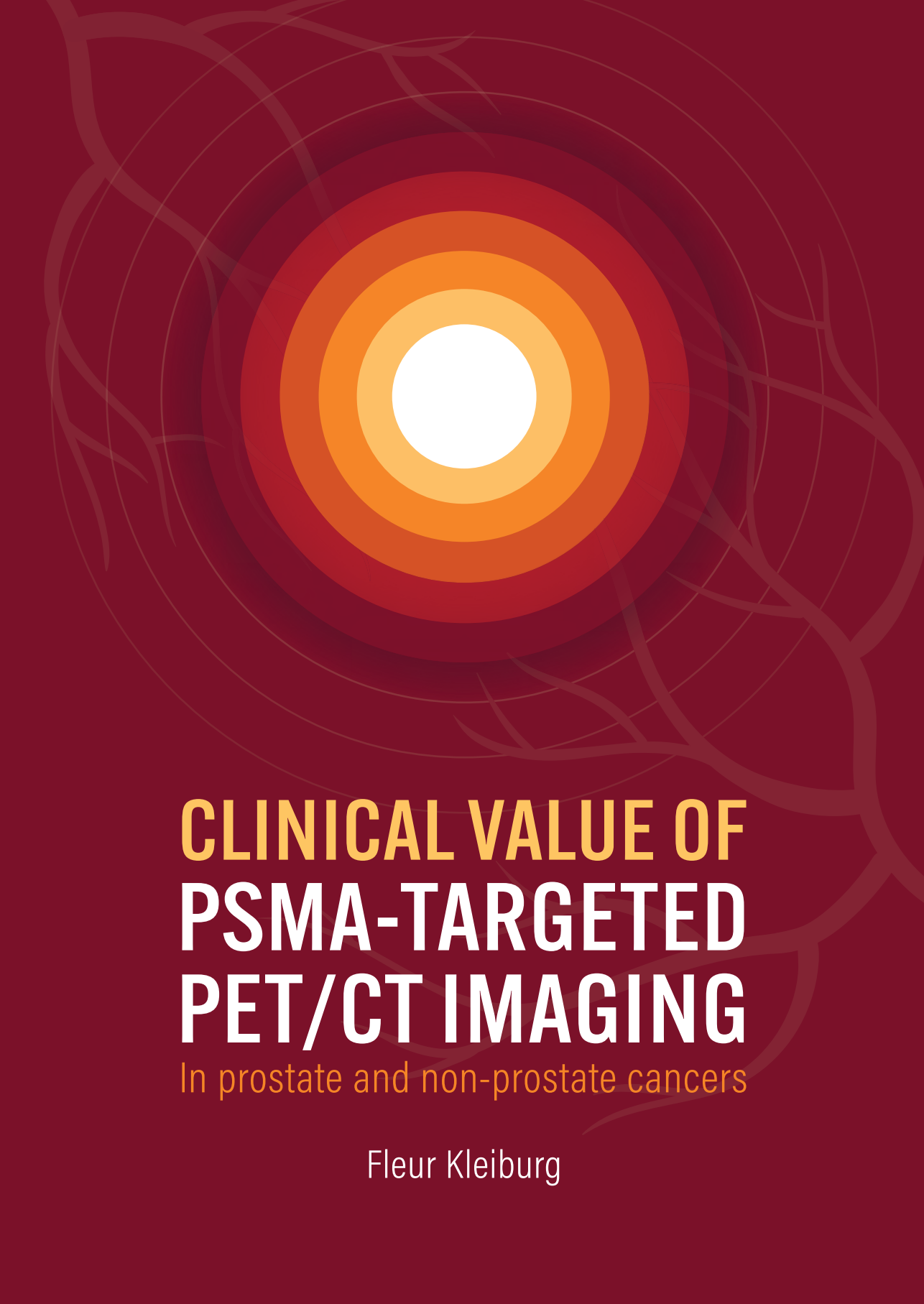


CLINICAL VALUE OF PSMA-TARGETED PET/CT IMAGING

In prostate and non-prostate cancers

Fleur Kleiburg

CLINICAL VALUE OF PSMA-TARGETED PET/CT IMAGING IN PROSTATE AND NON-PROSTATE CANCERS

Fleur Kleiburg

Dit proefschrift is goedgekeurd door:

Promotoren
prof.dr. L.F. de Geus-Oei
prof.dr. S. Manohar

Co-promotoren
dr. L. Heijmen
dr. T. van der Hulle

Cover design: Armando van Bruggen (bruggndesign.nl)
Printed by: Ipskamp Printing (ipskampprinting.nl)
Lay-out: Jacolijn de Krom (persoonlijkproefschrift.nl)
ISBN (print): 978-90-365-6987-3
ISBN (digitaal): 978-90-365-6988-0
URL: https://doi.org/10.3990/1.9789036569880

Curium (curiumpharma.com) kindly contributed to the printing costs of this thesis.

CLINICAL VALUE OF PSMA-TARGETED PET/CT IMAGING IN PROSTATE AND NON-PROSTATE CANCERS

PROEFSCHRIFT

ter verkrijging van
de graad van doctor aan de Universiteit Twente,
op gezag van de rector magnificus,
prof. dr. ir. A. Veldkamp,
volgens besluit van het College voor Promoties
in het openbaar te verdedigen
op dinsdag 20 januari 2026 om 14.30 uur

