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)^{4, 5}, later renamed translocator protein 18kDa⁶, that is expressed in activated microglia and astrocytes, and of the associated ligand PK11195 that could be radiolabelled with [¹¹C] led to a long series of positron emission tomography (PET) investigations in a broad range of neurological conditions⁷⁻¹¹. 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 conditions¹¹. Consequently, second generation TSPO radioligands were developed to overcome this limitation⁹; however one must note that findings from [¹¹C]PK11195 PET studies have never been contradicted by more recent ones performed with new TSPO tracers. Moreover, with one of those radiotracers, [¹¹C]PBR28, a single nucleotide polymorphism rs6971 of the TSPO gene was discovered¹², and while this issue affects more or less severely all 2nd generation TSPO radiotracers¹³, it does not affect the binding of [¹¹C]PK11195. Since then 3rd and 4th generation radiotracers that are also not affected by this polymorphism have been developed^{14, 15} (for review see¹¹). 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 TSPO¹⁶⁻¹⁹.

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 levels²⁰⁻²² (for review see²³). In schizophrenia, the results are not as clear, whereas the first PET studies showed increases in TSPO²⁴⁻²⁶ or positive correlation between symptoms and TSPO levels²⁷, later studies showed no difference between patients and controls²⁸⁻³³ or even significant decreases in schizophrenic patients³⁴. 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^{11, 23, 35, 36}); 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 inflammation³⁷ or medication status^{29, 33}.

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 astrogliosis (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^{38, 39}). 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 [¹¹C]TGN-020⁴⁰, C-X-C chemokine receptor type 4 (CXCR4), imaged with [⁶⁸Ga]^{41, 42} and Tissue transglutaminase (TG2)^{43, 44}, are all exclusively used in cancer imaging, including brain tumours. Numerous radiotracers have also been employed to image adenosine receptors 1 (A1)⁴⁵ and 2 (A2)⁴⁶, 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 [¹⁸F]CPFPX to image A1 receptors after stroke⁴⁷ and one clinical study using [¹¹C]TMSX to image A2 receptors in multiple sclerosis⁴⁸.

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 astrogliosis) 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^{49, 50}. Although they are expressed by different cell types, in particular the peripheral immune cells^{51, 52} and glial cells, i.e. oligodendrocytes and astrocytes^{53, 54}, they are mainly expressed by microglia⁵⁵, while their expression by neurons is still controversial⁵⁶. P2X7R are also expressed by tumour cells⁵⁷.

The involvement of P2X7R in several CNS disorders was initially highlighted by Burnstock⁵⁸. It was proposed that ATP released by injured neurons induce the microglial activation through P2X7R stimulation⁵⁹. 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^{60, 61}), and also in mood disorders (see for review⁶²).

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 [¹¹C]-labelled antagonists, A-740003⁶³ and GSK1482160⁶⁴. Since then, new radioligands from different chemical families labelled either with [¹¹C] or [¹⁸F] have been developed (Figure S1), and their main characteristics have recently been reviewed⁶⁵.

These ligands belong mainly to four different chemical families. Some of them have not yet been studied in humans, such as [¹¹C]GSK1482160 and its fluorinated derivative [¹⁸F]4A^{66, 67}. 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 [¹¹C]SMW139 in relapsing remitting multiple sclerosis⁶⁸. Several [¹¹C]-labelled and [¹⁸F]-labelled JNJ derivatives, although having better affinity and suitable properties for human use^{69,70}, were not able to discriminate healthy controls from patients, as assessed for [¹¹C]JNJ-54173717 in PD⁷¹. The fluorinated derivative [¹⁸F]JNJ-64413739 with promising properties *in vivo* in healthy humans^{72,73} 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 patients⁷⁴.

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 NI⁷⁵, it seems that, at least in several pathological conditions, the exploration of P2X7R offers little added value when compared to TSPO^{74,76}.

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^{77,78}. 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 oligodendrocytes⁷⁷. Furthermore, *P2ry12* gene was confirmed to be expressed by human microglia⁷⁹⁻⁸². Such disease-associated signatures have been identified in models of AD, amyotrophic lateral sclerosis and MS and have been termed microglial neurodegenerative phenotype (MGnD)⁸³ or disease-associated microglia (DAM)⁷⁸.

