MATERIAL AND METHODS

Image Acquisition

PET images were obtained using ^{68}Ga-labelled HBED-CC. ^{68}Ga^{3+} was obtained from a ^{68}Ge/^{68}Ga radionuclide generator (iThemba Labs, Cape Town) and complexed with the HBED-CC conjugate by means of a fully automated module (Scintomics) and good manufacturing practice-grade disposable cassettes and agent kit (ABX). The final product was formulated in isotonic phosphate-buffered saline with subsequent sterile filtration. The ^{68}Ga-PSMA ligand complex was applied to patients via an intravenous bolus (mean 149±27 MBq, range 85-215 MBq, interquartile range 130-165 MBq). Variation of injected radiotracer activity was caused by the short half-life of ^{68}Ga and variable elution efficiencies obtained during the lifetime of the ^{68}Ge/^{68}Ga radionuclide generator.

PET acquisition was started at a mean time of 55.7±12.2 min after tracer injection (range: 35-81 min, ICR: 49-59 min). All patients underwent ^{68}Ga-PSMA ligand PET/CT on a Biograph mCT scanner (Siemens Medical Solutions). A diagnostic CT scan was performed first in the portal venous phase after intravenous injection of contrast agent (Imeron 300), followed by the PET scan. All PET images were acquired in three-dimensional mode with an acquisition time of 3-4 min per bed position. Emission data were corrected for randoms, dead time, scatter, and attenuation and were reconstructed iteratively by an ordered-subsets expectation maximization algorithm (4 iterations, 8 subsets) followed by a postreconstruction smoothing gaussian filter (5 mm in full width at half maximum).

Validation Criteria

Imaging findings were validated in 35.5% (38/107) of patients with PET-positive results by at least one of the following procedures: (a) transrectal ultrasound-guided biopsy in patients with

suspected prostate/prostate bed relapse (n=3); (b) histological analysis after salvage radical prostatectomy and/or lymph node dissection (n=3); (c) correlative conventional imaging including contrast enhanced-CT, magnetic resonance imaging (MRI) or BS (n=2); or (d) clinical follow-up including contrast enhanced-CT, MRI, BS and repeated ^{68}Ga-PSMA ligand PET/CT or PET/MRI confirming the initial suspicious lesion(s) or showing disappearance of suspected metastatic sites after local/systemic treatment and corresponding PSA decline (n=30).

Supplemental Table 1: Univariate linear regression analyses: Influence of clinical and pathological features on

SUVs

| IV	RC	95% CI		P (SUV$_{max}$)	RC	95% CI		P (SUV$_{mean}$)
		Lower	Upper			Lower	Upper	
PSA	0.012	0.002	0.021	0.014	0.011	0.002	0.021	0.019
iPSA	0.002	-0.002	0.006	0.288	0.002	-0.002	0.006	0.228
ADT	0.538	0.224	0.852	0.001	0.520	0.199	0.840	0.002
GS	-0.461	-0.859	-0.062	0.024	-0.453	-0.860	-0.046	0.030
Type of RT	-0.280	-0.631	0.071	0.117	-0.289	-0.646	0.067	0.111
Age	0.002	-0.020	0.023	0.876	0.002	-0.020	0.023	0.884
Injected activity	-0.005	-0.011	0.001	0.133	-0.005	-0.011	0.002	0.151
Acquisition time	0.003	-0.010	0.016	0.672	0.004	-0.009	0.017	0.557
BMI	0.004	-0.039	0.047	0.855	0.002	-0.042	0.046	0.935

IV, independent variables; RC, regression coefficient; CI, confidence interval

Supplemental Table 2: Multivariable linear regression analyses: Influence of clinical and pathological features on

SUVs

IV	RC	95% CI		P (SUV$_{max}$)	RC	95% CI		P (SUV$_{mean}$)
		Lower	Upper			Lower	Upper	
PSA	0.006	-0.003	0.016	0.192	0.006	-0.004	0.016	0.231
iPSA	0.001	-0.003	0.006	0.508	0.002	-0.003	0.006	0.436
ADT	0.415	-0.016	0.847	0.059	0.392	-0.049	0.834	0.081
GS	-0.121	-0.607	-0.366	0.622	-0.097	-0.595	-0.401	0.699
Type of RT	-0.387	-0.861	0.086	0.107	-0.417	-0.901	0.068	0.091
Age	0.011	-0.014	0.036	0.390	0.011	-0.014	0.037	0.379
Injected activity	-0.005	-0.013	0.003	0.219	-0.005	-0.013	0.003	0.226
Acquisition time	-0.004	-0.017	0.010	0.564	-0.003	-0.017	0.011	0.670
BMI	0.007	-0.050	0.064	0.809	0.003	-0.055	0.062	0.912

