Final Report Summary - PET BRAIN (Mapping the brain with PET radiolabeled cannabinoid (CB1) ligands)

The major objectives of the PET BRAIN project (www.abdn.ac.uk/pet-brain/) were:

GOAL-1. The identification of novel radiolabeled CB1 (cannabinoid) receptor ligands, having suitable properties, for their use as PET tracers: by the end of the project we aimed at the identification of at least one novel structural sub-class of CB1 receptor radioligands which can be used for the PET imaging of brain.

GOAL-2. The development of radiolabeled CB1 receptor ligands for multimodal imaging of brain (combined PET/MRI, PET/near-infrared, PET/fluorescence), and more specifically: (a) the identification of at least one novel structural sub-class of CB1 radioligands with potential for multi-modal imaging; (b) the accomplishment of multi-modal imaging studies on at least one radiolabeled CB1-ligand on suitable animal models.

GOAL-3. The development of innovative synthetic methodologies for the introduction of 18F and 11C positron emitting radioisotopes in CB1 receptor ligands, which can be possibly used for other drug-like organic molecules too. By the end of the project (possibly within month-36) we aimed at developing at least two innovative synthetic methodologies for the introduction of 18F or 11C in drug-like organic molecules.

The major final goal of this project was therefore to identify and develop PET-tracer molecules to visualize CB1-receptor related processes in the brain of animal models, with the future perspective of using these novel molecular tools as diagnostic tools in human brains.

All the objectives above have been achieved in full. In terms of chemistry, different molecules based on structures originally discovered by the partner company Pharmaness have been identified as potential radioligands. These molecules are characterised by having the same core-structure: a pyrazole ring-core substituted with a thiophene moiety, a phenyl group and with different kinds of heterocycle rings linked to the pyrazole by an amide bond. A new class of pentafluorosulfanyl-substituted compounds was designed and synthesised. The corresponding CF3 analogues were also synthesised and compared to the SF5-compounds, showing that the latter have generally better affinity for the CB1 receptor and selectivity towards the CB2. Subsequently, a novel class of pyrazoles incorporating a triazole group were synthesised using a key "click"-cycladdition reaction. These compounds showed nanomolar Ki for the CB1 receptor and promising physico-chemical properties for being used as PET tracers. At least 6 different synthetic pathways for the synthesis of potential CB1 receptor radiotracers, suitable for PET imaging, have been designed and a number of new molecules have been synthesised in good yields. Within the frame of a Structure-Activity-Relationship study on tricyclic Rimonabant analogues, Pharmaness synthesised at least 20 novel structures, which were then tested in Aberdeen for their affinity towards CB1 and CB2 receptors. Importantly, a novel radiofluorination methodology, involving the used of 18F-FDR (fluoro-deoxy-ribose) as prosthetic group has been developed and used for the synthesis of CB1 receptor ligands.

Pharmacology studies have been very positive too. At least 20 new molecules synthesised in Aberdeen have been tested in vitro giving interesting results. Some of them, such as Zsa78 showed a good CB1 affinity and a good CB1 versus CB2 selectivity. Finally, a novel class of cyano-substituted pyrazoles was designed, synthesised and pharmacologically studied. About 15 molecules synthesised by Pharmaness were submitted to binding assays with [3H]CP55940. The assays were performed with suitably prepared mouse whole brain membranes or with CHO-hCB2 cell membranes. Preliminary results
indicate Ki values in the medium/high nanomolar range for the CB1 receptor, and in the micromolar range for the CB2 receptor.

The radiochemistry angle has been developed successfully. Based on the positive results above, a tailored synthetic strategy for the radioactive 11C version was designed and carried out in Aberdeen. The radioactive analogue of Zsa78 was synthesised using 11CH3I as source of 11C and following a methylation strategy with 11C-CH3I. The reaction was performed with a good radiochemical yield, and optimised HPLC conditions for the purification were identified. Among the cyano-pyrazoles, compound ZSA304, having nanomolar affinity for the CB1 receptor, was selected for 18F-radiosynthesis, which was successfully performed.

An in vivo imaging experiment using a wild-type rodent was successfully performed using 11C-ZSA78. Low uptake of the tracer in the brain was observed at any time point. There was some evidence of accumulation of the tracer at later times, but the activity (<0.2 %ID/g) was much lower than that which is generally required to obtain a good signal to noise ratio. Finally, full body and brain PET/CT scans were performed with 18F-ZSA304 on wild-type pre-clinical models showing very promising results for the imaging of the CB1 receptor in vivo with this tracer.

These CB1 radioligands will be used in the future in several different ways, including:
1. To see whether the potential CB1 ligand makes it to the brain, where CB1 receptors are predominantly located, and what other organs sequester the drug.
2. To measure the affinity of the CB1 radioligands, information that can be critical in dosimetry studies.
3. To visualize medically important CB1-receptor related processes in the brain, like pathological states (psychiatric disorders, depression, obesity, neuropathic pain, etc.) and drug addiction.

The main goal of the project, that was the identification of novel radiolabeled compounds having use as diagnostic brain imaging agents, has strong medical and social relevance. This ambitious goal has been fully achieved and the tracers resulting from the PET BRAIN project might be translated, in the future, into clinical and industrial development research programmes.