Data Supplement for Bloomfield 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 (doi: 10.1176/appi.ajp.2015.14101358)

On-line only supplementary Material

Comparison of 2TCM and 2TCM-1K performance

Performance of the 2TCM-1K and 2TCM models for describing [^{11}C]PBR28 PET data at region level was evaluated visually against the acquired data. The 2TCM-1K model provided a better fit of the tissue data for all the analyzed ROIs, all the groups of subjects and all the affinities compared to the 2TCM (Supplemental Figure 1). The relative difference of the mean (± SD) weighted residual sum of squares obtained with the 2TCM-1K, compared to the 2TCM model, was -55%±25%, while the relative difference of the residual sum of squares was -50%±30%. Weighted residuals obtained with 2TCM-1K were consistent with the assumptions about the measurement error (random and uncorrelated). In terms of parsimony criteria (1), the Akaike Information Criterion (AIC)* was smaller for the 2TCM-1K than that for the 2TCM in 99.4% of the regions. In term of outliers, the two models performed similarly (brain outlier fraction: 3% for 2TCM and 4% for 2TMC-1K). These were concentrated particularly in small regions (average volume <3 cm^3), indicating they are very likely characterized by high noise data. After correction for outliers, individual V_T estimate precisions were consistent for both models (for 2TCM CV V_T = 4%±5%; for 2TCM-1K CV V_T = 7%±5%). The models were solved using the weighted nonlinear least square estimator (WNLLS), as implemented in Matlab (The Mathworks Inc., Natick, MA). Initial microparameter values were set based on the literature (2), while weights for the individual data points were defined as the inverse of the variance of the PET measurement error. This was assumed to be additive, uncorrelated, normally distributed with a zero mean and variance equal to the decay-corrected activity divided by the length of the relative scan interval, multiplied by a scale factor, estimated *a posteriori* (3). The total distribution volume (V_T representing the ligand uptake in tissue including binding to microglia) was then determined

(regions with V_T estimates higher than 10 mL/cm^3 or with unreliable precision (CV > 50%) are non-physiological (2) and where therefore excluded from the analysis). In summary the model fit performance analysis indicated the 2TCM-1K to be superior to 2TCM for describing [^{11}C]PBR28 PET data at a regional level. This finding is consistent with the results reported by Rizzo and colleagues applying [^{11}C]PBR28 imaging in a healthy population (2). Vb was derived from the data for both models (2TCM and 2TCM-1K) and there were no differences between the groups (UHR vs Control 2TCM p= 0.950, 2TCM-1K p= 0.864; Schizophrenia vs Control 2TCM p= 0.996, 2TCM-1K p= 0.999) . Further to this, the values were consistent with the brain blood volume fraction (~6%± 2%).

Blood sampling for arterial input function

Discrete blood samples were manually withdrawn at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 minutes, centrifuged and used to determine the plasma over blood activity ratio (POB). Samples at 5, 10 and 15 minutes were used to calibrate the two sampling modalities. Samples taken at 5, 10, 20, 30, 50, 70 and 90 minutes were also analysed using HPLC to calculate the plasma fraction of authentic tracer free of metabolites (PPf). Both POB and PPf were fitted with an extended Hill model(4), while whole blood TACs were fitted using a multi-exponential(5). For each scan, a time delay was fitted and applied to the input functions (both parent and whole blood TACs) to account for any temporal delay between blood sample measurement and the target tissue data.

Akaike Inromation Criterion (AIC)

AIC is the Akaike Information criterion and it is defined as:

AIC = $nD \cdot \log(WRSS) + 2 \cdot nP$

where nD represents the number of data fitted by the model, $WRSS$ the weighted residual sum of squares and nP the number of model parameter.