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 phase⁸³. Other preclinical studies utilizing models of MS also demonstrated changes in P2Y12R expression depending on the disease state^{84,85}. 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 cortices⁸⁶.

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 P2Y₁₂ receptor belongs to the P2Y family of G protein-coupled receptors including eight subtypes: P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, and P2Y₁₄, which are activated by extracellular nucleotides. They are further subdivided into P2Y₁-like, G_q protein-coupled receptors (P2Y_{1,2,4,6,11}) and P2Y₁₂-like receptors (P2Y_{12,13,14}) which are coupled to G_i protein, and thus leading to reduced intracellular cAMP production upon activation. Besides its expression on microglia, P2Y₁₂ 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 P2Y₁₂ receptor have been identified^{51, 87}.

The group of Janssen *et al.* (2015) published one of the first reports on radioligands for the P2Y₁₂ receptor, describing the synthesis of a carbon-11 labelled P2Y₁₂ receptor antagonist⁸⁸. Later the same group used this compound for autoradiographic studies *i*) in a rat EAE model⁸⁴, *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 tissue⁸⁹. Their results confirmed specific binding of the [¹¹C]-P2Y₁₂R 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 brains⁸⁹. The work by Villa *et al.*⁸⁹ 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 P2Y₁₂R antagonist [¹¹C]AZD1283 has been described by two groups^{86, 90}. Although Maeda and colleagues could show disease-related changes of P2Y₁₂R expression in mouse models of Alzheimer's and tau pathologies using [¹¹C]AZD1283 autoradiography, no significant brain uptake was found *in vivo*⁸⁶. The same is true for Jackson and colleagues who investigated metabolism and *in vivo* uptake of [¹¹C]AZD1283 in mice and non-human primates (NHPs)⁹⁰. 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 P2Y₁₂R 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 SUVs⁹¹. 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 P2Y₁₂R 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 NI⁹². These functions, the specific microglial expression of CSF1R in the brain as well as its up regulation in activated microglia in many neurological diseases⁹³ made CSF1R a potential biomarker and hence an attractive imaging target of NI (Figure S3).

The first publication⁹⁴ 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 publication⁹⁴, later renamed [¹⁸F]FOMPyD⁹⁵) retained the specificity and selectivity for CSF1R. However, this radiotracer suffered from cumbersome radiochemistry conditions, and inconclusive blocking studies⁹⁵. In the meantime, another compound, [¹¹C]AZ683 ([¹¹C]-radiolabelled AZD6495 referred to in⁹⁶), had been synthesised and tested in rats and primate, but showed a low brain uptake⁹⁷. Soon after, Horti *et al.*⁹⁸ investigated the new CSF1R PET tracer, [¹¹C]CPPC⁹⁹, in several mouse NI models (LPS, EAE, AD) and in primates, as well as in post-mortem human brain tissue. This publication showed increased [¹¹C]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 [¹¹C]CPPC uptake, which they attributed to a low level of expression of CSF1R in basal condition vs high non-specific binding. Finally, they examined [¹¹C]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 [¹¹C]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 tracer⁹⁸. Following this characterisation study, the same group performed the first-in-man PET study¹⁰⁰, although without blocking experiments. However, the results of Horti *et al.*⁹⁸ must be examined in the context of another study by Knight *et al.*¹⁰¹ who investigated further the binding and specificity of [³H]CPPC for CSF1R *in vitro*. Worryingly, Knight *et al.*¹⁰¹ demonstrated by autoradiography of brain sections after central administration of LPS or in a model of glioblastoma in rat that [³H]CPPC binding was low and did not colocalise with immunostaining for CSF1R, microglia Iba1 or [³H]PBR28 (TSPO) binding. Similarly, saturation experiments showed that [³H]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 [³H]PBR28 demonstrated a

significant increase in displaceable and saturable binding in the ipsilateral side. Knight *et al.*¹⁰¹ also questioned the specificity of [³H]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 IC₅₀ in the nanomolar range for insulin receptor (70 nM), tyrosine protein kinase LCK (22 nM), and vascular endothelial growth factor receptor 2 (74 nM)¹⁰¹. Thus, CPPC might not be such a suitable CSF1R radiotracer.

