Supplemental Data

Radiosyntheses

All radiotracers for the study listed below were produced in-house at the Radiopharmacy Unit, Vienna General Hospital, applying standard procedures in accordance with the state of the art in radiopharmaceutical preparations. Quality control was performed according to the European Pharmacopoeia. 25 μg PSMA HBED-CC was labelled in acetate buffer at room temperature for 5 min using a dedicated kit (Telix Pharmaceuticals Ltd.) and 1.1 mL generator eluate (Galli Eo™, IRE Elit) under aseptic conditions. The obtained product [68Ga]Ga-PSMA HBED-CC ([68Ga]Ga-PSMA-11) was used without further purification. The radiosynthesis of 16β-[18F]fluoro-5α-dihydrotestosterone was performed as previously described with some modifications using an automated synthesizer (TracerLab FXFN, Nuclear Interface platform, GE Healthcare).(14) In brief, [18F]fluoride was produced on-site via 18O(p,n)18F reaction (GE PET trace, GE Medical Systems). After azeotropic drying in the presence of Kryptofix 2.2.2 and potassium carbonate, [18F]fluoride reacted with the precursor 16α- [[(trifluoromethyl)sulfonyl]oxy]-3,3-(ethylenedioxy)androstan-17-one (GMP-grade, ABX GmbH) in acetonitrile (40°C, 10 min). The crude product was separated from the reaction mixture by solid phase extraction (Sep-Pak® tC18 Plus), washed and eluted with ethanol to a second reaction vessel. Sodium borohydride was added for reduction and then the protective groups were removed by acid hydrolysis (2 N HCl, 85°C). After neutralization, the crude product was purified by radio-HPLC and solid phase extraction and subsequently formulated in physiological saline containing < 10% ethanol.

Immunohistochemical analysis

PSMA IHC was performed using a Rabbit Anti-Human PSMA Antibody (AC-0160; monoclonal [clone EP192]; Epitomics, Inc.: Burlingame, California, USA). AR IHC was performed using a Mouse Anti-Human AR Antibody (M3562; monoclonal [clone AR441]; DAKO, Agilent Technologies: Santa Clara, California, USA). Interpretation of marker expression in all tissue samples was performed by the same qualified uropathologist using high resolution scans performed with the Pannoramic 250 Flash II digital scanner (3DHistech) at 40x magnification.