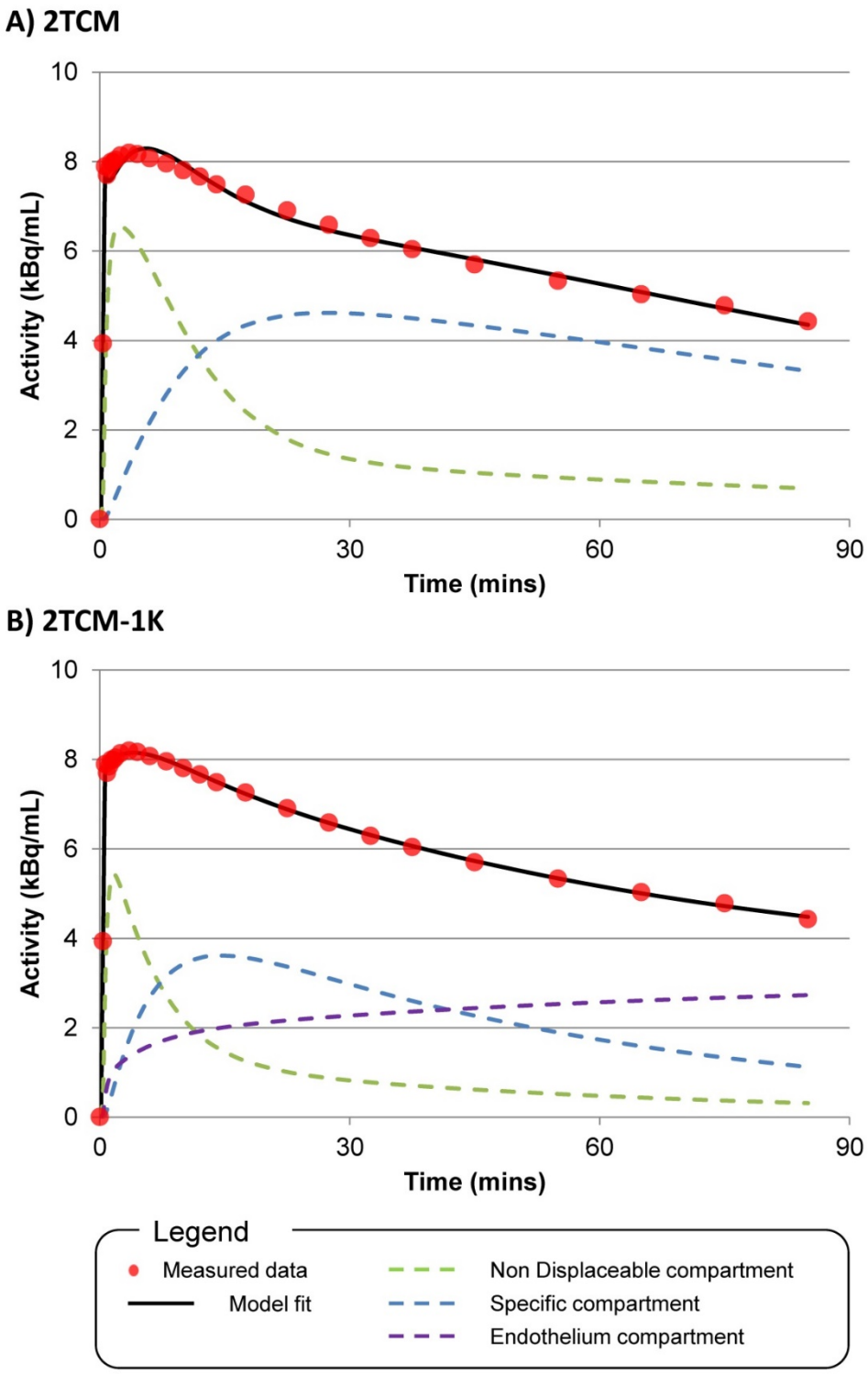
Anti-inflammatory medication

We selected one month as the minimum period without an anti-inflammatory as this is >one hundred time the plasma half-lives for non-prescription anti-inflammatory drugs available in the UK (http://www.mhra.gov.uk/home/groups/s-par/documents/websiteresources/con068576.pdf MHRA, NSAID Public Assessment Report accessed 18/05/2015). Thus we minimised the risk of acute effects, although it should be noted that it is not known if anti-inflammatory drugs have effects that persist following wash-out. In practice, of the 56 subjects in our study, one Control (MAB genotype) had taken an anti-inflammatory drug (Paracodol) one month prior to scanning. Removal of this subject did not alter the experimental outcome of this study (Grey matter DVR, F= 8.765; *p*= 0.007). No other subject had taken an anti-inflammatory within three months of scanning.