Despite these observations, Rubin *et al.*¹⁰² recently used [¹¹C]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 virus¹⁰³⁻¹⁰⁸, except for one study in VS-PWH¹⁰⁹.

Zhou *et al.*¹¹⁰ developed the ¹¹C-radiolabelling of the CSF1R reference inhibitor GW2580 and compared it directly with [¹¹C]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.*¹⁰¹, they found that [¹¹C]GW2580 was a slightly better tracer than [¹¹C]CPPC in terms of displaceable (specific) binding and consequently signal-to-noise ratio. More recently, Altomonte *et al.*¹¹¹ developed fluorinated analogues of [¹¹C]CPPC, identifying Psa374 as the best candidate. As Psa374 can also be labelled with ¹¹C, they used [¹¹C]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 [¹¹C]CPPC and [¹¹C]- or [¹⁸F]-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.*^{96, 112, 113} also performed the radiolabelling of identified or chemically derived CSF1R inhibitors. Unfortunately, BLZ945^{96, 114} was shown to be a Pgp substrate and displayed low selectivity towards CSF1R vs other kinases, while compound #5^{112, 115} displayed low brain uptake, and hybrid molecules such as compound #4¹¹³ 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.*¹¹⁶) and fluorinated derivative (compound #4 in An *et al.*¹¹⁷) of a recently described CSF1R inhibitor¹¹⁸. Interestingly, compound #1 developed by Lee *et al.*¹¹⁶ 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.*¹¹⁷, including their best candidate, compound #4, do not seem suitable PET tracers as the authors established that they were Pgp substrates.

Although [¹¹C]CPPC is certainly the most tested radiotracer for CSF1R and has now reached clinical investigation, it is important to note that most [¹¹C]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 [¹¹C]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 inflammation¹¹⁹. 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., [¹⁸F]SC58125 for COX-2 and [¹⁸F]SC63217 for COX-1. However, although *in vitro* uptake of [¹⁸F]SC58125 corresponding to COX-2 expression by LPS+IFN γ -stimulated macrophages was promising, both tracers had overall low and non-specific *in vivo* brain uptake in rat and NHP¹²⁰.

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 [¹⁸F]celecoxib in rat¹²¹, and a non-specific binding of [¹¹C]celecoxib in mouse brain¹²². Better results (heterogeneous uptake) were obtained in NHP, but requiring high injected dose rather incompatible with human use¹²³. The evaluation of [¹¹C]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 inflammation¹²⁴ or in a model of brain ischemia¹²² in mice. The radiolabelling with fluor-18 of another COX-2 inhibitor, valdecoxib¹²⁵, or its methoxy analogue [¹¹C]MOV¹²⁶ 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 indomethacin¹²⁷ and more recently Nimesulide¹²⁸.

Besides attempts to develop NSAID derivative tracers, other chemical families were considered such as azaindazole¹²⁹ and pyrimidine from which the [¹¹C]MC1 (¹¹C-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)¹³⁰. However, despite these promising properties, [¹¹C]MC1 showed low specific to non-displaceable binding in monkey brain¹³¹. This tracer could however be eventually useful to evaluate COX-2 inhibitors occupancy and/or in peripheral disorders such as rheumatoid arthritis¹³². Another tracer with high COX-2 affinity and selectivity, [¹¹C]TMI (¹¹C-3-(4-methylsulfonylphenyl)-4-phenyl-5-trifluoromethyl isoxazole) seemed also promising in NHPs¹³³ but no human evaluation has been published to date. Thus, it appears that promising PET results with various COX-2 PET tracers¹³⁴ 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 [¹¹C]ketoprofen methyl ester appeared promising, with a high *in vivo* selectivity for COX-1 vs COX-2 in mouse brain confirmed using KO animals¹³⁵. The brain uptake of this tracer was clearly increased after acute NI induced by LPS or quinolinic acid in mouse models¹³⁵ and also in a transgenic mouse model of Alzheimer's disease¹³⁶. In addition, an interesting complementarity between COX-1 and TSPO PET imaging was revealed in the LPS mouse model, demonstrating earlier uptake for [¹¹C]ketoprofen than for [¹¹C]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 [¹⁸F]FKTP-Me¹³⁷ but no data in NHP or human are yet available.

