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PET imaging quantifying ^{68}Ga-PSMA-11 uptake in metastatic colorectal cancer

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ABSTRACT

At diagnosis 22% of colorectal cancer (CRC) patients have metastases and 50% later develop metastasis. Peptide receptor radionuclide therapy (PRRT) with lutetium-177 (^{177}Lu)-PSMA-617 is employed to treat metastatic prostate cancer (PC). ^{177}Lu-PSMA-617 targets Prostate Specific Membrane Antigen (PSMA), a cell surface protein enriched in PC and the neovasculature of other solid tumors including CRC. We performed gallium-68 (^{68}Ga)-PSMA-11 PET-CT imaging of ten metastatic CRC patients to assess metastasis avidity. Eight patients had lesions lacking avidity and two had solitary metastases exhibiting very low avidity. Despite expression of PSMA in CRC neovasculature, none of the patients exhibited tumor avidity sufficient to be considered for ^{177}Lu-PSMA-617 PRRT.

Keywords: metastatic colorectal cancer; peptide receptor radionuclide therapy; positron emission tomography; theranostics

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cause of cancer-related death (1). At diagnosis 22% of patients have metastases and 50% develop metastasis during their lifetime (1).

Theranostics employs tumor-selective ligands conjugated to imaging radionuclides and cytotoxic agents for, respectively, cancer imaging and treatment (2). By targeting tumor cell surface antigens these agents are delivered selectively to malignancies (2). Using a diagnostic positron-emitting radionuclide and positron emission tomography (PET), tumor burden is quantified and response to therapy predicted based on tumor avidity (2). In peptide receptor radionuclide therapy (PRRT), therapeutic α or β emitting radionuclides, conjugated to the same PET imaging peptide, induce DNA damage and cell death (2). PRRT is a mainstay treatment for neuroendocrine tumors and emerging for metastatic prostate cancer (mPC) (2).

The PRRT target Prostate-Specific Membrane (PSMA) is enriched in mPC with low expression in normal tissues (3, 4). It is also elevated on endothelial cells of certain solid tumors including CRC where 75-80% of primary tumors and metastases express PSMA which correlates with poor outcome (5, 6). PSMA-11, a high specificity and affinity ligand for PSMA that incorporates a radiometal chelator (7), is used for PET imaging of mPC using gallium-68 (^{68}Ga)-PSMA-11 (8) and PRRT using lutetium-177 (^{177}Lu)-PSMA-617 (9).

Several case reports note CRC avidity during ^{68}Ga-PSMA-11 imaging for mPC, potentially supporting PSMA-targeted PRRT in advanced CRC (10, 11). Responding to a recent call for prospective studies in place of incidental case reports or series (12), we assessed metastatic CRC avidity for ^{68}Ga-PSMA-11 to determine whether avidity meets criteria for ^{177}Lu-PSMA-617 PRRT.

MATERIALS AND METHODS

Patients

Inclusion and exclusion criteria (Supplemental Table 1) and sample size (n= 10) were from PET imaging studies assessing tumor avidity (8, 13, 14). Recruitment would continue if initial results indicated that ≥30% of patients met TheraP trial (NCT03392428) criteria, defined below, to progress to PRRT.

PET Scans and Interpretation

The study has Human Research Ethics Committee approval (HREC/18/QPCH/51). Recruitment was from August to November 2018. Imaging used PSMA-11 (HBED-CC, ABX, Germany) ^{68}Ga labelled as described (15) with labeling efficiency >98%. PET and computed tomography (CT) images were reconstructed with maximum standardized uptake value (SUVmax) and tumor to liver (background) SUVmax ratio determined as described (16). 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) PET-CT or ceCT localized low ^{68}Ga-PSMA-11 avidity metastases, and SUVmax values were

compared between PSMA-PET, FDG-PET and ceCT. TheraP trial criteria to stratify patients as “likely responders” to PRRT required: (1) SUVmax ≥10 at all tumor sites not subject to partial volume artifact (i.e. > 10 mm diameter); (2) SUVmax >20 at the most avid site; and (3) PSMA avidity > FDG avidity at all sites, where recent FDG imaging was available (17).

Immunohistochemistry

The study has Human Research Ethics Committee approval (HREC/11/QRBW/453; P2139). Immunohistochemistry was performed on a tissue microarray of matched CRC primary tumors and metastases from 37 patients, using anti-PSMA clone 3E6 (Agilent) and Biocare Medical MACH1 Mouse HRP Polymer. Signal was quantified by a pathologist (CL) as nil, weak, moderate or strong based on, respectively, no, ≤2.5%, ≥2.5 to ≤4.5% or ≥4.5% positive tumor cells.

Statistical Methods

Statistics were performed using Prism (version 7, GraphPad). Data represent highest SUVmax of representative lesions per anatomical region. Quantification is consistent with STARD reporting guidelines (18).

