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LETTER TO THE EDITOR

In response to: The validity of ^{18}F-GE180 as a TSPO imaging agent

Nathalie L. Albert[1] · Marcus Unterrainer[1] · Matthias Brendel[1] · Lena Kaiser[1] · Markus Zweckstetter[2,3] · Paul Cumming[4,5] · Peter Bartenstein[1]

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Dear Sir,

We read with great interest the letter by Zanotti-Fregonara et al. calling into question the fitness of ^{18}F-GE180 as a PET tracer for the 18-kDa mitochondrial translocator protein (TSPO) in human brain [1]. They attribute to ^{18}F-GE180 poor imaging characteristics due to its low uptake in healthy brain and flat time–activity curves, concluding that the increased ^{18}F-GE180 uptake seen in lesions of patients with multiple sclerosis (MS) [2, 3] or glioma [4–6] is merely an "aspecific accumulation through a broken BBB" [1].

We dispute their claim based on our experience in patients with various central nervous system (CNS) disorders, particularly MS and glioma, and on evidence from the literature. Contrary to the statement by Zanotti-Fregonara et al. [1], we have observed that lesional uptake of ^{18}F-GE180 does *not* correlate with the local intensity of contrast enhancement on MRI (e.g. see Fig. 1). Notably, the presence of contrast enhancement on MRI is not necessarily associated with focally elevated ^{18}F-GE180 uptake. For example, a treatment-associated disruption of the blood–brain barrier (BBB) can occur in gliomapatients after radiotherapy in the absence of elevated ^{18}F-GE180 uptake (see Fig. 1b). This discordance demonstrates that BBB disruption is not a sufficient cause of accumulation of ^{18}F-GE180. Conversely, pronounced ^{18}F-GE180 uptake in the absence of contrast enhancement on MRI has been reported in patients suffering from diverse neurological diseases, such as untreated brain tumour (see Fig. 1c), MS [2], progressive supranuclear palsy [7], IgLON5-associated encephalitis [8], CNS vasculitis [9] and progressive multifocal leukoencephalopathy [10], indicating that increased ^{18}F-GE180 uptake can indeed occur without evident BBB disruption.

Certainly, MRI with a high molecular weight contrast agent may not capture all potential microleakages of the BBB. We also acknowledge that additional factors (e.g. reduced P-glycoprotein activity) might enhance BBB permeability to ^{18}F-GE180 under disease conditions. We suppose that some impairment or perturbation of the BBB in lesions is permissive to ^{18}F-GE180 uptake, despite clear evidence of BBB breakdown on MRI. Conceivably, this may be related to the molecular weight of gadolinium contrast agents (>500 Da), such that there is an imperfect relationship between BBB integrity on MRI and permeability to a lower molecular weight PET tracer. However, specific binding of ^{18}F-GE180 'beyond the BBB' can be postulated, keeping in mind the constellation of cases with 'macrodisruption' of the BBB on MRI, but without increased ^{18}F-GE180 uptake.

The specificity of ^{18}F-GE180 for TSPO has been confirmed in mouse models that show an extraordinarily high correlation between ^{18}F-GE180 binding in vivo and immunostaining for the microglial markers CD68 and Iba1 [11–14]. Such correlation studies have not yet been performed in humans and the abundant tracer uptake in mouse brain parenchyma stands in contrast to the low BBB permeability reported for ^{18}F-GE180 in healthy human brain, which is less than 10% of that reported for other TSPO tracers [15]. However, given the clearly elevated ^{18}F-GE180 uptake in human brain lesions (even in areas without apparent BBB disruption), we consider that ^{18}F-GE180 PET is a highly promising tool to provide clinically valuable information about inflammatory activity and extent of disease.