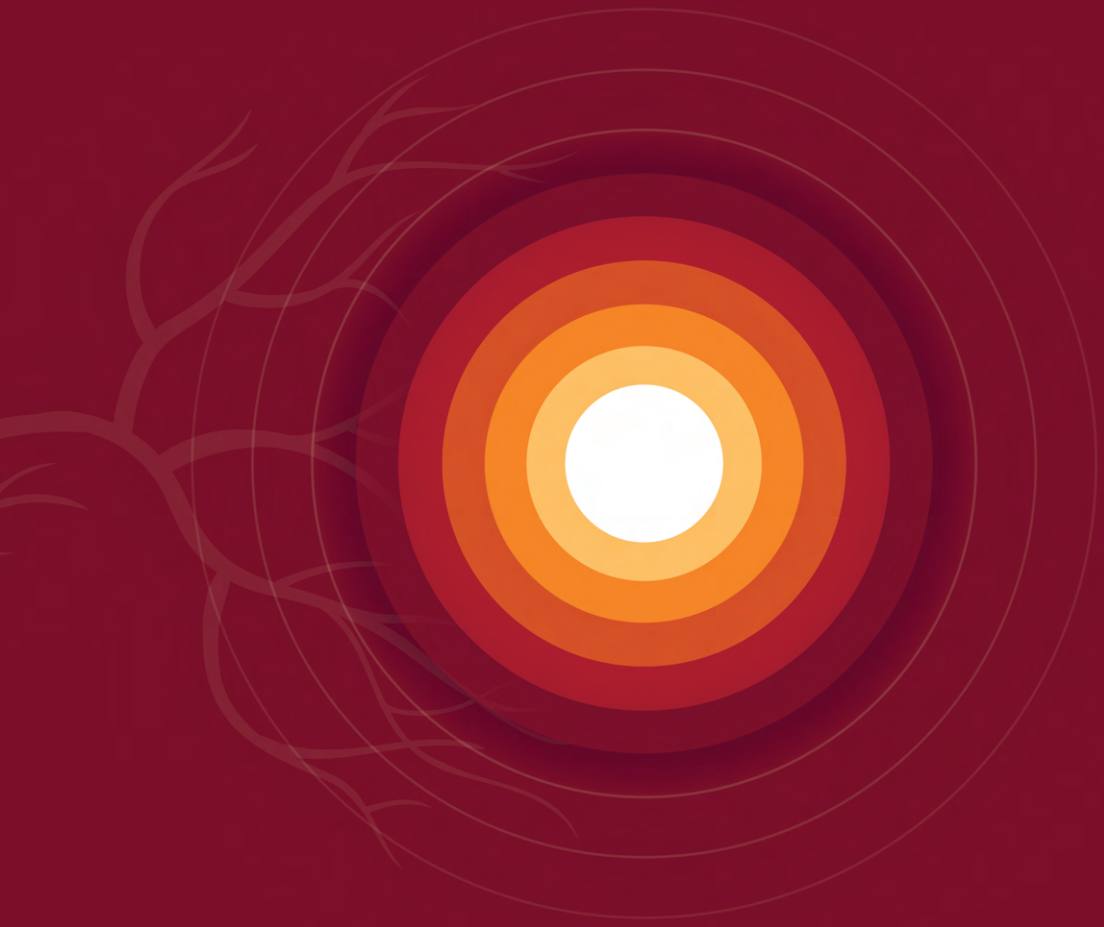
door

Fleur Kleiburg
geboren op 18 augustus 1994
in Amstelveen, Nederland

PROMOTIECOMMISSIE:

Voorzitter / secretaris:	prof.dr. J.J.L.M. Cornelissen
Promotoren:	prof.dr. L.F. de Geus-Oei University of Twente, TNW, Biomedical Photonic Imaging; Leiden University Medical Center, Radiology; Delft University of Technology, Radiation Science and Technology
	prof.dr. S. Manohar University of Twente, TNW, Multi-Modality Medical Imaging
Co-promotoren:	dr. L. Heijmen Leiden University Medical Center, Radiology
	dr. T. van der Hulle Leiden University Medical Center, Medical Oncology
Leden:	prof.dr. R.H.J.A. Slart University of Twente, TNW, Biomedical Photonic Imaging
	prof.dr. J.J. Fütterer University of Twente, EEMCS, Robotics and Mechatronics
	prof. dr. R.J.A. Van Moorselaar Amsterdam University Medical Center, location VUmc, Urology
	dr. J.M. De Feijter Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Medical Oncology
	prof. dr. R.J. Bennink Leiden University Medical Center, Radiology

TABLE OF CONTENTS



CHAPTER 1

General introduction and outline of thesis

GENERAL INTRODUCTION

In nuclear medicine, prostate-specific membrane antigen (PSMA) has emerged as an important target for both imaging and treatment of prostate cancer. PSMA is a transmembrane glycoprotein that is expressed on the cell surface of prostate epithelial cells [1]. Interestingly, its expression is significantly upregulated in prostate cancer cells compared to normal prostate tissue, and higher levels of PSMA expression have been associated with increased tumour aggressiveness and patient mortality rates [2]. Although the exact function of PSMA is not fully understood, several studies suggest its involvement in pathways that regulate cell proliferation, invasion and migration [3]. The characteristics of PSMA, including a large extracellular domain that can bind and internalise antibodies or ligands, make it an optimal target for imaging and treatment of prostate cancer (Figure 1). As a result, various radiotracers have been developed for these purposes.

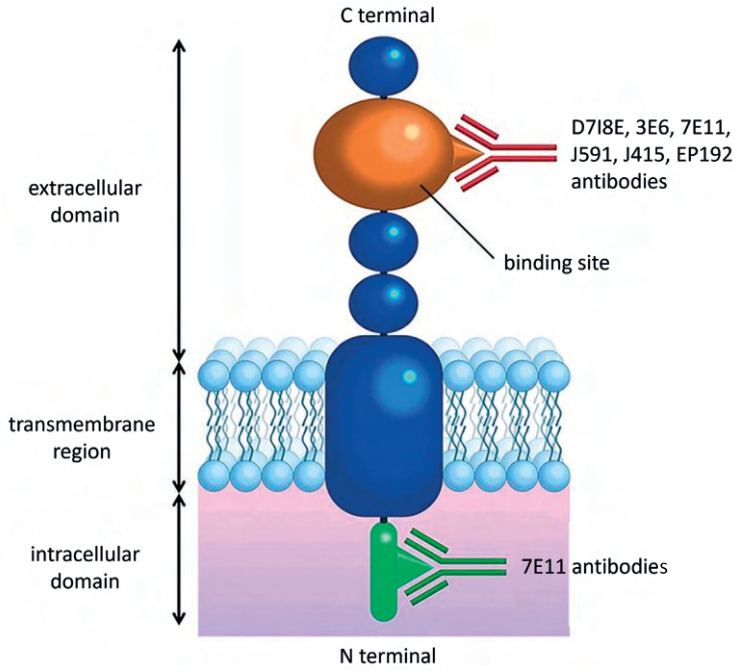


Figure 1. Schematic structure of PSMA [4]. PSMA is a transmembrane and largely extracellular glycoprotein with enzymatic activity as glutamate carboxypeptidase II or folate hydrolase. The majority of developed anti-PSMA antibodies bind to the extracellular domain.