Pike *et al.* developed the highly promising COX-1 PET tracer [¹¹C]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). [¹¹C]PS13 showed a significant uptake in NHP brain which was blocked by COX-1 inhibitors^{131, 138, 139}. These promising properties were then confirmed in healthy humans, in terms of stability, target quantification¹⁴⁰, and even the ability to measure the NSAIDs site occupancy *in vivo*¹⁴¹. The possibility to use the fluorinated analogue [¹¹C]PS13 is being investigated by the same research group¹⁴²; 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* comparison¹⁴³.

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-A¹⁴⁴. 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 ¹¹C¹⁴⁵. Despite the high specificity of [¹¹C]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-B¹⁴⁶. This feature makes [¹¹C]-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 sensitivity¹⁴⁷. $[^{11}\text{C}]$ L-deprenyl-D2 rapidly became the reference PET radiotracer being used to study astrogliosis in different pathologies such as epilepsy¹⁴⁸⁻¹⁵⁰, traumatic brain injury¹⁵¹ or amyotrophic lateral sclerosis¹⁵²⁻¹⁵⁵. In humans, age is a factor contributing to the variability among subjects given the established increased brain expression of MAO-B in normal aging^{146, 156, 157}. In animals, anaesthesia should be carefully chosen considering the inhibitory effect of sevoflurane on radioligand binding to MAO-B¹⁵⁸.

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 Institute¹⁵⁹⁻¹⁶¹. An excellent review on this specific point has recently been published¹⁶². The complex relationship between fibrillar A β 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 β deposition, with main areas of increased $[^{11}\text{C}]$ L-deprenyl-D2 binding being in the frontal, parietal, temporal and medial temporal lobes¹⁶³⁻¹⁶⁶. 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^{167, 168}. 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 metabolism¹⁶⁹. 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 function¹⁷⁰. Nevertheless, further investigations are warranted given the differences in the pathological cascade between variants of AD recently described¹⁷¹. 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 cortex¹⁷²⁻¹⁷⁴. Overall, imaging MAO-B activity agrees with the view of a beneficial role for astrocytes in contributing to A β clearance but also that excess A β 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^{175, 176}. Despite the great success of [¹¹C]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 [¹¹C]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^{177, 178} and the corresponding deuterium-substituted ([¹⁸F]fluorodeprenyl-D2) for which a clinical proof-of-concept has been recently established^{179, 180}, many other potential new radiotracers for MAO-B activity were proposed in the last decade, with varying degrees of success¹⁸¹⁻¹⁸⁴. The most successful developments relate to two new PET tracers for MAO-B activity, [¹⁸F]SMBT-1 on the one hand and [¹¹C]SL25.1188 on the other. [¹⁸F]SMBT-1 is the most recent and demonstrated high selectivity and reversible binding¹⁸⁵. 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 [¹⁸F]SMBT-1 would benefit from a full kinetic analysis with metabolite-corrected arterial input function to formally validate its use as a surrogate imaging biomarker¹⁸⁶⁻¹⁸⁸. However, the relationship between astrocytes and amyloid- β load (and probably tau) may not be the same across different brain regions reflecting a complex regional relationship rather than a global response^{187, 189}. The other alternative radiotracer is the [¹¹C]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^{190, 191}. Radiochemical optimization was needed for first-in-human testing and a formal pharmacokinetic and modelling validation study revealed a similar binding pattern than [¹¹C]L-deprenyl-D2 PET, which is also the case in NHPs, and [¹¹C]SL25.1188 is a predictor of the known concentration of MAO-B *post-mortem* in human^{192, 193}. Interestingly, a case-control study in patients suffering from major depressive disorder (MDD) demonstrated that [¹¹C]SL25.1188 distribution volume was significantly increased in the prefrontal cortex of patients with major depressive episodes¹⁹⁴. The same group also reported preliminary results in post-traumatic stress disorders (PTSD) with or without comorbid MDD¹⁹⁵. 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 drugs¹⁹⁶. In a recent study extending beyond strict neuro-psychiatric applications, researchers investigated patients who had undergone traumatic brain injuries. Utilizing [¹¹C]SL25.1188 PET scans, the study unveiled a persistent presence of astrogliosis in cortical areas. Specifically, an

increase in [¹¹C]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 targeting¹⁹⁷.

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 derivative¹⁹⁸. This new radioligand named [¹⁸F]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 deprenyl¹⁹⁹.

