Supplement Material

^{18}F-DCFBC PET/CT Imaging Protocol

^{18}F-DCFBC PET/CT imaging was performed on a 3D time of flight (TOF) mode Philips Gemini TF camera (Philips, Cleveland OH), with an 18 cm coronal and a 57 cm axial FOV. Data were reconstructed with an relaxed list mode ordered subset expectation maximization (LMOSEM) TOF based algorithm using 3 iterations and 33 subsets (8). The scanner uses CT based attenuation correction; along with random, normalization, dead time and a model-based scatter correction (9) for anatomical correlation and attenuation correction purposes.

^{18}F-DCFBC was produced following the microwave assisted manual synthesis procedure (**supplement material**) at the nuclear-pharmacy of Leidos Biomedical Research, Inc. at NCI Frederick (*Frederick, MD*).

Each patient received an IV bolus injection of ^{18}F-DCFBC, mean dose 287.21 MBq [7.76 mCi] (range 244.20-296.0 MBq [6.4-8.0 mCi]), followed by a dynamic PET/CT imaging of the lower pelvis for the first 30 minutes, and static whole body PET/CT images at ~60 min and 120 min post-injection (2 min/bed position). Low dose transmission CT scans (120 KV, 60 mAs) were acquired prior to each PET scans for anatomical correlation and co-registration purposes.

Vital signs were obtained prior to ^{18}F-DCFBC injection, and at 10 and 30 minutes post-injection and directly after the last PET scan. The patients were queried regarding potential subjective adverse events during the scan and immediately after, and an additional telephone query was performed the following day.

MRI Protocol

All MRI studies were performed using an endorectal coil (BPX-30, Medrad, Pittsburgh, PA) and a 16-channel anterior cardiac coil (SENSE, Philips Medical Systems, Best, The Netherlands) on a 3T magnet (Achieva, Philips Medical Systems, Best, the Netherlands) without prior bowel preparation. The endorectal coil was inserted using a semi-anesthetic gel (Lidocaine, Akorn Inc., Lake Forest, IL) while the patient was in the left lateral decubitus position. The balloon surrounding the coil was distended with perfluorocarbon (Fluorinert FC-770, 3M, St. Paul, MN) to a volume of approximately 45 mL. The MRI protocol included tri-planar T2W turbo spin echo (TSE), diffusion weighted (DW) MRI (ADC maps and b2000 DW MRI), axial pre-contrast T1W, axial 3D T1-weighted fast field echo dynamic contrast-enhanced MRI (DCE MRI) sequences (10).

Method for Preparation of ^{18}F-DCFBC

a. The ^{18}F-fluoride loaded QMA cartridge obtained from an outside vendor (Cardinal Health) is eluted with aqueous potassium carbonate and Kryptofix 2.2.2® solution and the eluate is collected in the reaction vessel (Biotage microwave vial).

b. The ^{18}F-fluoride and potassium carbonate / Kryptofix 2.2.2 are azeotropically dried with multiple additions of acetonitrile at 120˚ C under a nitrogen flow and vacuum.

c. An acetonitrile solution of 4-formyl-N,N,N-trimethylanilinium triflate is added to the reaction vessel.

d. The reaction mixture is microwaved for 4 min at 100 °C.

e. An aqueous solution of sodium borohydride is added and the resulting mixture is stirred for 5 min at RT.

f. Aqueous hydrobromic acid is added and the resulting mixture is microwaved for 3 min at 100 °C.

g. The reaction mixture is passed through a C-18 Plus solid phase extraction cartridge.

h. The reaction vessel is washed with 5-10 mL of HPLC water and eluted through the C-18 Plus.

i. The cartridge is eluted with acetonitrile into a microwave reaction vessel (Biotage) containing ~ 600 µg precursor, 2-[3-(1-carboxy-2-mercapto-ethyl)-ureido]-pentanedioic acid and 150 µL tetrabutyl ammonium hydroxide (1 M in water).

j. The mixture is microwaved for 3 min at 80 °C.

k. The mixture is diluted with HPLC water(4-5 mL) and applied to semipreparative HPLC column (Waters Atlantis T3 reverse phase C18 5µ 250 X 10mm) eluted with 30% acetonitrile : 70% phosphate buffer (pH 3.2) at a flow rate of 4 mL/minute until the product is observed and collected.

l. The ^{18}F-DCFBC fraction is collected in HPLC water. The water reservoir is pressurized to load the ^{18}F-DCFBC onto the Waters C-18 Light solid phase extraction cartridge. The cartridge is then washed with 5 mL of water (SS-WFI) and 5-10 mL of air.

m. The ^{18}F-DCFBC is eluted from the C-18 Light cartridge with 1 mL of ethanol followed by 10 mL of 0.9% sodium chloride, via a sterilizing 0.22µ filter into a sterile vial containing 4 mL 0.9% sodium chloride. (figure 5)

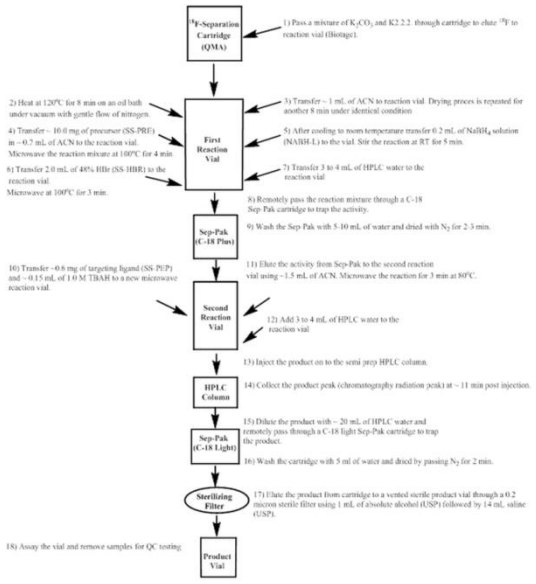

Figure 5 (supplement figure): Schematic Flow Chart of the Process for Radiosynthesis of ^{18}F-DCFBC