IV, independent variables; RC, regression coefficient; CI, confidence interval

Supplemental Figure 1. Detection efficacy of ^{68}Ga-PSMA ligand PET/CT in relation to androgen deprivation therapy in patients with EBRT or brachytherapy as primary radiation therapy.
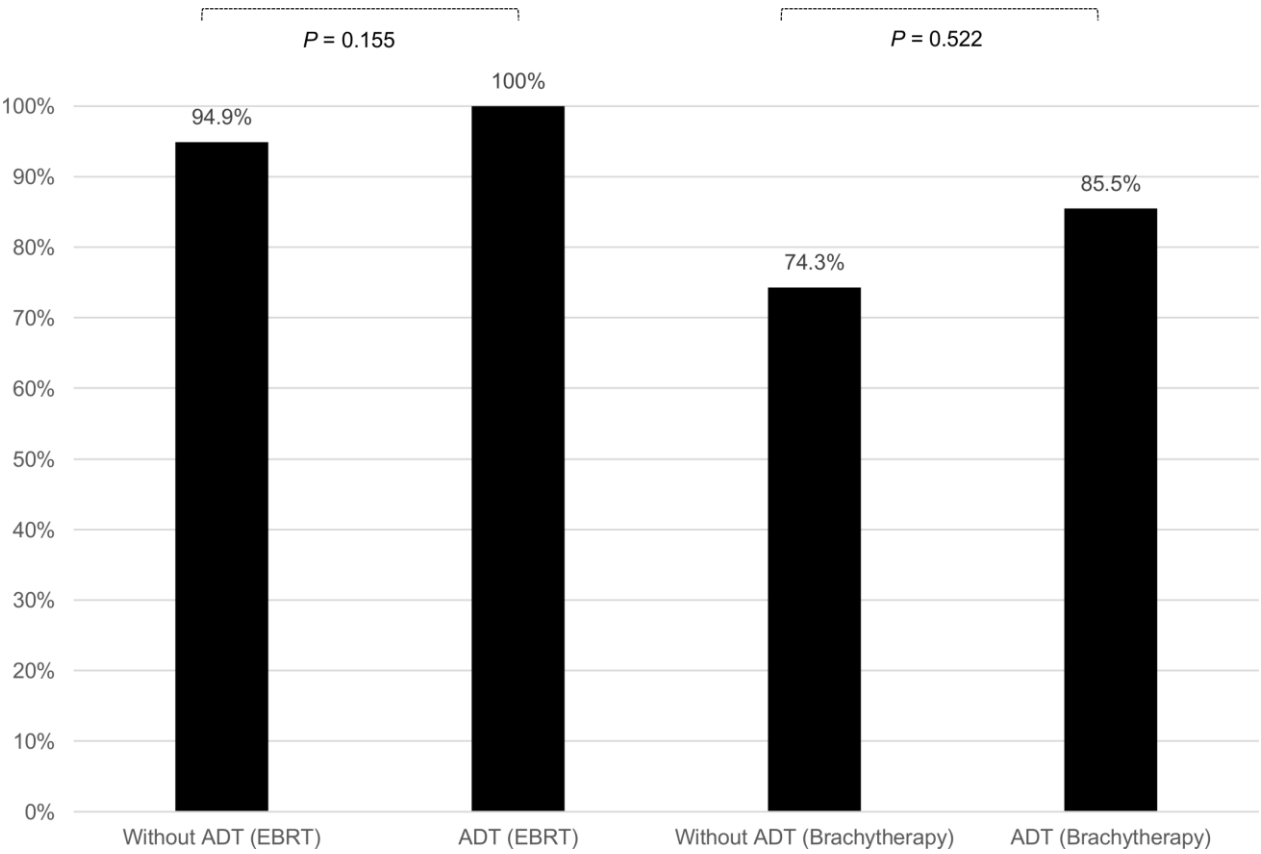
Supplemental Figure 2. Set of fused PET/CT images of a 77-year-old patient with metastasized prostate cancer demonstrating ^{68}Ga-PSMA ligand positive pararectal lymph node metastasis (*white arrow; A*) and ^{68}Ga-PSMA ligand positive local recurrence (*red arrow; C*) after brachytherapy as a primary treatment (GS 7, PSA nadir below detection limit, staging PSA level 21.71 ng/mL). After subsequent chemotherapy, a follow-up ^{68}Ga-PSMA ligand PET/CT scan (B-D) did not reveal any ^{68}Ga-PSMA ligand positive suspicious finding, suggesting therapy response in accordance with a significantly decreasing PSA (0.6 ng/mL) and validating the nature of initially ^{68}Ga-PSMA ligand positive lesions as true positive.