RESULTS

^{68}Ga-PSMA-11 PET imaging of 10 patients with metastatic CRC (Supplemental Table 2 and 3) resulted in no adverse events. Maximum intensity projections of

participants are shown in Figure 1 with SUVmax values in Figure 2 including TheraP criterion 1 and 2 (19). Metastases of all patients fell significantly short of satisfying criterion 1 and 2, except for liver of patient 3 and lymph node of patient 8. Liver metastases of patient 3 met criterion 1 but not criterion 2. Patient 3 had synchronous lung and omental metastases which also had insufficient avidity to satisfy criterion 1 and 2. Primary tumor and pelvic lymph node metastases of patient 8 exhibited avidity greater than criterion 1 but not criterion 2. This patient also had locoregional lymph node metastases which failed to satisfy criterion 1 and 2. Two of three patients with primary tumors (patient 6 and 7) failed to satisfy both criterion 1 and 2. Bone metastases of patient 6 had the greatest avidity of all lesions and satisfied criterion 1 but fell just short of satisfying criterion 2. Locoregional and retroperitoneal lymph nodes and adrenal metastases of patient 6 failed to satisfy both criterion 1 and 2.

Also of note, patient metastases lacked consistency in tumor to liver SUVmax ratios (Figure 3) and no patient satisfied criterion 3 of PSMA avidity > FDG avidity (Supplemental Table 4). Supplemental Table 5 lists the lesions of each patient, detected by FDG-PET, ceCT and PSMA-PET, including the number missed by PSMA-PET. The time period between PSMA-PET imaging and FDG-PET or ceCT scans is provided in Supplemental Table 6. Eight of ten patients (patient 2, 3, 4, 5, 6, 7, 8, 10) had lesions detected by FDG-PET or ceCT but missed by PSMA-PET. Liver and lymph node metastases of patient 1 and 8 had heterogeneous uptake with only a portion of lesions avid. Although patient 6 had bone metastases with significantly higher avidity during PSMA PET than other soft tissue and visceral lesions, avidity was still significantly lower

than FDG-PET avidity. Supplemental Figure 1 provides representative images of pelvic lymph node metastases with negligible avidity during PSMA-PET compared to high FDG avidity for patient 7 and 8. No lesions were detected by PSMA-PET that were not also identified during FDG-PET. Patients 2, 3, 4, 9 and 10 had previously received neoadjuvant, adjuvant or palliative chemotherapy, with patient 3 receiving palliative chemotherapy eight weeks before PSMA-PET, which likely had minimal effect on avidity because tumor response was poor. For the remaining four patients at least seven months had elapsed since chemotherapy.

Because resected tumors from the ten patients were unavailable to explore the reason for the lack of tumor avidity, we performed immunohistochemistry for PSMA in matched primary tumors and metastases from an independent cohort of 37 patients (Supplemental Table 7 and 8). PSMA was exclusive to endothelial cells of tumor vasculature which consistently comprised ~5% of the cells in tumors. Representative images of tumor regions displaying moderate (≥2.5 to ≤4.5% positive cells) and strong (≥4.5% positive cells) PSMA expression (Supplemental Figure 2A), demonstrate that tumor expression was consistently very low. Quantitative analyses indicated that the invasive edge of 79% of primary tumors and 87% of central regions of primary tumors had nil or weak PSMA expression (Supplemental Figure 2B) with levels consistent between tumor regions (Supplemental Figure 2C). In metastases, the invasive edge of tumors and the central region of 95% of tumors displayed nil or weak PSMA expression (Supplemental Figure 2D) and expression was also consistent between these regions of

metastases (Supplemental Figure 2E). These data suggest that the low observed PSMA ligand avidity is due to consistently low PSMA expression in CRC tumors.

DISCUSSION

Responding to the recent call for prospective trials to assess the utility of PSMA-targeted theranostic agents for cancers beyond PC (12), this study indicates that PSMA PET has low avidity in metastatic CRC with heterogeneous or non-existent uptake in lesions. A range of factors may contribute to low tumor avidity, the most likely of which is low PSMA expression on tumor vasculature. Although PSMA expression has been reported on colorectal neovasculature (5, 6), PSMA mRNA is 10-20 times lower in CRC than PC (19), with our immunohistochemistry confirming low PSMA protein levels in CRC vasculature.

While it is also possible that low avidity was due to a lack of homing of ^{68}Ga-PSMA-11 to CRC tumors this is unlikely because we employed a protocol that identifies mPC allowing sufficient time for radioligand circulation, antigen binding and internalization by PSMA-expressing cells (8). Other potential contributing factors include heterogeneous neovascularization and microvessel density in CRC lesions (20), tumor co-opting of normal vessels lacking PSMA expression (21), and vascular mimicry with tumor blood-conducting channels lined by malignant cells (22).

We estimated that PSMA-PET for metastatic CRC could be beneficial if tumor avidity was sufficient to progress ≥ 30% of patients to PRRT. However, none of the patients had sufficient avidity to progress onto PRRT. Because our sample size is small we cannot be definitive that PSMA-PET is not justified for CRC. However, we note that using binomial probability there was only a very small chance (3%) that none of 10 patients would have sufficient tumor avidity to warrant PRRT, justifying our decision not to continue recruitment beyond 10 patients.

CONCLUSION

^{68}Ga-PSMA-11 PET-CT is not sufficiently sensitive to detect metastatic CRC. Further research is required to identify cell surface receptors as theranostic targets for imaging and treatment of CRC metastasis.

DISCLOSURE

The study was supported by the Redcliffe Private Practice Fund, the Royal Brisbane and Women's Hospital Foundation, and the Mater Foundation. No other potential conflict of interest was reported.