PSMA-targeted imaging

PSMA-targeted positron emission tomography/computed tomography (PET/CT) is an imaging technique that uses PSMA small-molecule ligands labelled with a radioactive isotope such as gallium-68 (^{68}Ga) or fluorine-18 (^{18}F) [5]. After intravenous injection, these ligands bind to prostate cancer cells via the PSMA receptor, and are internalised and accumulated. Both ^{68}Ga and ^{18}F are unstable radioactive isotopes that decay by emitting a positron [6]. The emitted positron travels a short distance until it interacts with an electron, resulting in the annihilation of both positron and electron. This produces a pair of gamma photons that travel in the exact opposite direction. PET scanners can detect these photons and locate their source, allowing accurate detection of prostate cancer lesions (Figure 2). The CT scan is used to correlate the source of radiation with an anatomical location.

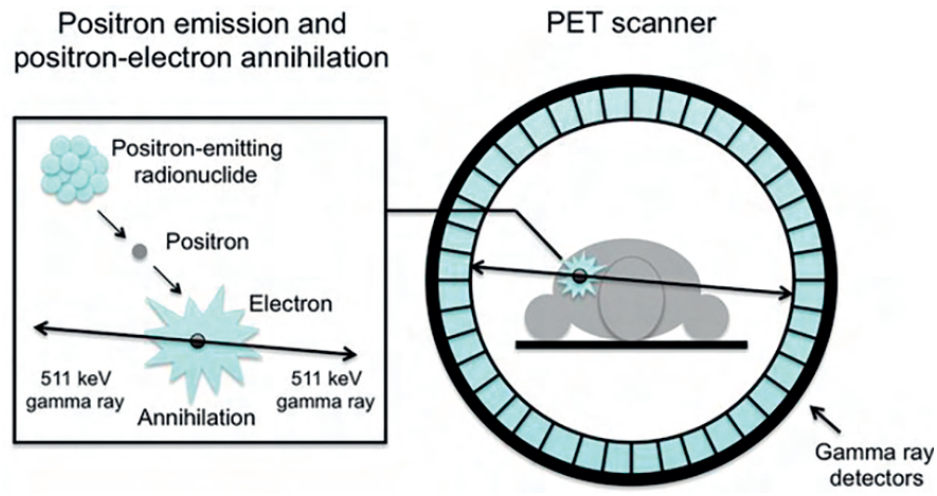


Figure 2. Schematic overview of the detection of positron-emitting radionuclides by a PET scanner.

Several PSMA-targeting radiotracers have been developed and approved for clinical use in prostate cancer patients, including [^{68}Ga]Ga-PSMA-11, [^{18}F]DCFPyL and [^{18}F]PSMA-1007 [5]. Although [^{68}Ga]Ga-PSMA-11 is currently the most widely used radiotracer, the use of ^{18}F-labelled radiotracers is expected to increase. This shift is driven by several advantages: (1) a longer half-life (110 minutes vs. 68 minutes), which allows more time for distribution and permits longer imaging windows; (2) lower positron energy and range, combined with a higher positron yield, resulting in improved spatial resolution; and (3) greater scalability, as ^{18}F is produced using a cyclotron rather than a generator, facilitating large-scale production [6, 7]. Although

there are differences between the radiotracers in terms of radionuclide labelling, physiological distribution, uptake time and excretion route, there is no evidence to suggest that one specific radiotracer has superior diagnostic accuracy over another [5]. Figure 3 shows an example of a [^{18}F]PSMA-1007 PET/CT scan.

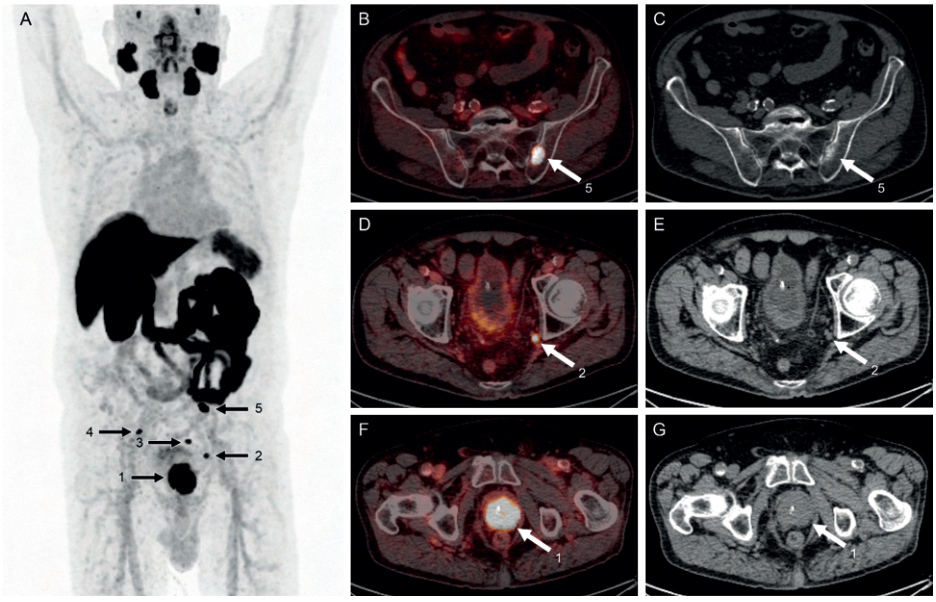


Figure 3. Staging [^{18}F]PSMA-1007 PET/CT of a patient with prostate cancer in the prostate (1), lymph node (2) and bone (3-5). Physiological uptake is observed in the small intestines, kidneys, liver, spleen, salivary glands and lacrimal glands. A: Maximum intensity projection. B, D, F: fused PET and CT images. C, E, G: CT images.