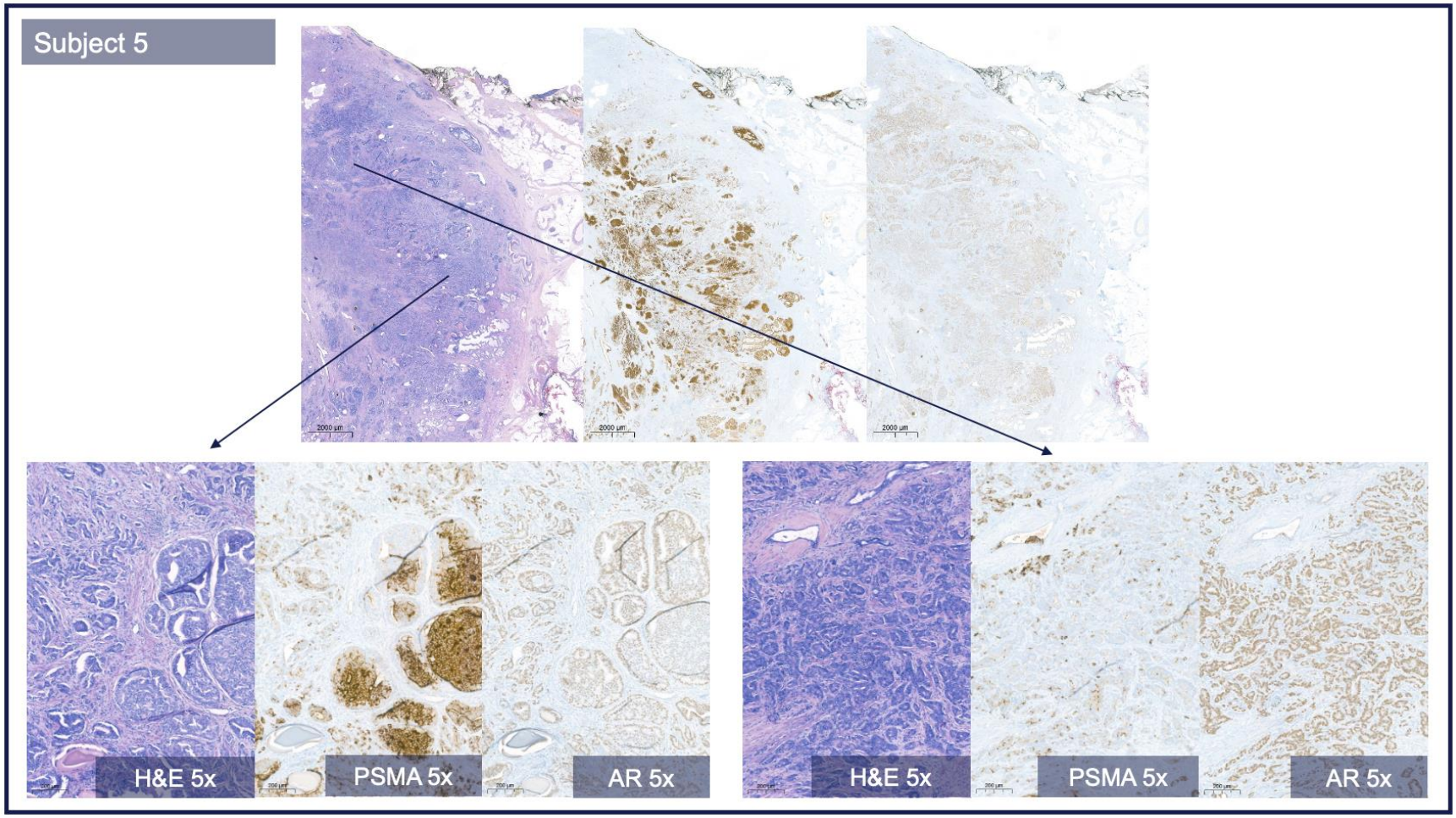
Imaging protocols – sequence parameters

FDHT PET/MRI

For the integrated 3T MRI following sequences and parameters were performed: Pelvis: T2w turbo spin echo (tse) axial, in-plane resolution: 0.8x0.8x5mm; Acq. Matrix 235x512 FoV phase: 262.5mm; TR: 3600ms; TE: 103ms. T1w turbo spin echo (tse) coronal: Matrix size: 346x385, in-plane resolution: 0.9x0.9x5mm FoV phase: 350mm; TR: 600ms; TE: 12ms. Diffusion weighted imaging: Acq. Matrix size: 108x192, in-plane resolution: 2.0x2.0x5mm; FoV: 180mm; with b-values: 0 and 600s/mm^2; TR: 9200ms; TE: 85ms. Partial body MRI simultaneous with PET: T2w HASTE: Matrix size: 256x256, in-plane resolution: 1.5x1.5x6mm; FoV: 380mm; TR: 1400ms; TE: 121ms. T1 VIBE Dixon: Matrix size: 175x320, in-plane resolution: 1.3x1.3x3mm; FoV: 320mm; TR: 4.02ms; TE: 1.23/2.46ms. Diff. stir: Acq. Matrix 105x168, b values 50 and 800, TR: 600ms; TE 68ms.

Ga-PSMA PET/MRI

The integrated 3T MRI was performed with the following sequences and parameters: T2 tse tra pelvis: Matrix size 235x512, in-plane resolution 0.8x0.8x5mm, Acq. Matrix 235x400, TR 3100ms, TE 106. T2 tse tra p2: Matrix size 320x320, in-plane resolution 0.6x0.6x3.5, FoV 200mm, TR 7500ms, TE 101ms. T2 space tra p2: Matrix 300x320, in-plane resolution 0.7x0.7x1mm; FOV 291x320mm, TR 1600ms, TE 88ms. T2 tse sag p2: Matrix 310x320, in-plane resolution 0.6x0.6x3.5mm, FoV 200mm, TR 7500ms, TE 101ms. T2 tse cor p2 320: Matrix 320x320, in-plane resolution 0.6x0.6x3.5 mm, FoV 200mm, TR 7500ms, TE 101ms. Diffusion weighted imaging: ep2d diff b0 800 tra p2: Matrix size 132x132, in-plane resolution 1.5x1.5x3.5mm, FoV 200mm, TR 4200ms, TE 87ms. T1 vibe tra dyn dixon 2 means: Matrix 154x192, in-plane resolution 1.4x1.4x3.5mm, FOV 260mm, TR 4.75ms, TE1 1.34ms and TE2 2.57ms. Whole body MRI simultaneous with PET: T1 vibe fs tra GK KM: Matrix size 195x320, in-plane resolution 1.2x1.2x3mm, FOV 380, TR 4.56ms, TE 2.01. T2w HASTE: Matrix size: 256x256, in-plane resolution 1.5x1.5x6mm, FoV 380mm, TR 1400ms, TE 121ms.



Supplemental figure 1 - Immunohistochemical (IHC) stains of subject 5. Staining images are magnified 5-fold. This figure shows a tissue specimen with heterogeneous AR and PSMA expression in two distinct morphological areas. On the left magnification, there is no AR expression in the center of the cribriform glands, but strong PSMA expression (rated with "3+"). On the right magnification the glands show strong AR expression (rated with "3+"), while PSMA stains weaker (rated with "2+") and even partially negative.

H&E = Hematoxylin and Eosin stain; PSMA = prostate-specific membrane antigen, AR = androgen receptor