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KEY POINTS

QUESTION: Can PSMA expression on CRC neovasculature be targeted using ^{68}Ga-PSMA-11 with high sensitivity and avidity to qualify patients for ^{177}Lu-PSMA-617 therapy?

PERTINENT FINDINGS: This prospective pilot study assessed the tumor avidity of ten patients with metastatic CRC using ^{68}Ga-PSMA-11. Overall, ^{68}Ga-PSMA-11 was insensitive in detecting CRC metastases. Identified lesions had avidity that was insufficient to warrant PSMA-targeted therapy.

IMPLICATIONS FOR PATIENT CARE: Theranostic ligands targeting specific receptors on metastatic CRC cells should be sought in place of targeting PSMA expressed by tumor neovasculature.

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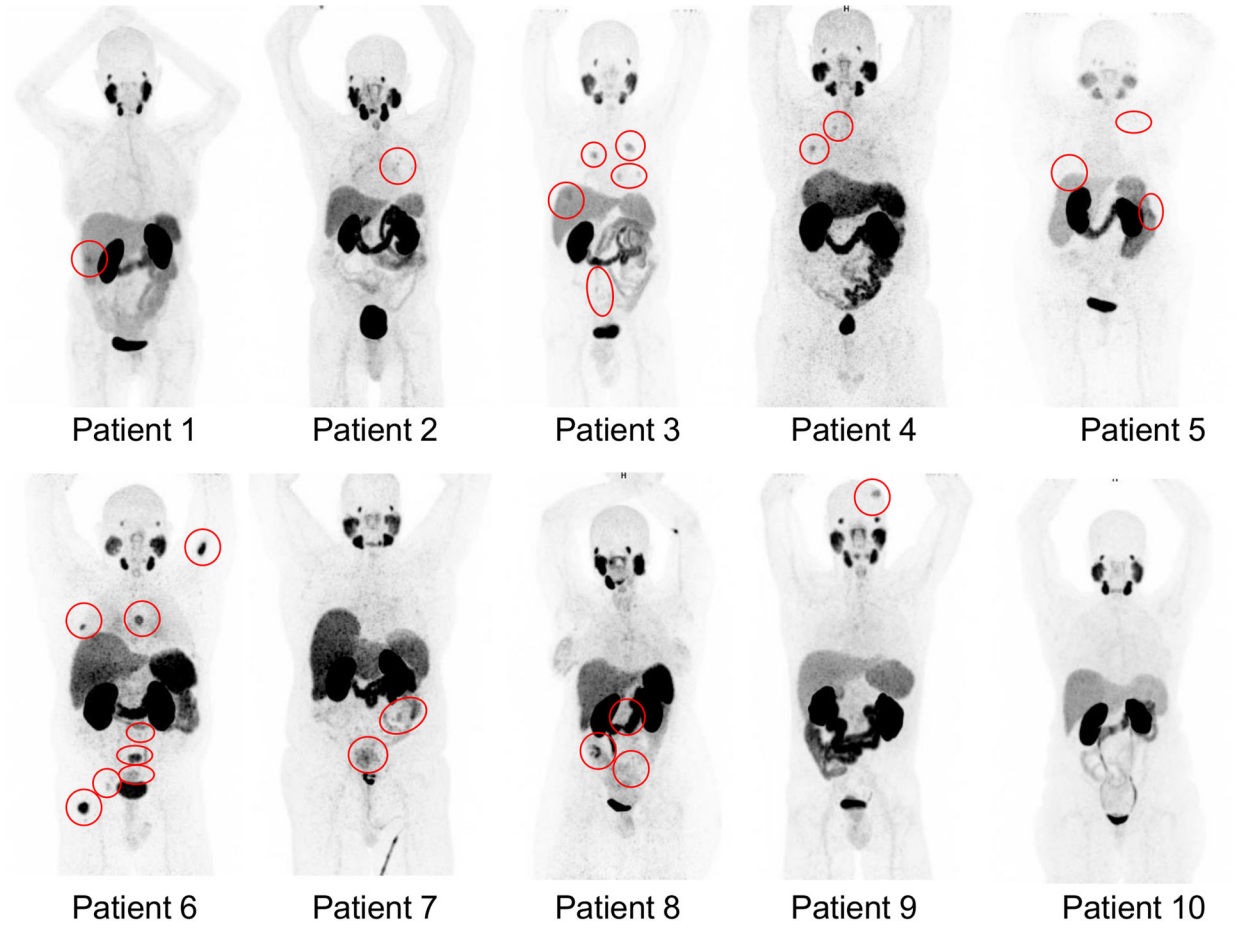


FIGURE 1. ^{68}Ga-PSMA-11 PET maximum intensity projection images of patients with metastatic CRC. Red circle, avid lesion.

