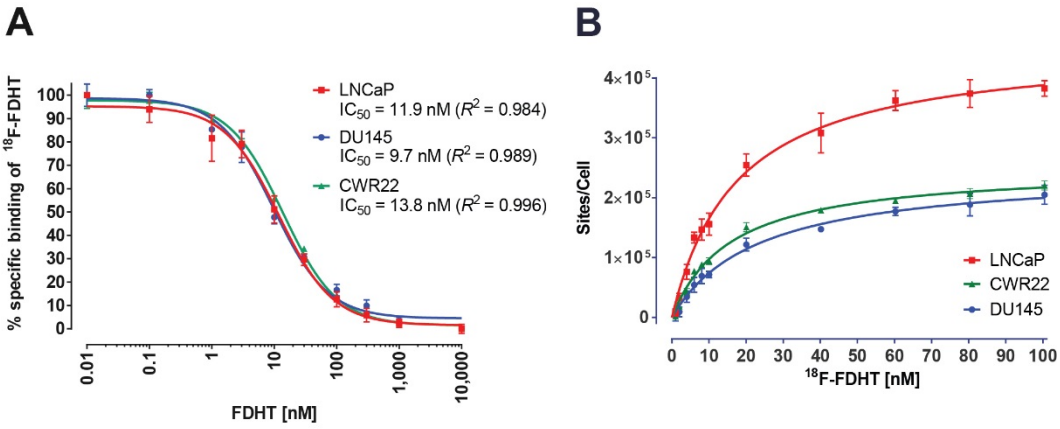
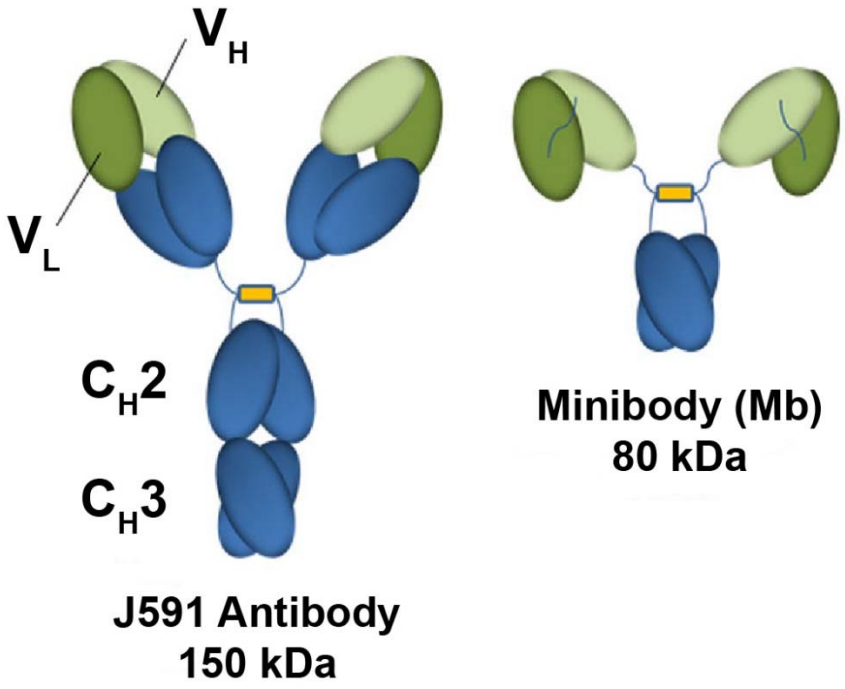


C

	LNCaP	CWR22	DU145
B_{max}	456,000	251,000	246,000
K_d	17.6	23.2	15.9
R^2	0.982	0.988	0.976

Supplemental Figure 1. Characterization of ^{18}F-FDHT in LNCaP, DU145, and CWR22 cells. (A) Homologous competitive binding study and IC_{50} values obtained by non-linear regression analysis. (B) Saturation binding curves. (C) Binding parameters determined by non-linear regression analysis of data in (B).



Supplemental Figure 2. Whole antibody (J591) vs. minibody (IAB2M).

Supplemental Table 1. Bayesian analysis for prediction of disease-positive sites

Modality	Tissue	Total positive sites on scans	Total # biopsied sites	Total biopsy+ lesions (out of total biopsies)	Total scan+ sites biopsied	Scan+ and biopsy+	Total number unbiopsied sites that are scan+	Estimated total number of biopsy+ sites out of unbiopsied scan+ group	95% CI
J591	Bone	491	21	18*	19	18*	470	425	352-465
BS	Bone	339	21	18*	15	14	318	281	220-314
CT	Bone	301	21	18*	16	14	280	233	176-270
FDG	Bone	207	21	18*	16	14	186	155	116-180
CT	Soft	124	25	22	20	18	99	86	67-97
J591	Soft	90	25	22	16	14	65	54	40-64
FDG	Soft	88	25	22	15	13	63	52	37-61

*One site was initially negative on pathology, but showed progression on follow-up; repeat biopsy performed clinically was positive for disease.[61]

Bayesian statistical analysis. Since all of the patients underwent all of the scans, the Bayesian analysis best estimate and 95% confidence interval for detecting positive lesions should be the same if all the tests were equally accurate. However, the studies are ranked in order of J591>bone scan>CT>FDG for detecting bone lesions. In soft tissue, however, the order is CT>J591>FDG. The study and analysis provide a benchmark for comparing imaging performance of PSMA-targeting agents being proposed as prostate cancer imaging biomarkers.