PSMA-targeted therapy

While radioligands for diagnostic purposes deliver a low radiation dose, with no side effects for the patient, the aim of PSMA-targeted radioligand therapy (PSMA-RLT) is to use higher amounts of radiation to cause DNA damage in the prostate cancer cells, leading to cell death [8] (Figure 4). This can be achieved using radioactive isotopes such as lutetium-177 (^{177}Lu), a beta emitter, or actinium-225 (^{225}Ac), an alpha emitter, which cause DNA strands to break. PSMA-RLT is generally well tolerated, but can cause side effects such as fatigue, dry mouth and anaemia. In 2022, [^{177}Lu]Lu-PSMA-617 (Pluvicto®) became the first PSMA-RLT to be approved for clinical use by both the European Medicines Agency and U.S. Food and Drug Administration.

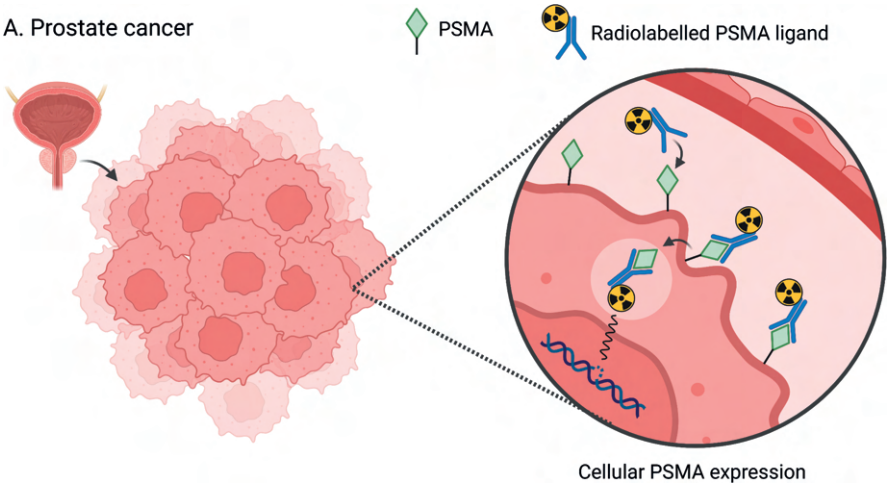


Figure 4. Illustration of PSMA-targeted radioligand therapy in prostate cancer. The radiation from the injected radiolabelled PSMA ligand causes DNA damage. Image created with BioRender.com.

PSMA-TARGETING STRATEGIES IN PROSTATE CANCER

Prostate cancer diagnosis

Prostate cancer is one of the most common cancers in men, with yearly approximately 1.5 million diagnoses and 400 thousand deaths worldwide [9]. It typically develops at an older age, with a median age at diagnosis of 70 years [10]. The majority of prostate cancer progresses slowly, and patients diagnosed with localised or regional disease have a 5-year survival rate of nearly 100% [10]. However, in case of more aggressive prostate cancer that has metastasised, 5-year survival rates decrease to 45%. Prostate cancer screening aims to detect aggressive prostate cancer at an early stage, while leaving indolent prostate cancer that will not cause any symptoms during a man's lifetime undetected.

While magnetic resonance imaging (MRI) remains the gold standard for prostate cancer diagnosis and imaging of localised disease, in high-risk patients whole-body imaging is needed to screen for metastases. For these high-risk patients, with either a PSA level > 20 ng/mL, Gleason score ≥ 8 or T-stage ≥ 3 (Table 1) PSMA-targeted PET/CT imaging is recommended in the EAU guidelines [11]. Patient are staged according to the TNM classification system (Table 1).

Table 1. Prostate cancer staging using the TNM classification system.

T1	Clinically inapparent tumour neither palpable nor visible by staging
T2	Tumour confined within prostate
T3	Tumour extends through the prostate capsule
T4	Tumour invades adjacent structures, such as the rectum, bladder or pelvic wall
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M0	No distant metastasis
M1	Distant metastasis
	M1a = distant lymph nodes
	M1b = bones
	M1c = other organs

Prostate cancer treatment

A representation of the disease course and treatment options for prostate cancer patients is shown in Figure 5. In localised prostate cancer, treatment options depend on risk stratification. For low-risk disease, which may never cause symptoms, active surveillance is typically preferred to avoid treatment side effects. For intermediate- and high-risk disease, standard curative-intent treatment options are radical prostatectomy or radiation therapy. In cases of locally advanced prostate cancer (T-stage ≥ 3), radiation therapy is often combined with two to three years of androgen deprivation therapy (ADT) to improve treatment efficacy.

Patients that present with metastases or develop metastases after local treatment, are classified as having metastatic hormone-sensitive prostate cancer (mHSPC). Unfortunately, mHSPC has no curative treatment options. In this stage, lifelong ADT remains the backbone of treatment by continuously lowering the testosterone levels that drive cancer cell growth, and the addition of novel androgen receptor-targeting agents (ARTAs) such as abiraterone and enzalutamide has significantly improved patient survival. In high-volume metastatic disease (as defined by the CHAARTED trial [12]), docetaxel chemotherapy can be added, whereas radiation therapy to the prostate can be considered for low-volume disease [13] to improve disease control.

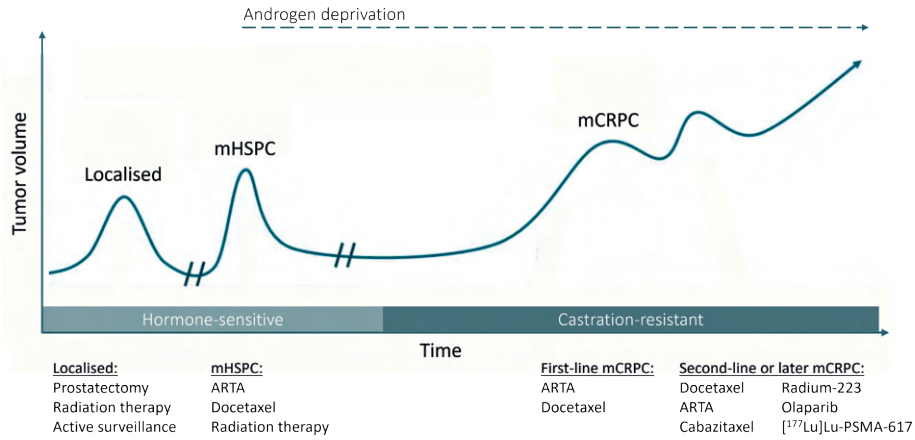


Figure 5. Schematic overview of prostate cancer progression and treatment options. In case of metastatic disease, patients receive continuous androgen deprivation therapy (ADT). mHSPC = metastatic hormone-sensitive prostate cancer. mCRPC = metastatic castration-resistant prostate cancer. ARTA = androgen receptor-targeting agents.