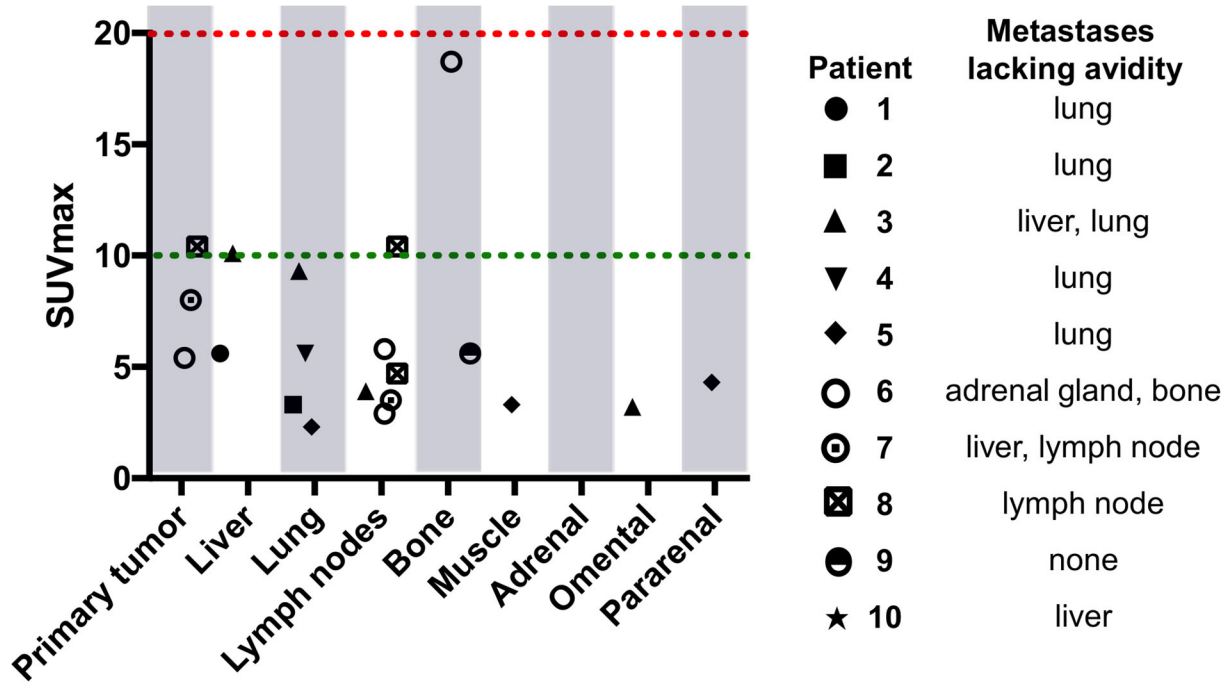


FIGURE 2. SUVmax of ^{68}Ga-PSMA-11 metastatic CRCs. Green line, TheraP criterion 1 SUVmax ≥10 required at all sites. Red line, criterion 2 SUVmax >20 required at the most avid site.