2.2 Imidazoline type-2 binding sites (I2BS)

Historically, a non-adrenergic imidazoline binding was identified in astrocytes, and increased in AD²⁰⁰. 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 effects²⁰¹.

Pharmacological exploration of I2BS started in 2003 with the tritium labelling of BU224 and related compound 2BF1²⁰², which then enabled the testing of multiple compounds by an *in vitro* binding assay²⁰³ (Figure S6). This led to the identification and tritium-labelling of the lead compound BU99008 in 2012²⁰⁴. After successful radiolabelling and biodistribution studies in large animals (pig²⁰⁵, monkey²⁰⁶), the first-in-man studies, including competition scans to assess selectivity and specificity²⁰⁷, along with biodistribution and dosimetry²⁰⁸, were reported in 2018.

Increased [³H]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 [³H]BU99008 and [³H]Deprenyl but an interaction of [³H]BU99008 with MAO-B binding sites cannot be ruled out and could explain the apparent detection of multiple binding sites and different affinity status²⁰⁹. So far, clinical [¹¹C]BU99008 PET studies were performed in neurodegenerative diseases. In AD, a reduced uptake of [¹¹C]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 areas²¹⁰. These observations support a bi-phasic hypothesis with high astrocyte reactivity initially triggered by early A β deposition, followed by astrocyte dystrophy in the advanced stages of the disease^{210, 211}. The same

pattern emerged from two pilot studies in PD, with widespread increases of binding in the early stage²¹² and no difference or decreases of binding in advanced Parkinson, compared to controls^{212, 213}. However, astrogliosis seemed to increase with aging²¹³.

Few alternative radioligands are available. [¹¹C]TEIMD, and [¹¹C]metrazoline were radiolabelled and evaluated in mice²¹⁴. [¹¹C]FTIMD biodistribution was examined in normal rats^{215, 216} and in monkeys²¹⁷. A fluorinated radiotracer, [¹⁸F]BU99018 or FEBU, was evaluated in rodents and showed high specific-binding in the hypothalamus²¹⁸. The same group later used [¹¹C]BU99008 in a genetic rat model of obesity, and reported increased uptake in the hypothalamus²¹⁹, using ultra-high specific activity^{215, 220}.

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 ([¹¹C]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 (CB₂R)

Cannabinoid receptors (CBRs) are part of the endocannabinoid system and belong to the G protein-coupled receptor superfamily. There are two types of CBRs, CB₁ and CB₂, with CB₁R primarily found in the brain and expressed on neurons, but its expression can also occur in cells of the periphery. CB₂ 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^{221, 222}. In fact, over-expression of CB₂R has been linked to NI and activation of microglia and brain macrophages^{222, 223}, thus making it an interesting target for therapeutic intervention and PET radioligand development (Figure S7). However, it should be mentioned that neuronal CB₂R expression has been detected in the brain²²⁴. In particular, Onaivi and colleagues have shown CB₂R expression in the brain of rats and mice exposed to chronic mild stress or in model of drug abuse^{225, 226}. Further findings support an impact of CB₂R in anxiety, depression, schizophrenia, and additional neuropsychiatric disorders^{227, 228}.