Once metastatic prostate cancer progresses despite castration-level testosterone, it is classified as castration-resistant prostate cancer (mCRPC). mCRPC is associated with a poor prognosis, with a median overall survival of only 2 years from mCRPC diagnosis [14]. In this setting, ARTAs and docetaxel chemotherapy remain key treatment options. In the approximately 20% of mCRPC patients that reach third-line treatment [14], additional therapies such as radium-223 and cabazitaxel chemotherapy can be considered. Since 2022, following the results of the VISION trial, [^{177}Lu]Lu-PSMA-617 is a new life-prolonging treatment option for patients who have progressed on both an ARTA and docetaxel chemotherapy [15]. Each treatment strategy increases overall survival with approximately 4-5 months, so shared decision-making weighing the survival benefit against treatment toxicity is important. Ongoing research is focused on optimising treatment sequencing, exploring combination therapies, and developing novel agents to improve outcomes for mCRPC patients.

Current indications for PSMA PET/CT in prostate cancer

In routine clinical practice, PSMA PET/CT is recommended for three main indications [5]. The first is for the initial staging of newly diagnosed patients with high-risk prostate cancer to screen for metastases, as mentioned previously. PSMA PET/CT has replaced conventional imaging, i.e. CT and bone scans, for this purpose mainly as a result of the phase 3 proPSMA study [16]. This study showed

that PSMA PET/CT had a 27% better accuracy (92% versus 65%), a higher rate of changes in patient management (28% versus 15%) and a lower rate of equivocal findings (7% versus 23%) compared to conventional imaging. PSMA PET/CT has demonstrated the ability to detect lesions as small as 3-5 mm, which are difficult to detect with conventional imaging [17, 18].

Secondly, in patients with biochemical recurrence or persistence after curative intent local treatment, a PSMA PET/CT scan is recommended for restaging. In this setting, PSMA PET/CT has demonstrated a high detection rate of recurrent lesions, especially when PSA levels have risen above 2.0 ng/mL [19].

Thirdly, PSMA PET/CT is used to assess eligibility for PSMA-RLT in patients with advanced mCRPC. As PSMA is a theranostic target, i.e. both imaging and therapy are directed at the same protein, the amount of tracer uptake on PSMA PET/CT can be used as a surrogate measure of PSMA expression. This helps to assess the likelihood of achieving a therapeutic dose with PSMA-RLT and subsequently, the likelihood of a tumoural response.

Potential novel applications for PSMA PET/CT in prostate cancer

Beyond its established indications, PSMA PET/CT presents additional opportunities to improve prostate cancer management, which will be addressed in this thesis. Compared to conventional imaging modalities, PSMA PET/CT scans provides extensive information on tumour characteristics that goes beyond anatomical location and morphological features such as tumour shape and volume. It also provides molecular and biological characteristics, including tracer intensity and heterogeneity, which can reflect tumour aggressiveness. By extracting and analysing these imaging biomarkers on PSMA PET/CT, parameters may be identified that can predict course of disease and patient outcomes. Improved insight into tumour biology and patient prognosis prior to treatment initiation may help guide decisions on whether to initiate or withhold treatment in the shared decision making process. With this, we can increasingly move from “one-size-fits-all” treatment decisions to personalised treatment decisions.

In addition, PSMA PET/CT may play a valuable role in evaluating response to systemic treatments. Accurate assessment of treatment response is important to ensure therapies are continued only when they effectively reduce tumour burden, and discontinued when disease progresses, thereby avoiding unnecessary toxicity. Currently, treatment response evaluation typically relies on PSA testing and

periodic conventional imaging. However, compared to these techniques, PSMA PET/CT offers a more accurate reflection of tumour burden, enabling a more precise evaluation of treatment response or progression. By identifying ineffective treatments earlier, new potentially effective treatments can be initiated earlier and PSMA PET/CT may increase the chance for longer survival.

PSMA-TARGETING STRATEGIES IN NON-PROSTATE CANCERS

Neovascular PSMA expression in non-prostate cancers

Despite its name, PSMA is not only expressed in prostate cancer, but also in a wide range of other tumours. Interestingly, non-prostate malignancies have a different PSMA expression pattern, where generally PSMA is expressed on the endothelial cells of the tumour-associated neovasculature instead of on the cancer cells itself [20]. In the vasculature of aligning normal tissues, PSMA expression is absent. The mechanisms behind PSMA expression are not completely understood, but the expression pattern suggests that PSMA has a role in tumour-induced neoangiogenesis and regulation of vascular growth factors.

The increasing availability of PSMA PET/CT imaging has revealed PSMA uptake in a wide variety of non-prostatic malignant and benign diseases with different anatomical locations. An overview is shown in Table 2 [21, 22]. The prevalence and intensity of neovascular PSMA expression vary among tumour types. For instance, in soft tissue and bone sarcomas, PSMA expression has been reported in 20% of cases, with higher expression levels associated with more aggressive tumour subtypes [23]. In colorectal and gastric cancer, PSMA expression was detected in 85% and 66% of tissues, respectively, with higher expression correlating with higher tumour grade [24].

The presence of PSMA expression in non-prostate tumours highlights the need for careful interpretation of PSMA PET/CT images by nuclear medicine physicians to avoid incorrectly identifying lesions as metastatic prostate cancer. On the other hand, it presents an opportunity to expand the applications of PSMA theranostics beyond prostate cancer.