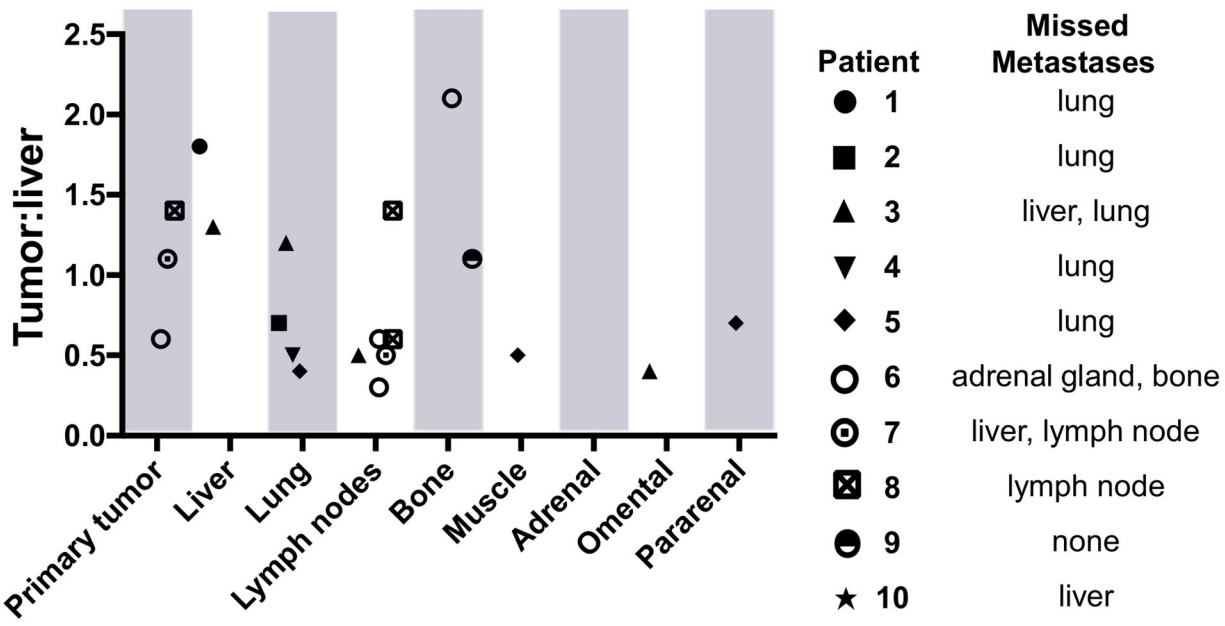
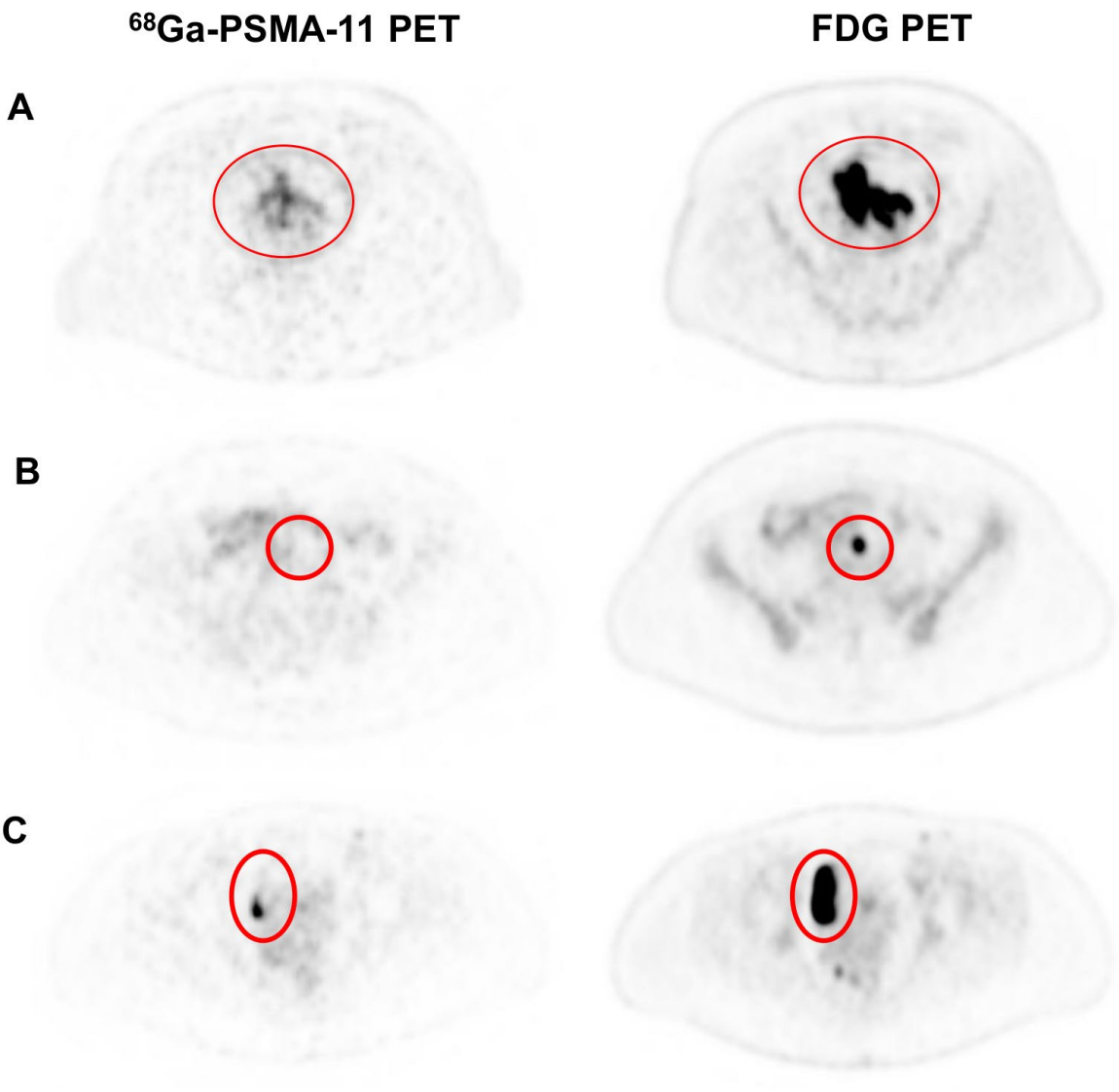


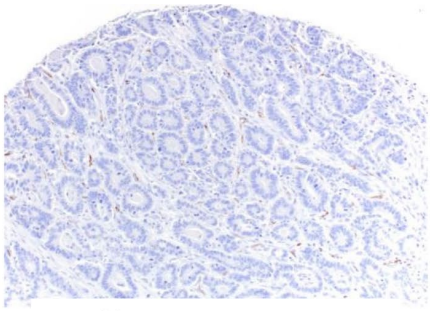
FIGURE 3. CRC tumor to liver (background) SUVmax.

SUPPLEMENTARY FIGURE LEGENDS

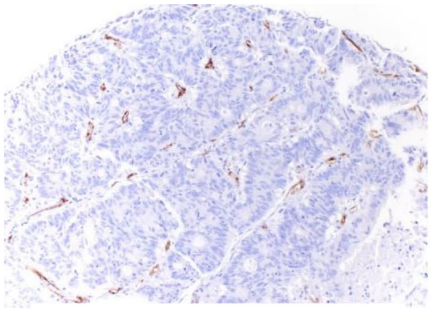


SUPPLEMENTARY FIGURE 1. Representative ^{68}Ga-PSMA-11 PET and FDG PET axial images demonstrating low SUVmax in malignant lesions of patients imaged with ^{68}Ga-PSMA-11 PET (left) versus high SUVmax of the same lesions during FDG PET (right). **A** Primary sigmoid colorectal cancer of patient 7; **B** Pelvic lymph node metastasis of patient 8; **C** Lateral side wall pelvic lymph node metastasis of patient 8.