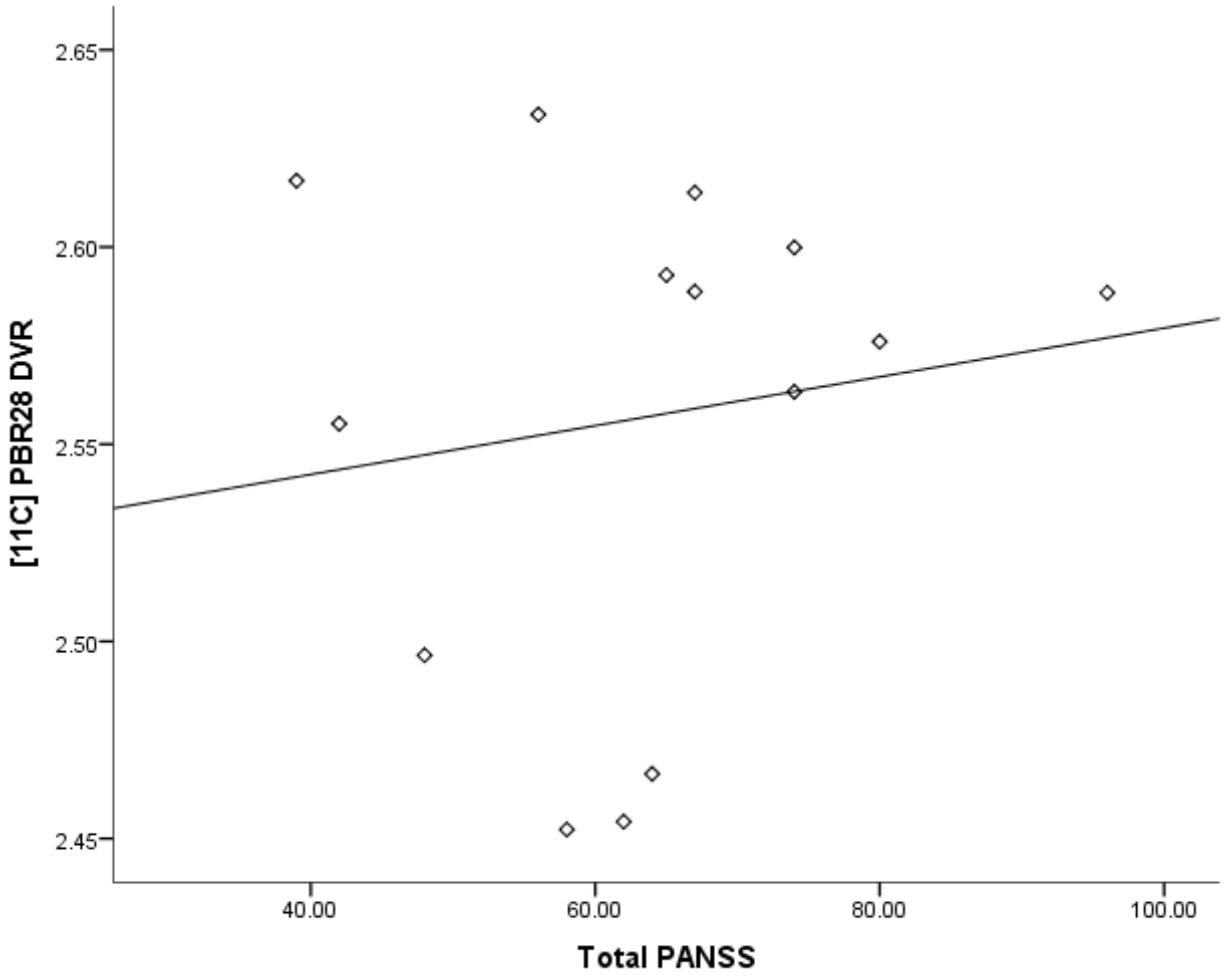
Statistical analyses

In this manuscript, we used whole brain to normalise TSPO PET data. The analyses were conducted using a multivariate analysis approach, where whole brain was used, alongside age and TSPO genotype, as covariates in an ANCOVA producing a marginal mean (termed 'DVR'). This is rather than the division method.

Supplemental Figure 1 – 2TCM and 2TCM-1K fit comparison for [^{11}C]PBR PET data in schizophrenia: application to a cortical region in a representative HAB subject. The correspondence between the model fit (black line) and measured date (red circles) is closer in the 2TCM-1K than the 2TCM model.