As suggested by Zanotti-Fregonara et al., pharmacological displacement studies should be used to test the specificity of

✉ Nathalie L. Albert
Nathalie.Albert@med.uni-muenchen.de

1 Department of Nuclear Medicine, University Hospital, LMU Munich, Munich, Germany

2 Department for NMR-Based Structural Biology, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

3 German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany

4 Department of Nuclear Medicine, Inselspital, University Hospital Bern, Bern, Switzerland

5 School of Psychology and Counselling and IHBI, Queensland University of Technology, Brisbane, Australia

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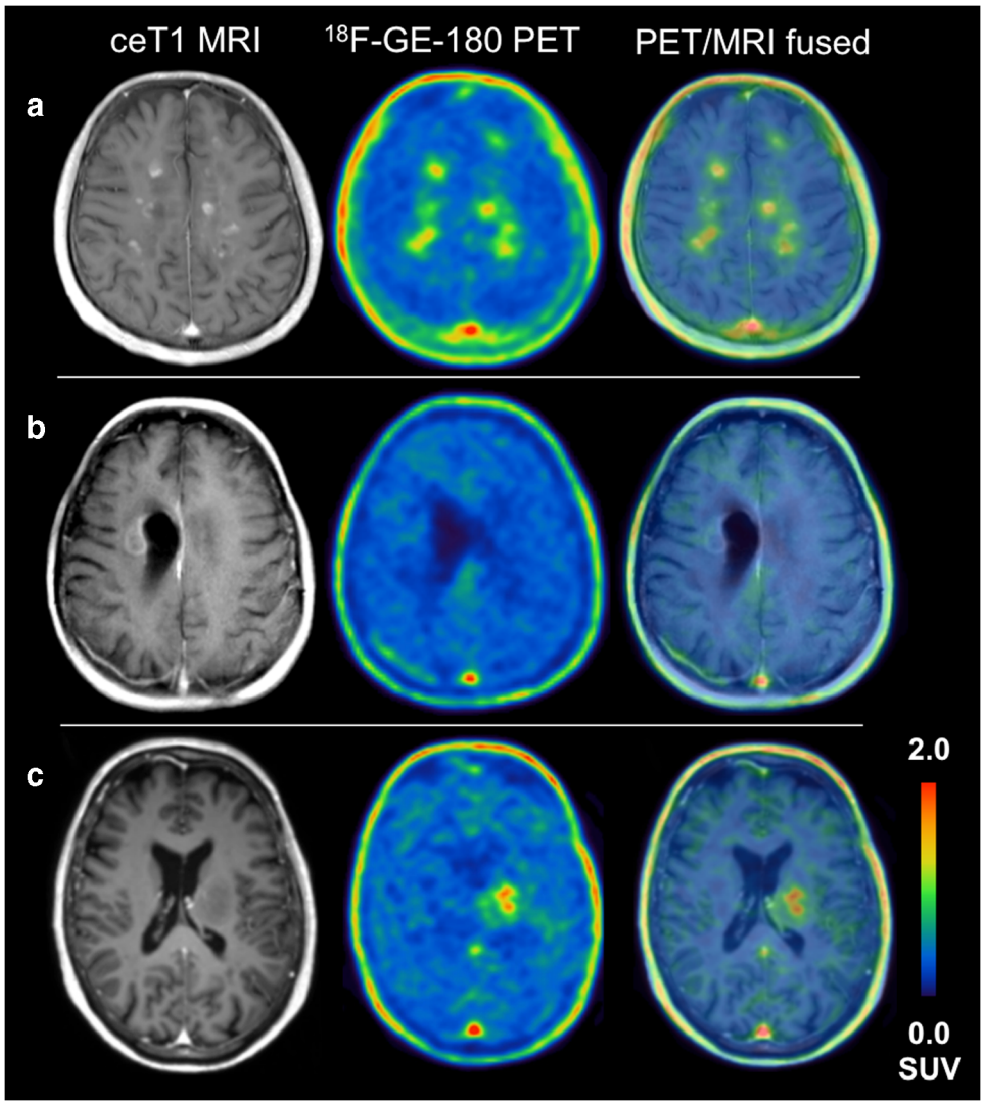

Fig. 1 Three patient examples with contrast-enhancement on MRI and ^{18}F-GE180 PET. **a** A patient with highly active relapsing–remitting multiple sclerosis shows no tight correlation between the intensity of contrast enhancement on MRI and ^{18}F-GE180 uptake. **b** A patient after re-irradiation of recurrent glioblastoma shows distinct contrast-enhancement on MRI but no focally elevated ^{18}F-GE180 uptake. **c** A patient with newly diagnosed anaplastic astrocytoma WHO grade III, IDH wildtype, shows distinctly elevated ^{18}F-GE180 uptake but no relevant contrast-enhancement on MRI

^{18}F-GE180 binding in humans. Indeed, Sridharan et al. were able to show successful competitive displacement of the TSPO tracer ^{18}F-GE180 by the TSPO ligand XBD173 in MS patients [16], finding that half of the signal was displaceable in vivo which indicates specific binding to TSPO. This clearly requires further substantiation, particularly by evaluation of displacement in lesions with disrupted BBB.