A

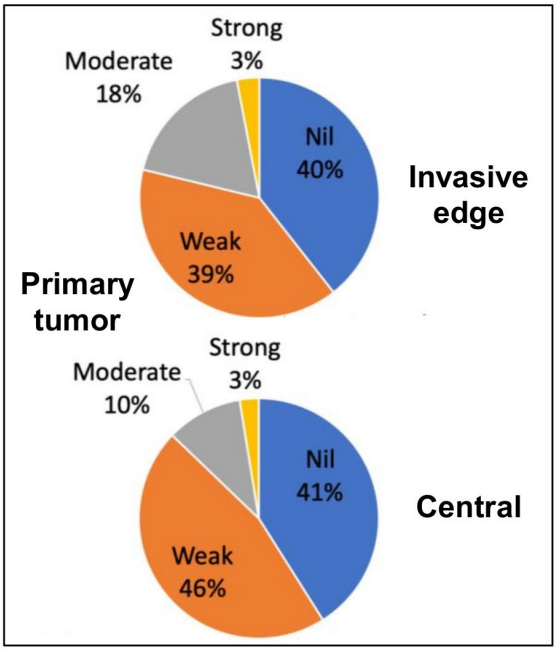


2.5% Moderate staining



4.5% Strong staining

B



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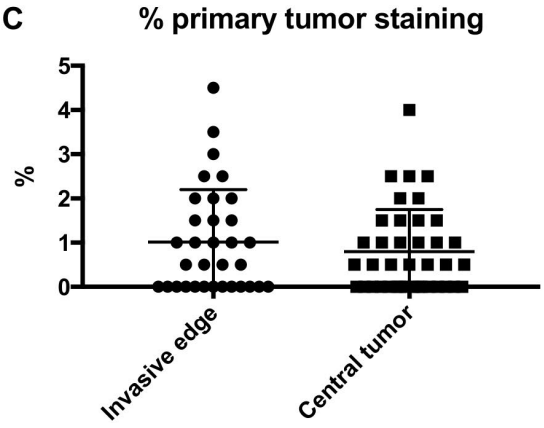

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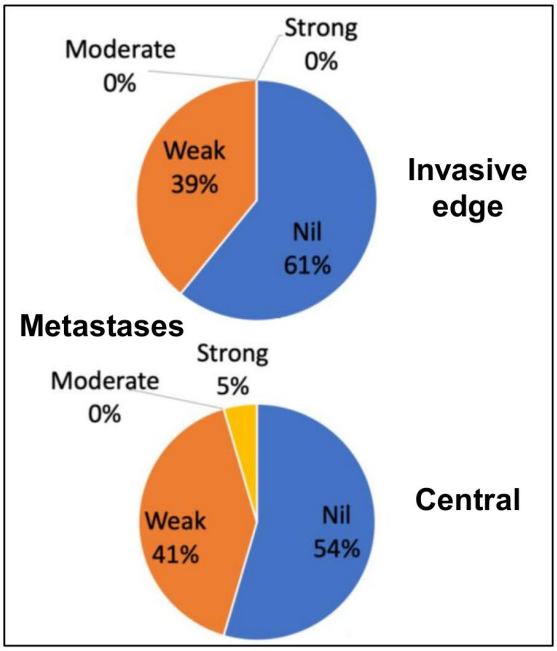

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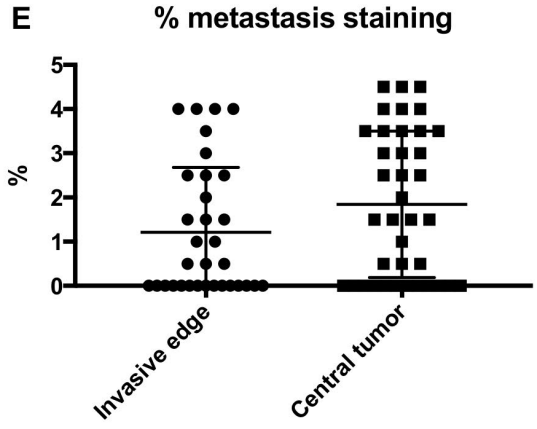

SUPPLEMENTARY FIGURE 2. A Representative images of PSMA staining of primary CRC tumor cores displaying 2.5% of cells with moderate staining and 4.5% of cell with strong staining. **B** Intensity of PSMA staining of central tumor and invasive edge cores of primary CRC tumors. **C** Percentage of whole tumor staining in central tumor and invasive edge cores of primary CRC tumors with mean +/- SD displayed. **D** Intensity of PSMA staining of central tumor and invasive edge cores of CRC metastases. **E** Percentage of whole tumor staining in central tumor and invasive edge cores of CRC metastases with mean +/- SD displayed.