Supplemental Figure 2 – Total grey matter [^{11}C]PBR28 DVR correlation with PANSS symptoms in patients with schizophrenia.



Total grey matter [^{11}C]PBR28 distribution volume ratios were not significantly correlated with symptoms in patients with schizophrenia (r= 0.538, *p*= 0.071), measured on the positive and negative syndrome scale (PANSS).

Supplemental Table 1 – [^{11}C]PBR28 Distribution volume ratios (DVR) using 2TCM analysis methods

Regional DVR of [^{11}C]PBR28	**Control (SD)**	**UHR (SD)**	***p*-value**	**Control (SD)**	**Schizophrenia (SD)**	***p*-value**
Total grey Matter	4.169 (0.010)	4.204 (0.015)	0.031*	4.676 (0.015)	4.738 (0.011)	0.001**
Frontal lobe	4.093 (0.029)	4.140 (0.043)	0.290	4.623 (0.050)	4.682 (0.034)	0.024*
Temporal lobe	4.230 (0.041)	4.225 (0.061)	0.940	4.807 (0.047)	4.693 (0.033)	0.256

Microglial activity, as measured by [^{11}C]PBR28 distribution volume ratio with a 2TCM analysis, is elevated in subjects at ultra high risk of psychosis (df=21 p=0.031) and patients with schizophrenia (df=21 p=0.001). The mean regional distribution volume ratios are shown for each group together with those for matched controls. The results of the ANCOVA covarying for age and translocator-protein genotype are shown for each case-matched control comparison (*p<0.05; **p<0.01).

Supplemental Table 2 – Descriptive data from normalization parameters tested.

Controls vs. Schizophrenia

Mean of parameter	Control	Stdev	Schizophrenia	Stdev	F	*p*-value
AUC Cp	137.09	26.94	139.52	36.78	0.04	0.843
Cerebellum	3.16	1.14	2.55	0.66	6.54	0.097
Brain	2.74	0.94	2.12	0.65	4.13	0.051
White Matter	1.91	0.66	1.34	0.44	4.09	0.011*
POB	0.004	0.001	0.004	0.002	1.11	0.303
PPf	11.99	3.62	12.66	2.94	0.29	0.594

PPf: parent plasma fraction. POB: Plasma-over-blood ratio.

Controls vs. Ultra High Risk (UHR)

Mean of parameter	Control	Stdev	UHR	Stdev	F	*p*-value
AUC Cp	138.71	26.48	151.74	31.62	0.05	0.258
Cerebellum	2.52	0.92	2.07	0.77	0.41	0.174
Brain	2.18	0.77	1.80	0.64	0.19	0.161
White Matter	1.62	0.64	1.22	0.43	0.70	0.063
POB	0.004	0.001	0.004	0.002	1.11	0.303
PPf	11.99	3.62	12.66	2.94	0.29	0.594

PPf: parent plasma fraction. POB: Plasma-over-blood ratio.

Of the parameters assessed between groups, we see a reduction in White Matter between schizophrenia and respective controls. Statistical analysis refers to two-tailed independent T-test (*p<0.05).

Supplemental Table 3 – Age correlations with volume of distribution (V_T) and distribution volume ratios (DVR).

Measure	**r statistic**	***p*-value**
Total grey matter V_T	0.336	0.016*
Frontal lobe V_T	0.368	0.008**
Temporal lobe V_T	0.329	0.018*
Frontal lobe DVR	0.307	0.032*

Statistical analysis refers to Pearson's two-way product moment correlation coefficient (*p<0.05; **p<0.01).

Supplemental Table 4 – Scan Parameters for [^{11}C]PBR28

	Control (SD)	**UHR (SD)**	***p*-value**	**Control (SD)**	**Schizophrenia (SD)**	***p*-value**
Injected dose (MBq)	326.6 (26.6)	327.6 (26.7)	*0.982*	326.3 (25.5)	318.9 (33.8)	0.910
Injected mass (µg)	2.8 (1.3)	3.4 (2.1)	*0.390*	2.5 (0.8)	2.5 (1.1)	0.905
Specific activity (GBq/µmol)	49.3 (22.1)	43.4 (21.4)	*0.418*	50.0 (18.4)	52.7 (20.9)	0.981
PPf (%)	8.5 (2.1)	9.9 (3.5)	*0.376*	12.0 (3.6)	12.7 (3.0)	0.635
POB (ratio)	0.0034 (0.0017)	0.0033 (0.0013)	*0.667*	0.0039 (0.001)	0.0044 (0.002)	0.511

PPf: parent plasma fraction. POB: Plasma-over-blood ratio.