Due to the relatively low expression of CB₂ receptors in the brain under normal conditions, and in contrast the abundance of CB₁, an important aspect for the development of radioligands targeting CB₂R is their selectivity over CB₁R. The first PET radioligand with high specificity, selectivity and first-in-human application is the 2-oxoquinoline derivative [¹¹C]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, CB₂R overexpressing and diseased rodents or NHPs before the first-in-human application²²⁹⁻²³². Interestingly it was a group from the Hamamatsu University School of Medicine that showed increased binding of [¹¹C]NE40 at early times points in a rat model of stroke as well as in a senescence-accelerated mouse model and observed that CB₂R expression was linked to activated microglial cells^{233, 234}. Surprisingly, later the Leuven group did not detect the anticipated up-regulation of CB₂R in the brains of AD patients but rather a decrease of CB₂R bioavailability compared to healthy controls²³⁵. Although [¹¹C]NE40 showed a high-affinity (K_i-CB₂R = 9.6nM and a 100-fold selectivity over CB₁R), these unexpected findings could be due to an insufficient selectivity over CB₁R, 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 CB₂R rather than a change in microglial expression. Other oxoquinoline, oxadiazole or indole derivatives have also been studied with PET by the Leuven group²³⁶⁻²³⁹, however [¹¹C]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-11²⁴⁰. *In vivo* preclinical studies with this radioligand showed increased binding of [¹¹C]A-836339 in mouse brains from LPS-treated animals as well as in two different mouse models of Alzheimer's disease^{240, 241}. 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 ischemia²⁴². Interestingly, activation of CB₂R by the selective CB₂ agonist JWH133, induced a decrease in binding of the TSPO PET radioligand [¹⁸F]DPA-714 in the ischemic model, linking its effect to NI. Despite the differences in the animal models, [¹¹C]A-836339 (named [¹¹C]MDTC in the publication) has recently been published in a first-in-human study resuming its safety and pharmacokinetic data²⁴³. Brain V_T 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 CB₂R have been developed so far²⁴⁴⁻²⁵⁶, however only a few have been investigated by *in vivo* PET and demonstrated significant CB₂R binding in preclinical models of NI or CB₂R overexpression. Of those [¹⁸F]LU14 and [¹⁸F]LU13, with nano- and sub-nanomolar binding affinity, demonstrated specific

and significant binding in a rat model with local CB₂R overexpression^{257, 258}. Others as [¹¹C]RS-016, [¹¹C]RSR-056, and [¹⁸F]29 were shown to have specific and increased binding in the brain of LPS-treated mice in comparison to controls or, as for [¹⁸F]RoSMA-18-d6, in animals with cerebral ischemia²⁵⁹⁻²⁶².

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)²⁶³ and are implicated in numerous physiological CNS functions^{264, 265}.

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 S1PRs²⁶⁶, and was first iodinated by Novartis in 2011 to yield the radiotracer [^{123/124}I]BZM055²⁶⁷. In the following years, independent groups reported the fluorine-18 labelling of other molecules (a 6-substituted indole-3-carboxylic acid amide compound²⁶⁸, the antagonist W146²⁶⁹, two new sphingosine analogues, and a siponimod derivative²⁷⁰). But none of these initial tracers were further developed (Figure S8).

Following the development of a binding assay for S1PR ligands using [³²P]S1P²⁷¹, 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 (EAE²⁷² and LPS²⁷³). Three different radiotracers were used in these seminal studies. Importantly, vascular diseases (stenosis, balloon injury, atherosclerosis) were also explored in these initial studies with [¹¹C]TZ3321²⁷⁴⁻²⁷⁶.

The clinical translation of [¹¹C]TZ3321, also named [¹¹C]CS1P1, was carefully prepared through *i*) autoradiographic studies performed on human tissues with its tritiated derivative^{277, 278}, *ii*) a dosimetry study in rodents²⁷⁹ and *iii*) the implementation of radiosynthesis under Good Manufacturing Practices (GMP)²⁸⁰. The phase 1 evaluation (safety and dosimetry) of this tracer was finally reported in 2022²⁸¹. The fluorine-18 labelling of the same molecule was recently implemented, giving rise to the radiotracer [¹⁸F]FS1P1, which shares the same structure as [¹¹C]CS1P1²⁸².

In parallel, a wealth of new tracers is continuously reported by the same research group (Pr Zhude Tu, St. Louis, USA), targeting S1P1²⁸³ or S1P2²⁸⁴, and evaluated in rodents^{285, 286} and in non-human primates (Figure 3)^{287, 288}. Of note, a monoclonal antibody against S1P1 receptor was labelled with ^{99m}Tc for immunopET²⁸⁹.

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 [³H]CS1P1 in type 2 schizophrenia subjects compared to type 1 (n=5 each), matching the known differential expression of S1P1 receptor²⁷⁸.

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 [¹¹C]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 BBB²⁹⁰. 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>).