Supplemental Table 1. Inclusion and exclusion criteria of ^{68}Ga-PSMA-11 PET-CT pilot study participants

Inclusion criteria:	Exclusion criteria:
1. ≥ 18yo, male or female 2. Histologically confirmed colorectal non-mucinous adenocarcinoma (not signet ring/mucinous) 3. Metastatic colorectal cancer 4. Patient of RBWH or Redcliffe Hospital Health Services 5. No current or recent chemotherapy, external beam radiation, immunotherapy, radiological, angiogenesis inhibitors up to four weeks prior for all therapies except angiogenesis inhibitors bevacizumab for at least eight weeks; 6. Adequate hemopoietic (*58*): i. Absolute neutrophil count >1.5x10^9/L ii. Platelets >150x10^9/L iii. Hemoglobin >5.6 mmol/L 7. Adequate hepatic function (total bilirubin not more than twice the upper limit of normal (ULN), aspartate transaminase/alanine transaminase not more than three times ULN) (*58*) 8. Adequate renal function (serum creatinine not more than twice ULN, Cockcroft clearance >50 ml/min) (*58*) 9. No known problems of peripheral venous access 10. Able to provide informed, signed consent	1. Concurrent malignancies, except non-melanoma skin tumors or stage 0 (in situ) cervical carcinoma (*59*) 2. Cardiac disease with NYHA classification III or IV or any other illness significantly affecting the patient's clinical condition 3. Eastern Cooperative Oncology Group performance status two or greater (*58,59*) 4. Participation would delay imminent conventional treatment 5. Pregnant or breast feeding (*59*) 6. Patient without capacity 7. Lives a distance from Brisbane Herston Imaging Research Facility (HIRF) requiring extensive travel 8. Administered a radioisotope within five half-lives before intended ^{68}Ga-PSMA-11 imaging (*59*) 9. Patients with allergies to PSMA agent 10. Patient with a concurrent or history of PC or raised PSA indicating prostatic malignancy 11. Unable to lie flat for imaging

Supplemental Table 2. Characteristics of study participants

Characteristic	Total number of patients
Age - mean (SD)	62.1 (+/-10.1)
Male:female	6:4
Primary tumor site	Right colon n=3 Left colon n=1 Sigmoid n=1 Rectosigmoid n=1 Rectum n=3 Not identified n=1
Location of metastases	Abdominal/pelvic LN n=4 Bone n=2 Liver n=4 Lung n=4 Peritoneal/omental n=1 Soft tissue n=1
Histological grade	Mod diff n=8 Poorly diff n=2
Therapy before imaging	Adj CTx n=2 LCCRTx n=1 Pall CTx n=2

*Adj CTx, adjuvant chemotherapy; LCCRTx, long course neoadjuvant chemoradiotherapy; LN, lymph nodes; mod diff, moderately differentiated; n, number of patients; pall CTx, palliative chemotherapy; poorly diff, poorly differentiated.

Supplemental Table 3. Patient demographics

Patient	Gender	Age	Primary location	Metastasis sites	Tumor histological grade	TNM stage	Microscopic inv	Mismatch repair proteins	BRAF/KRAS	Neoadj/adj prior to imaging	Surgical resection
1	F	69.9	R colon	Liver	Mod-diff	T4aN1bM1aR0	VI, LI, TB	Intact	NT	Nil	R hemi
2	M	76.3	Not identified	Lung	Mod-diff	TxNxM1a (biopsy only)	Biopsy only	NT	NT	Nil	Nil
3	M	65.3	Rectal	Lung Liver Iliac LN Omental	Mod-diff	T4aN0M1aR2	PI, TB	Intact	NT	Pall CTx	TATME
4	M	71.0	Rectal	Lung	Mod-diff	T3N2bM1cR0	VI, TIL	NT	KRAS mutation	Pall CTx	APR R hep
5	F	68.0	R colon	Lung Thoracic wall Pararenal	Mod-diff	T4aN1bM1cR0	VI, LI	Intact	NT	Nil	R hemi
6	M	59.3	Rectosigmoid	Primary tumor Locoregional LN Adrenal Retroperitoneal LN Bone	Mod-poorly diff	TxNxM1c (biopsy only)	Biopsy only	NT	BRAF mutation	Nil	Nil
7	M	49.8	Sigmoid	Primary tumor Mesenteric LN	Mod-diff	T4aNxM1c (biopsy only)	Biopsy only	Intact	NT	Nil	Nil

				Retroperitoneal LN Liver							
8	F	43.8	R colon	Primary tumor Locoregional LN Pelvic LN	Poorly-diff	TxNxM1c	Biopsy only	Intact	Intact	Nil	Nil
9	M	55.8	L colon	Bone	Mod-diff	T3N1aM1b	TB	NT	NT	Adj CTx	HAR Pulm lob
10	F	61.6	Rectal	Liver	Mod-diff	TxNxM1a	Biopsy only	Intact	KRAS mutation	Adj CTx, neoadj LCCRTx	Nil

*adj, adjuvant; APR, abdominoperineal resection; CTx, chemotherapy; HAR, high anterior resection; hemi, hemicolectomy; hep, hepatectomy; LCCRTx, long course chemoradiotherapy; LI, lymphatic invasion; LN, lymph nodes; mod diff, moderately differentiated; neoadj, neoadjuvent; NT, not tested; pall CTx, palliative chemotherapy; PI, perineural invasion; poorly-diff, poorly differentiated; pulm lob, pulmonary lobectomy; TATME, transanal total mesorectal excision; TB, tumor budding; TIL, tumor infiltrating lymphocytes; VI, venous invasion.