Table 2. An overview of reported PSMA-avid malignant and benign disease (adapted from De Galiza Barbosa et al. [21] and Srinivasan et al. [22]). Underlined disease will be discussed in this thesis.

Region	Malignant	Benign
Nervous system	Glioma	Meningioma Schwannoma Neurofibroma
Head and neck	Squamous cell carcinoma Adenoid cystic carcinoma Thyroid cancer	(Para)thyroid adenoma
Thoracic	Breast cancer Lung cancer Mesothelioma	Sarcoidosis Tuberculosis
Abdominal	Pancreatic carcinoma Gastric adenocarcinoma Colon carcinoma Urothelial cell carcinoma Hepatocellular carcinoma Cholangiocarcinoma Renal cell carcinoma Gastrointestinal stromal tumour Ovarian cancer Vulvar carcinoma	Adrenal adenoma Liver and splenic haemangioma
Haematological	Lymphoma Multiple myeloma	
Skeletal, soft-tissue and vascular	Leiomyosarcoma Liposarcoma Malignant nerve sheath tumour Undifferentiated pleomorphic sarcoma Angiosarcoma Osteosarcoma Ewing sarcoma	Angiolipoma Haemangioma Osteochondroma Osteomyelitis Paget's disease Fibrous dysplasia Dermatofibroma

Potential novel applications for PSMA theranostics in non-prostate cancers

As PSMA-RLT demonstrated therapeutic efficacy with limited toxicity in prostate cancer patients, this has led to a growing interest in its potential efficacy in other PSMA-expressing malignancies. Current literature consists mainly of case reports and small case series, with some promising results [25]. For example, treatment with [^{177}Lu]Lu-PSMA-617 resulted in clinical improvement in a patient with glioblastoma after three cycles of 3.7 GBq [26], and in a patient with adenoid cystic carcinoma after one cycle of 7.5 GBq [27]. However, some reports have described patients in who intra-therapeutic dosimetry demonstrated only moderate radioligand uptake, such as two cases of hepatocellular carcinoma [28], which would limit therapeutic efficacy. This suboptimal uptake may be due to neovascular PSMA expression (Figure 6) rather than cellular PSMA expression, which can affect radioligand binding and retention. In carefully selected patients, PSMA-RLT has the potential to improve survival and/or quality of life. Currently, however, there are no criteria to identify those who will achieve sufficient radioligand uptake and clinical benefit from PSMA-RLT. This is particularly relevant in cancers with poor prognosis and limited treatment options, where novel therapeutic strategies are urgently needed.

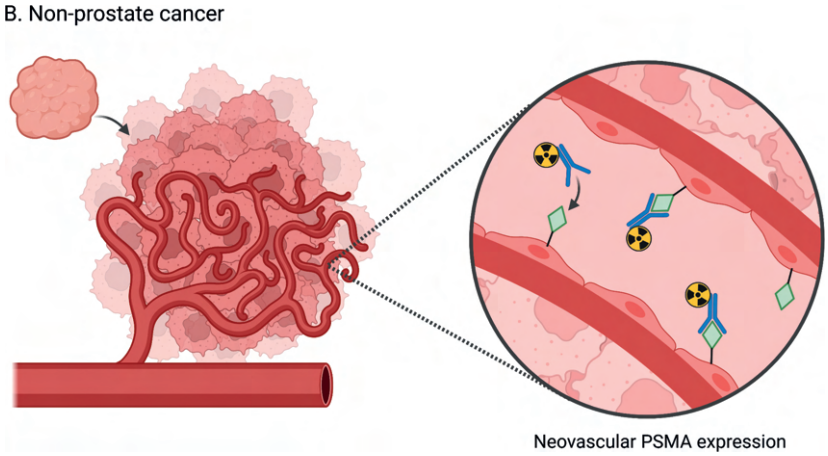


Figure 6. Illustration of endothelial PSMA expression in a non-prostatic tumour, targeted by a radiolabelled PSMA ligand. Image created with BioRender.com

AIM AND OUTLINE OF THESIS

This thesis consists of two parts. Part 1 focuses on new applications of PSMA PET/CT in prostate cancer. Part 2 focuses on PSMA expression and PSMA PET/CT imaging in non-prostate cancers.

Chapter 2 analyses stage migration with PSMA PET/CT compared to conventional imaging in high-risk prostate cancer patients that are referred for curative-intent radiation therapy. The study aimed to validate and extend the findings of the proPSMA study by focusing on a very high-risk population, hypothesising that the use of PSMA PET/CT in this cohort would lead to higher rates of upstaging than previously reported.

Since the CHAARTED criteria for high- and low-volume metastatic disease were established using conventional imaging, in clinical settings where PSMA PET/CT is now standard for staging it has become uncertain how to stratify mHSPC patients and how to select the optimal treatment intensification strategy. Chapter 3 evaluates the prognostic value of baseline PSMA PET/CT and clinical parameters in mHSPC patients. The aim was to improve survival prediction and personalised treatment decision-making, and to provide a basis for the development of a PSMA PET/CT-based risk stratification model for mHSPC.

Also in mCRPC patients, PSMA PET/CT has the potential to improve survival prediction at baseline and therefore, personalised treatment decision-making. As mCRPC represents a heterogeneous disease, improved survival prediction can help weighing the expected treatment effects against toxicity and patient burden. Chapter 4 describes the prognostic value of PSMA PET/CT parameters and clinical parameters in mCRPC patients receiving ARTAs or chemotherapy. Additionally, a preliminary lesion-level analysis was done to assess whether a selection of literature-based PET parameters could predict which lesions were likely to respond or progress after 3-4 months of treatment.

In addition to predicting survival, we hypothesise that PSMA PET/CT can improve treatment response evaluation in mCRPC patients receiving systemic therapies. Chapter 5 assesses PSMA PET/CT for treatment response evaluation in comparison to PSA, with the hypothesis that PSMA PET/CT is able to detect disease progression earlier than PSA, thereby improving patient outcome and reducing toxicity and costs.