The second major concern raised by Zanotti-Fregonara et al. refers to the presence or lack of A147T polymorphism allelic effects on ^{18}F-GE180 binding in healthy human brain, stating that "all in vivo studies, except one …, did not find a genotype-related difference despite a 15:1 binding affinity difference between HABs and LABs measured in vitro … " [1]. We note that the 15:1 allelic affinity difference cited by Feeney et al. [17] was based on a "personal communication" with D. Owen, and the supporting in vitro binding data have not been published. Be that as it may, of the two human ^{18}F-GE180 studies with compartmental analysis, the study by Fan et al. [18] did indeed suggest a higher specific binding for HAB carriers ($BP_{ND} = 2$) than for MAB carriers ($BP_{ND} = 1$) in healthy brain, according to the analysis of Cumming et al. [15]. Although the study by Feeney et al. did not confirm the finding of allelic differences in small groups of healthy humans [17], the recent study by Sridharan et al. did show just such a difference in MS patients [16]. Our studies in lesions of MS and glioma patients have not revealed a conspicuous dependence of ^{18}F-GE180 uptake on allelic status, however, we have to point to the extremely high variability of ^{18}F-GE180 uptake intensity in individual tumours and MS lesions. We suggest that differences in allelic status may be masked by large effects of tumour aggressiveness or inflammatory activity on TSPO expression. Studies correlating ^{18}F-GE180 signal with histological quantification of TSPO expression in genotyped individuals should resolve this issue.

The statement by Zanotti-Fregonara et al. that "In general, tracers with good imaging properties are able to differentiate

the three different populations …" (of binding affinity status) is another point of contention. In our opinion, a clinically optimal tracer should *not* be sensitive to the A147T polymorphism, but should indicate increased TSPO expression independent of the individual's allelic status. TSPO ligands can share the same binding pocket on TSPO, while retaining allelic sensitivity for their molecular interaction with TSPO [19]. In line with the low sensitivity of PK11195 for the human A147T polymorphism, structural analysis of the complex of (*R*)-PK11195 with wild-type and A147T-mutant TSPO has revealed very similar conformations [20]. A better understanding of allelic dependence on affinity of ^{18}F-GE180 and other TSPO PET tracers might be obtained through cellular and animal studies in which the human polymorphism is introduced using CRISPR/CAS-methodology.

To sum up, we agree that relevant methodological issues regarding the biochemical nature of the ^{18}F-GE180 PET signal in lesions remain to be resolved. While its low uptake in the parenchyma makes ^{18}F-GE180 unfavourable for quantitation of TSPO in healthy human brain, it has very high uptake in MS lesions (presumably in relation to the inflammatory state), in glioma (presumably correlating with tumour viability and grading), and in other neurological diseases, even in the absence of contrast enhancement on MRI. Preliminary data indicate pharmacological displaceability of the ^{18}F-GE180 signal in patients with MS, but there has not yet been a formal demonstration of this in areas of BBB disruption. To better understand the ^{18}F-GE180 signal, we concur with the recommendation of Zanotti-Fregonara et al. that ^{18}F-GE180 PET should be spatially compared with histopathological analysis of tissue samples from stereotactic biopsies, and we are planning just such a study in brain tumour patients. Altogether, we consider that ^{18}F-GE180 PET provides information on TSPO binding specifically in areas of active inflammation or tumour growth, providing highly valuable clinical information in patients with various CNS diseases.

Compliance with ethical standards

Conflicts of interest N.L.A. is a member of the Neuroimaging Committee of the EANM. P.B. has received a speaker honorarium from GE Healthcare. All other authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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