Supplemental Table 4. ^{68}Ga-PSMA-11 SUVmax and tumor:liver background

Patient	Tumor/metastatic site	PSMA SUVmax	Tumor:liver b/g ratio	FDG SUVmax
1	Liver	5.6◆	1.8	10.8
2	Lung	3.3■	0.7	-
3	Lung	9.3	1.2	-
	Liver	10.1	1.3	
	Iliac LN	3.9	0.5	
	Omental	3.2	0.4	
4	Lung	5.6	0.5	-
5	Lung	2.3	0.4	15
	Thoracic wall	3.3	0.5	13
	Pararenal	4.3	0.7	20
6	Primary tumor	5.4	0.6	-
	Locoregional LN	2.9	0.3	
	Adrenal	NA	NA	
	Retroperitoneal LN	5.8	0.6	
	Bone	18.7	2.1	
7	Primary tumor	8.0	1.1	43.7
	Mesenteric LN	3.5	0.5	6.3
	Retroperitoneal LN	NA	NA	3.3
	Liver	NA	NA	8.3
8	Primary tumor	10.4	1.4	23.7
	Locoregional LN	4.7	0.6	19.1
	Pelvic LN	10.4	1.4	23.4
9	Bone	5.6	1.1	-
10	Liver	NA	NA	22.0

*NA, lesions not avid; LN, lymph node; ◆, only partial lesion avidity; ■, some lesions not avid; -, imaging not available or not performed.

Supplemental Table 5. Lesion count of ^{68}Ga-PSMA-11, FDG AND ceCT

Patient	Tumor/metastatic site	FDG no. of lesions	ceCT no. of lesions	PSMA no. of lesions	Number of missed lesions by PSMA	% missed lesions
1	Liver	1	1	1◆	0	100
2	Lung	25	13	9	16	64
3	Lung	OD	16	10	6	37.5
	Liver		4	1	3	75
	Iliac LN		1	1	0	0
	Omental		1	1	0	0
4	Lung	NP	21	2	19	90.4
5	Lung	3	6	2	4	66.7
	Thoracic wall	1	1	1	0	0
	Pararenal	1	1	1	0	0
6	Primary tumor	1	1	1	0	0
	Adrenal	1	1	0	1	100
	Bone	12	2	6	6	50
7	Primary tumor	1	1	1	0	0
	Mesenteric LN	3	3	1	2	66.7
	Retroperitoneal LN	5	5	0	5	100
	Liver	1	0	0	1	100
8	Primary tumor	1	1	1	0	0
	Locoregional LN	1	1	0	1	100
	Pelvic LN	2	2	0	2	100
9	Bone	1	1	1	0	0
10	Liver	9	9	0	9	100

*no., number; LN, lymph nodes; ◆, only partial lesion avidity; OD, outdated scan, NP, scan not performed.

Supplemental Table 6. Days from ^{68}Ga-PSMA-11 PET

Patient	FDG PET-CT	ce-CT
1	+1	-27
2	-90	+1
3	-1206	-82
4	NA	-16
5	-41	-54
6	-8	-13
7	-13	-28
8	-7	-38
9	-45	-1
10	-252	-216
Median	**-41**	**-27.5**

*NA, not applicable – scan not performed

Supplemental Table 7. Total number of primary tumor and metastasis sites of independent cohort of patients included in TMA

Site	Total number of samples
Primary tumors	
L colon	14
R Colon	9
Rectal	14
Metastasis sites	
Bladder	1
Bone	1
Brain	3
Liver	26
Lung	6
Omentum	2
Ovary	1
Soft tissue	3
Spleen	1

Supplemental Table 8. Individual patient demographics of TMA

Patient	Primary tumour site in TMA	Individual metastasis sites in TMA	Initial T stage	Initial N stage	Initial M stage
1	Rectal	Liver	3	1	0
2	Rectal	Liver, Brain	3	0	0
3	Rectal	Lung, Lung	3	0	0
4	L colon	Liver	3	1	1
5	L colon	Liver	2	1a	1a
6	R colon	Liver, Liver	3	1b	0
7	Rectal	Liver	4a	2a	1
8	L colon	Liver	3	2b	1
9	Rectal	Lung	2	0	1
10	Rectal	Lung	3	0	0
11	L colon	Liver	3	0	0
12	Rectal	Liver	3b	2b	1a
13	Rectal	Liver, Lung, Spleen, Bladder	3	0	0
14	L colon	Liver	3	0	0
15	R colon	Liver	4a	2a	1
16	Rectal	Bone, Liver	3c	1	0
17	Rectal	Liver	2	0	0
18	R colon	Liver	4a	1b	1a
19	R colon	Liver	4a	0	1
20	L colon	Liver	3	1b	1
21	R colon	Omentum	4a	1a	0
22	L colon	Liver	3	0	0
23	Rectal	Brain	3	0	0
24	Rectal	Liver	2	1a	0
25	L colon	Soft tissue	4	0	0
26	L colon	Ovary	3	1a	
27	L colon	Omentum	4	1b	1b
28	R colon	Liver	4a	0	1
29	L colon	Liver	2	1a	1
30	Rectal	Brain	3	0	0
31	L colon	Lung	3	0	0
32	R colon	Lung	4b	1b	0
33	L colon	Liver	4b	1b	1a
34	L colon	Soft tissue	4	2	1
35	R colon	Soft tissue	4a	1a	1
36	Rectal	Liver	3	0	0
37	R colon	Liver	3	0	1a