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Figure legends

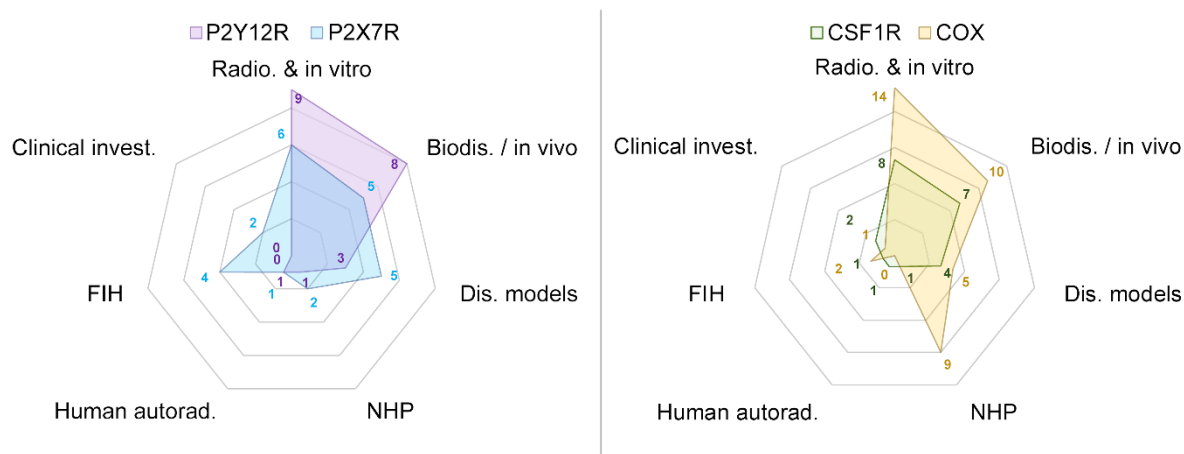


Figure 1: Development stages of radiotracers for the currently explored microglial targets (purinergic receptors P2Y12R and P2X7R on the left, Colony-Stimulating Factor-1 Receptor (CSF1R) and cyclooxygenase (COX) on the right) based on the numbers of published articles in each of the category, listed 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.

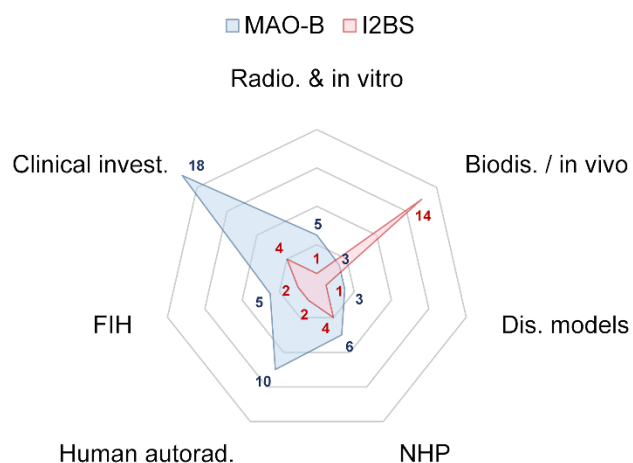


Figure 2: Development stages of radiotracers for the currently explored astrocytic targets based on the numbers of published articles in each of the category, listed as follow: **Radio. & in vitro**, Radiochemistry and *in vitro* studies; **Biodis. / in vivo**, Biodistribution and/or preclinical imaging 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.

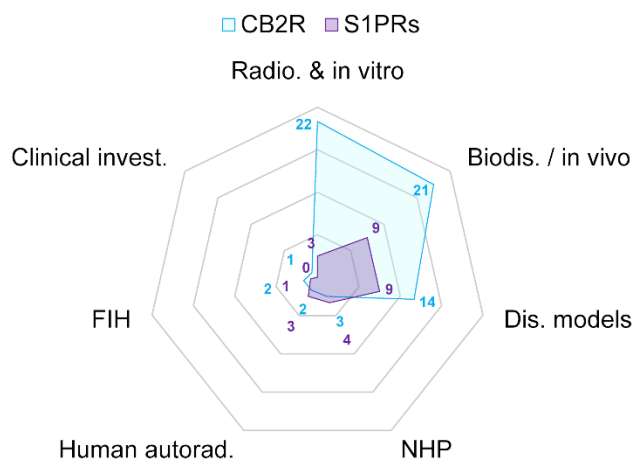


Figure 3: development stages of radiotracers for CB2R and S1PRs targets based on the numbers of published articles in each of the category, listed as follow: **Radio. & in vitro**, Radiochemistry and *in vitro* studies; **Biodis. / in vivo**, Biodistribution and/or preclinical imaging in naive animals; **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.