Time Activity Curve Results of ^{18}F-DCFBC PET/CT

The two main findings extracted from the time activity curves are the following. First of all, one cannot use the iliac to measure the input bolus function because the DCFBC compound binds to the arterial wall making it difficult to extract the blood concentration of the compound. The other finding is the rapid uptake of the compound into the tissues in the prostate. In order to perform a formal pharmacokinetic compartment model analysis, one needs to have an accurate input function which cannot be measured directly using the dynamic data due to the DCFBC binding to the arterial wall. But one can measure the rate at which the compound clears from the blood. This was done by postulating a blood clearance function of the following form

$$I(t) = \alpha\, e^{-t \ln(2)/T_{1/2}} + \beta \quad t > 0$$
$$I(t) = 0 \quad t < 0$$

where $I(t)$ is the input bolus function as measured by the contour of the iliac artery, α is the amplitude of the arterial blood concentration component, $T_{1/2}$ is the half life of the clearance of the DCFBC compound from the blood and β is the steady state binding of the DCFBC compound to the arterial wall of the iliac. This is a three parameter parametric fit which was performed using the root (CERN, Geneva Switzerland) data analysis software package (figure 6). The black curve is the input bolus measured in the iliac in units of SUV and the red curve is the result of the three parameter parametric fit. Each subject's input function TAC was fit and the results of the parametric fit is as follows

$$\overline{\alpha} = 4.7 \pm 0.7\ SUV$$
$$\overline{T_{1/2}} = 9.9 \pm 2.5\ min$$
$$\overline{\beta} = 3.8 \pm 0.6\ SUV$$

Where $\overline{\alpha}$, $\overline{T_{1/2}}$ and $\overline{\beta}$ are the mean ± standard deviation of the amplitude, the half life of the compound clearance from the blood and arterial binding respectively as measured from the fit parameters to the parametric fits to the input function of all the subjects.

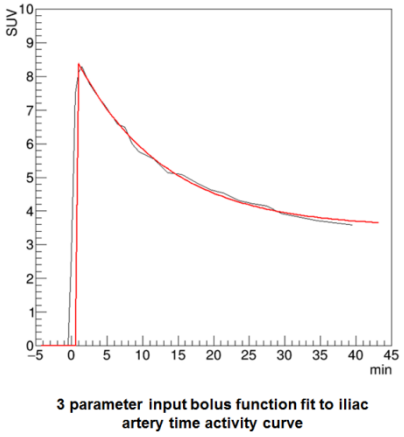

Figure 6 (supplement figure): An example of the three parameter input bolus function fit to the iliac artery time activity curve.

In order to quantify the uptake time of the DCFBC compound into the three prostate tissue regions of tumor, BPH and normal, the average activity after 2 minutes was measured. The time at which the TAC reached at least 90% of this mean was then measured for each TAC as a function of tissue type. The results are

$$Tumor\ 90\%\ rise\ time = 2.9\ \pm 1.5\ min$$
$$BPH\ 90\%\ rise\ time = 2.0 \pm 1.1\ min$$
$$Normal\ 90\%\ rise\ time = 2.7\ \pm 1.6\ min$$

where the 90% rise time mean $\pm$ standard deviations are reported for each tissue type in minutes. To best visualize the rapid rise time and equilibration of the compound in the various prostate tissues, the individual TAC were plotted for each tissue type independently on top of the input bolus function. TAC the prostate tissue has a faster uptake compared to the bladder (figure 7). This may imply that early imaging, between 10 and 15 minutes, could help reduce the background of the very high bladder uptake, typically a factor of 10 greater than the prostate tissue uptake and still properly measure the uptake of the DCFBC compound into the prostate tissues.

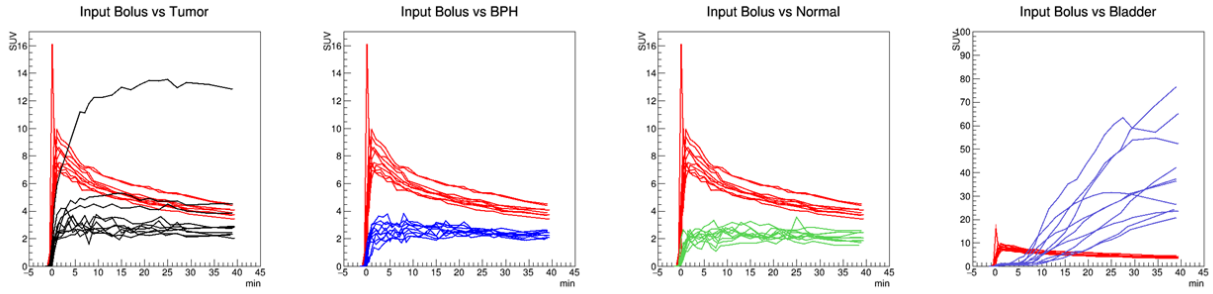

Figure 7 (supplement figure): Comparison of time activity curves for tumor (black), BPH (blue), normal prostate tissue (green) and bladder (blue) regions contoured in the prostate region versus the input injection bolus as measured in the iliac artery (red).