Chapter 6 provides an overview of the current literature on PSMA as a potential target for molecular imaging and treatment in sarcomas. Given the clinical heterogeneity, poor prognosis and limited treatment options in many sarcoma subtypes, a novel target such as PSMA could offer a valuable opportunity. The review discusses mRNA expression of the FOLH1 gene, which encodes PSMA, PSMA immunohistochemistry, PSMA-targeted imaging and PSMA-targeted therapy in sarcomas.

Chapter 7 presents the results of a prospective clinical trial we conducted to evaluate the feasibility of PSMA-RLT in patients with metastatic soft tissue sarcoma. PSMA expression was assessed in tumour tissue obtained by biopsy or surgical resection. Patients with high PSMA expression underwent PSMA PET/CT imaging. The primary objective was to determine the proportion of patients with sufficient radiotracer uptake on PSMA PET/CT, defined as $SUV_{max} > 8$, who could potentially be considered for PSMA-RLT. With this, we aimed to provide insights for potential future exploration of PSMA theranostics in soft tissue sarcomas.

Besides sarcomas, also several gastrointestinal malignancies have demonstrated PSMA expression. Chapter 8 describes the result of a prospective trial investigating PSMA PET/CT imaging in patients with colon, gastric and pancreatic cancer. The primary objective was to assess the feasibility of using PSMA PET/CT to target and visualise primary colon, gastric and pancreatic cancer.

Frequently used abbreviations in this thesis

Sorted in alphabetical order

^{18}F	Fluorine-18
^{68}Ga	Gallium-68
^{177}Lu	Lutetium-177
ADT	Androgen deprivation therapy
ALP	Alkaline phosphatase
ARTA	Androgen receptor targeted agent (e.g. abiraterone and enzalutamide)
Bq	Becquerel
C.I.	Confidence interval
CT	Computed tomography
DmaxVox	Maximum distance between two voxels
Hb	Haemoglobin
HR	Hazard ratio
HVD	High-volume disease
IQR	Interquartile range
LVD	Low-volume disease
mCRPC	Metastatic castration-resistant prostate cancer
MDT	Multidisciplinary team
mHSPC	Metastatic hormone-sensitive prostate cancer
OR	Odds ratio
OS	Overall survival
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PR	Partial response
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
PSMA-TV	Total tumour volume
RLT	Radioligand therapy
SD	Stable disease
SUV	Standardised uptake value
TL-PSMA	Total lesion uptake
VOI	Volume of interest

REFERENCES

1. Chang SS. Overview of prostate-specific membrane antigen. Rev Urol. 2004;6 Suppl 10:S13-8.
2. Hupe MC, Philippi C, Roth D, Kumpers C, Ribbat-Idel J, Becker F, et al. Expression of Prostate-Specific Membrane Antigen (PSMA) on Biopsies Is an Independent Risk Stratifier of Prostate Cancer Patients at Time of Initial Diagnosis. Front Oncol. 2018;8:623.
3. Sheehan B, Guo C, Neeb A, Paschalis A, Sandhu S, de Bono JS. Prostate-specific Membrane Antigen Biology in Lethal Prostate Cancer and its Therapeutic Implications. Eur Urol Focus. 2022;8(5):1157-68.
4. Tateishi U. Prostate-specific membrane antigen (PSMA)-ligand positron emission tomography and radioligand therapy (RLT) of prostate cancer. Jpn J Clin Oncol. 2020;50(4):349-56.
5. Fendler WP, Eiber M, Beheshti M, Bomanji J, Calais J, Ceci F, et al. PSMA PET/CT: joint EANM procedure guideline/SNMMI procedure standard for prostate cancer imaging 2.0. Eur J Nucl Med Mol Imaging. 2023;50(5):1466-86.
6. Sanchez-Crespo A. Comparison of Gallium-68 and Fluorine-18 imaging characteristics in positron emission tomography. Appl Radiat Isot. 2013;76:55-62.
7. Werner RA, Derlin T, Lapa C, Sheikbahaei S, Higuchi T, Giesel FL, et al. (18)F-Labeled, PSMA-Targeted Radiotracers: Leveraging the Advantages of Radiofluorination for Prostate Cancer Molecular Imaging. Theranostics. 2020;10(1):1-16.
8. Jang A, Kendi AT, Sartor O. Status of PSMA-targeted radioligand therapy in prostate cancer: current data and future trials. Ther Adv Med Oncol. 2023;15:17588359231157632.
9. Ferlay J EM, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2024). Global Cancer Observatory: Cancer Today.: Lyon, France: International Agency for Research on Cancer; [14-04-2025]. Available from: https://gco.iarc.who.int/today.
10. IKNL. Prostaatkanker in Nederland, een overzicht op basis van de Nederlandse Kankerregistratie (1989 tot en met 2021) [14-04-2025]. Available from: https://iknl.nl/prostaatkanker-in-nederland.
11. EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer. European Association of Urology 2022.
12. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737-46.
13. Parker CC, James ND, Brawley CD, Clarke NW, Hoyle AP, Ali A, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet. 2018;392(10162):2353-66.

14. Freedland SJ, Davis M, Epstein AJ, Arondekar B, Ivanova JI. Real-world treatment patterns and overall survival among men with Metastatic Castration-Resistant Prostate Cancer (mCRPC) in the US Medicare population. Prostate Cancer Prostatic Dis. 2023.

15. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177-PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2021;385(12):1091-103.

16. Hofman MS, Lawrentschuk N, Francis RJ, Tang C, Vela I, Thomas P, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. Lancet. 2020;395(10231):1208-16.

17. Incesu RB, Preisser F, Pompe RS, Nohe F, Mandel P, Maurer T, et al. Diagnostic Accuracy of Prostate-specific Membrane Antigen Positron Emission Tomography/Computed Tomography for Primary Lymph Node Staging Before Radical Prostatectomy. Eur Urol Focus. 2025.

18. Giesel FL, Fiedler H, Stefanova M, Sterzing F, Rius M, Kopka K, et al. PSMA PET/CT with Glu-urea-Lys-(Ahx)-[(6)(8)Ga(HBED-CC)] versus 3D CT volumetric lymph node assessment in recurrent prostate cancer. Eur J Nucl Med Mol Imaging. 2015;42(12):1794-800.