Radiolabeled Anti-PSMA Antibody as a Therapeutic: PSMA has been explored as a target for therapy using ^{90}Y or ^{177}Lu-radiolabeled anti-PSMA antibodies. In animal models, radiolabeled J591 showed significant anti-tumor activity in PSMA-expressing human prostate cancer cells (i.e., LNCaP) and >80% cure in mice with fractionated dose of ^{177}Lu-J591 (*1*). In a phase I dose escalation study, patients with androgen-independent prostate cancer were treated with doses of 370-2590 MBq/m^2 of ^{177}Lu J591, or 10-70 mCi/m^2 (*2*). Sixteen patients received up to three doses. Dose-limiting activity or maximum tolerated dose was a single dose of 2590 MBq/m^2 (70 mCi/m2) beyond which myelosuppression was seen. PSA-based responses included progressive disease in 14 patients (PSA increase of ≥25%) after treatment, ≥50% PSA declines in 4 patients lasting 3-8 months, and PSA stabilization in 16 patients (< 25% increase from baseline) of ≥ 28 days. The median duration of PSA stabilization was 60 days with a range of 28-601+ days. None of the 7 patients with measurable disease had an objective tumor response, nor a ≥50% PSA decline.

A phase II study in metastatic prostate cancer patients with a single infusion of 2405 or 2590 MBq/m^2 (65 or 70 mCi/m^2) of ^{177}Lu-labeled J591 (20 mg) assessed responses with PSA decline, changes in measurable disease, and survival analysis. Fifteen patients each were treated with a dose of 2405 MBq/m^2 (65 mCi/m^2) or 2590 MBq/m^2 (70 mCi m^2). A PSA decline of 50% or more was seen in 10.6% of patients, while 36.2% had a 30% or more decline. Overall, a PSA decline of any level was seen in 59.6% of participants following a single treatment dose (*3, 4*). For therapeutic doses, the liver was found to be the critical organ receiving 210 ± 60.3 cGy/GBq (7.77 ± 2.23 rads/mCi). The

bone marrow dose was estimated to be 32 ± 10.1 cGy/GBq (1.17 ± 0.37 rads/mCi) based on the assumption that 36% of bone marrow volume represents plasma volume.

In an initial dose escalation study using ^{90}Y-DOTA-HuJ591 (20 mg) as a therapeutic followed a week after imaging with ^{111}In-DOTA-HuJ591 (185 MBq, or 5 mCi) for pharmacokinetic and biodistribution determinations, 29 subjects received doses varying from 185-740 MBq/m^2 (20 mCi/m^2), with eligibility to receive three re-treatments based on adequate platelet and neutrophil recovery. The maximum tolerated dose level was 647.5 MBq/m^2 (17.5 mCi/m^2) with two patients experiencing thrombocytopenia with non-life-threatening bleeding episodes requiring platelet transfusions at the higher dose of 740 MBq/m^2 (20 mCi/m^2). No human anti-humanized antibody response was seen. About 21% of these patients experienced PSA stabilization (*5*).

These initial studies have provided proof of concept for the use of radiolabeled anti-PSMA targeting as a therapeutic. More recently, the concept is being explored with novel smaller molecules targeting PSMA.

REFERENCES

1. Smith-Jones PM, Vallabhajosula S, Navarro V, Bastidas D, Goldsmith SJ, Bander NH. Radiolabeled monoclonal antibodies specific to the extracellular domain of prostate-specific membrane antigen: Preclinical studies in nude mice bearing LNCaP human prostate tumor. *J Nucl Med.* 2003;44:610-617.

2. Bander NH, Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ. Phase I trial of (177)lutetium-labeled J591, a monoclonal antibody

to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J Clin Oncol.* 2005;23:4591-4601.

3. Tagawa ST, Jeske S, Milowsky MI, et al. Phase II trial of (177)lutetium radiolabeled anti-prostate-specific membrane antigen (PSMA) monoclonal antibody J591 (Lu-177-J591) in patients (PTS) with metastatic castrate-resistant prostate cancer (metCRPC). *Cancer Biother Radiopharm.* 2008;23:525-525.

4. Tagawa ST, Milowsky MI, Morris M, et al. Phase II study of Lutetium-177-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for metastatic castration-resistant prostate cancer. *Clin Cancer Res.* 2013;19:5182-5191.

5. Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ, Bander NH. Phase I trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. *J Clin Oncol.* 2004;22:2522-2531.