No statistical difference was seen with any scan parameters, parent plasma faction or POB. Statistical analysis refers to two-tailed independent T-test.

.

Supplemental Table 5 – [^{11}C]PBR28 Distribution volumes (V_T) using 2TCM and 2TCM-1K analysis methods

Regional V_T of [^{11}C]PBR28 (2TCM)	**Control (SD)**	**UHR (SD)**	***p*-value**	**Control (SD)**	**Schizophrenia (SD)**	***p*-value**
Total grey Matter	4.444 (0.245)	3.929 (0.357)	0.162	4.925 (0.348)	4.488 (1.211)	0.393
Frontal lobe	4.362 (0.242)	3.870 (0.352)	0.175	4.867 (1.039)	4.437 (1.223)	0.762
Temporal lobe	4.493 (0.238)	3.962 (0.346)	0.138	5.055 (0.404)	4.445 (1.218)	0.691
Regional V_T of [^{11}C]PBR28 (2TCM-1K)						
Total grey Matter	2.145 (0.157)	1.975 (0.228)	0.461	2.740 (0.210)	2.361 (0.306)	0.228
Frontal lobe	2.113 (0.158)	1.974 (0.230)	0.551	2.764 (0.212)	2.406 (0.310)	0.260
Temporal lobe	2.019 (0.148)	1.896 (0.215)	0.569	2.543 (0.207)	2.322 (0.302)	0.471

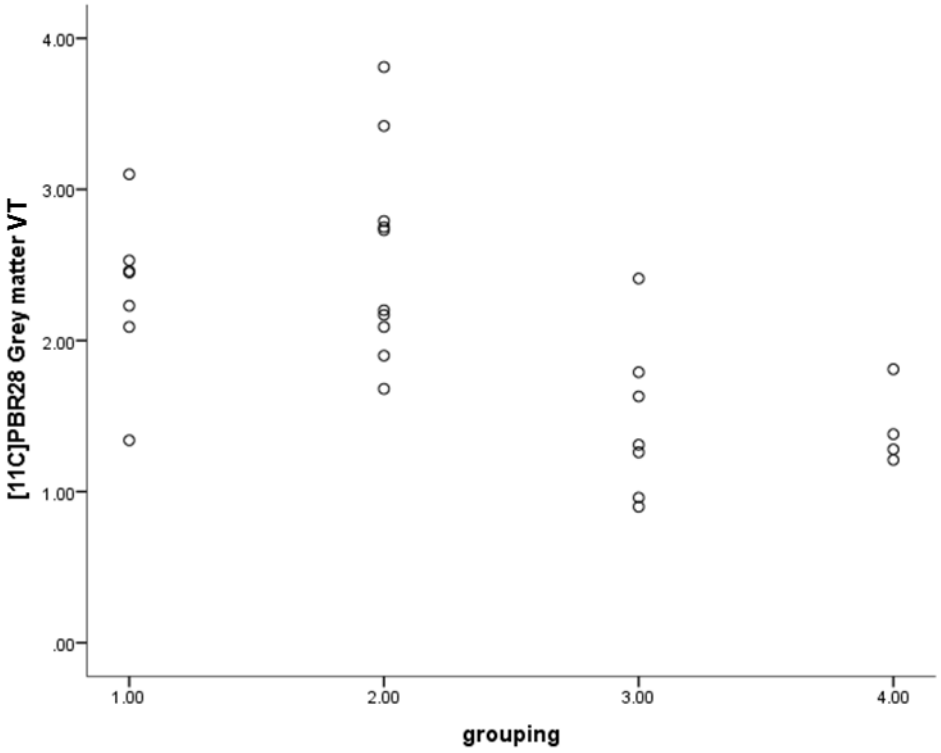
Microglial activity, here measured by PBR28 V_T with a 2TCM and 2TCM-1K analysis, did not differ between groups (UHR df=21 $p>0.05$; Schizophrenia df=21 $p>0.05$). The mean regional V_Ts are shown for each group together with those for matched controls. The results of the ANCOVA covarying for age and translocator-protein genotype are shown for each case-matched control comparison.

Supplemental Table 6 – HAB and MAB DVR comparison for stratified Ultra High risk (UHR) and control subjects.

Grey matter DVR	Control	Stdev	UHR	Stdev	F	*p*-value
HABs	2.428	0.016	2.494	0.020	6.091	0.028*
MABs	1.427	0.011	1.462	0.009	5.847	0.046*

Statistical analysis refers to ANCOVA where genotype, age and whole brain were included as covariates and grey matter as the region of interest.

Supplemental figure 3 – Group 1 = control HAB, 2 = UHR HAB, 3 = control MAB, 4 = UHR MAB.



Supplemental Table 7 – Exploratory analysis of varying the region used for normalization on the [^{11}C]PBR28 DVR in frontal and temporal grey matter regions.

Region of interest	Normalization region	Control (SD)	Schizophrenia (SD)	*p*-value
Frontal lobe- grey matter	Cerebellum	2.345 (0.100)	2.710 (0.070)	0.001**
Frontal lobe- grey matter	White matter	2.400 (0.111)	2.695 (0.074)	0.014*
Temporal lobe- grey matter	Cerebellum	2.232 (0.120)	2.599 (0.084)	0.006**
Temporal lobe- grey matter	White matter	2.208 (0.145)	2.592 (0.096)	0.015*

The results of the ANCOVA covarying for age and translocator-protein genotype are shown for each case-control comparison (*p<0.05; **p<0.01). Significant regional elevations of microglial activity, as measured by [^{11}C]PBR28 distribution volume ratio, are seen in patients with schizophrenia when using cerebellar and white matter normalization approaches, consistent with the findings with the whole brain normalization.

Supplemental Table 8 – Review of 2TCM regional variability in our data and published studies.

Study	**Region of interest**	**% variability between subjects**
Our data	Grey matter	31.32
	Frontal	31.46
	Temporal	31.84
[^{18}F]FEPPA (6)	Hippocampus	132.00
	mPFC	27.66
	dlPFC	30.93
	Temporal	31.90
	Striatum	29.12
[^{11}C]PBR28 (7)	Whole brain	27.03
	Frontal	29.24
	Temporal	28.57
	White matter	27.27

References

1. Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control,. 1974 19, :716-723.

2. Rizzo G, Veronese M, Tonietto M, Zanotti-Fregonara P, Turkheimer FE, Bertoldo A. Kinetic modeling without accounting for the vascular component impairs the quantification of [lsqb]11C[rsqb]PBR28 brain PET data. J Cereb Blood Flow Metab. 2014.

3. Bertoldo A, Vicini P, Sambuceti G, Lammertsma AA, Parodi O, Cobelli C. . Evaluation of compartmental and spectral analysis models of [/sup 18/F] FDG kinetics for heart and brain studies with PET. Biomedical Engineering, IEEE Transactions on. 1998; 45(12): 1429-1448.

4. Edison P, Brooks DJ, Turkheimer FE, Archer HA, Hinz R. Strategies for the generation of parametric images of [11C]PIB with plasma input functions considering discriminations and reproducibility. NeuroImage. 2009;48:329-338.

5. Tonietto M, Rizzo G, Veronese M, Zanotti-Fregonara P, Fujita M, and Bertoldo A. . Optimal metabolite curve fitting for [11 C]PBR28. NeuroReceptor Mapping, Amsterdam, 21-24 May. 2014.

6. Kenk M, Selvanathan T, Rao N, Suridjan I, Rusjan P, Remington G, Meyer JH, Wilson AA, Houle S, Mizrahi R. Imaging Neuroinflammation in Gray and White Matter in Schizophrenia: An In-Vivo PET Study With [18F]-FEPPA. Schizophrenia Bulletin. 2015;41:85-93.

7. Park E, Gallezot J-D, Delgadillo A, Liu S, Planeta B, Lin S-F, O'Connor K, Lim K, Lee J-Y, Chastre A, Chen M-K, Seneca N, Leppert D, Huang Y, Carson R, Pelletier D. 11C-PBR28 imaging in multiple sclerosis patients and healthy controls: test-retest reproducibility and focal visualization of active white matter areas. European Journal of Nuclear Medicine and Molecular Imaging. 2015;42:1081-1092.