19. Fendler WP, Calais J, Eiber M, Flavell RR, Mishoe A, Feng FY, et al. Assessment of 68Ga-PSMA-11 PET Accuracy in Localizing Recurrent Prostate Cancer: A Prospective Single-Arm Clinical Trial. JAMA Oncol. 2019;5(6):856-63.

20. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, Gaudin PB. Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. Cancer Res. 1999;59(13):3192-8.

21. de Galiza Barbosa F, Queiroz MA, Nunes RF, Costa LB, Zaniboni EC, Marin JFG, et al. Nonprostatic diseases on PSMA PET imaging: a spectrum of benign and malignant findings. Cancer Imaging. 2020;20(1):23.

22. Srinivasan R, Cook GJR, Patel N, Subesinghe M. Prostate specific membrane antigen (PSMA) avid nonprostatic benign and malignant disease: a pictorial review. Clin Radiol. 2024;79(9):639-56.

23. Heitkotter B, Trautmann M, Grunewald I, Bogemann M, Rahbar K, Gevensleben H, et al. Expression of PSMA in tumor neovasculature of high grade sarcomas including synovial sarcoma, rhabdomyosarcoma, undifferentiated sarcoma and MPNST. Oncotarget. 2017;8(3):4268-76.

24. Haffner MC, Kronberger IE, Ross JS, Sheehan CE, Zitt M, Muhlmann G, et al. Prostate-specific membrane antigen expression in the neovasculature of gastric and colorectal cancers. Hum Pathol. 2009;40(12):1754-61.

25. Uijen MJM, Derks YHW, Merkx RIJ, Schilham MGM, Roosen J, Prive BM, et al. PSMA radioligand therapy for solid tumors other than prostate cancer: background, opportunities, challenges, and first clinical reports. Eur J Nucl Med Mol Imaging. 2021;48(13):4350-68.

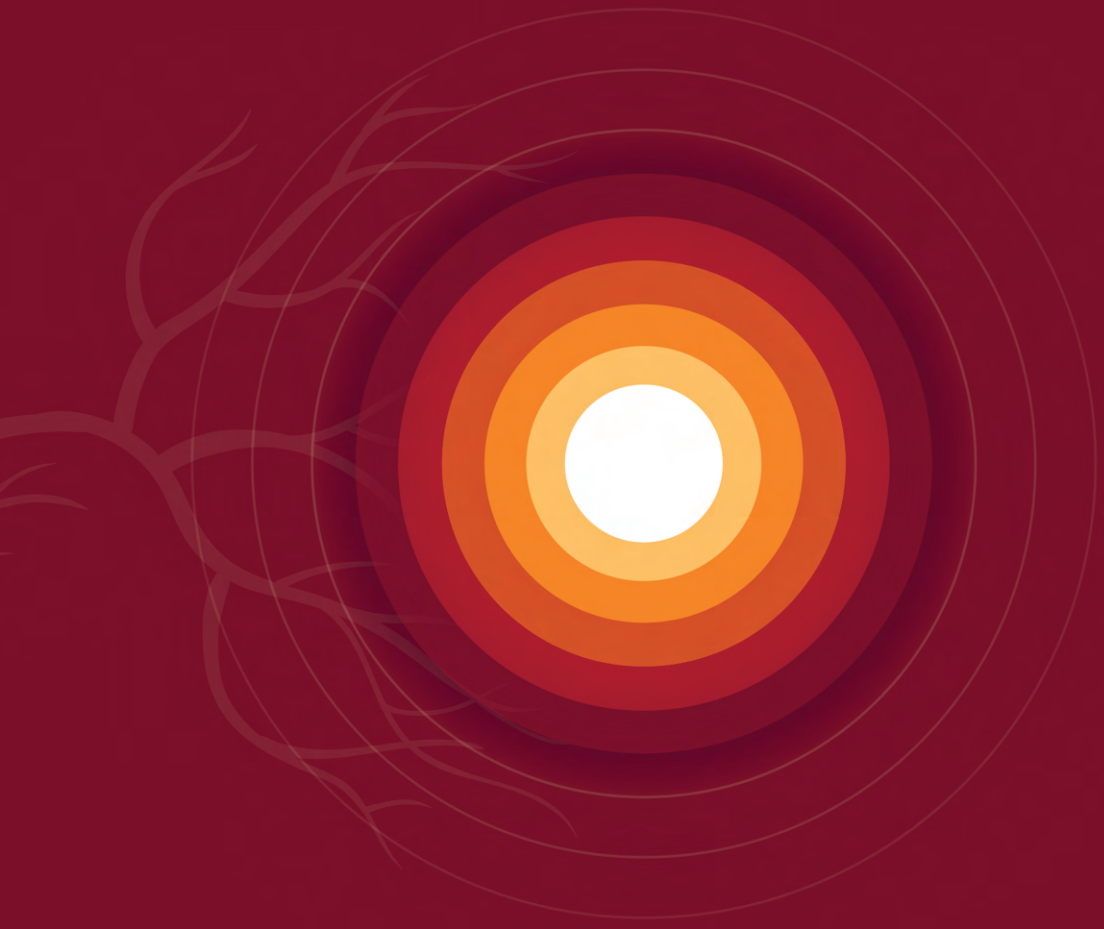
26. Kumar A, Ballal S, Yadav MP, ArunRaj ST, Haresh KP, Gupta S, et al. 177Lu-/68Ga-PSMA Theranostics in Recurrent Glioblastoma Multiforme: Proof of Concept. Clin Nucl Med. 2020;45(12):e512-e3.

27. Has Simsek D, Kuyumcu S, Agaoglu FY, Unal SN. Radionuclide Therapy With 177Lu-PSMA in a Case of Metastatic Adenoid Cystic Carcinoma of the Parotid. Clin Nucl Med. 2019;44(9):764-6.

28. Hirmas N, Leyh C, Sraieb M, Barbato F, Schaarschmidt BM, Umutlu L, et al. (68)Ga-PSMA-11 PET/CT Improves Tumor Detection and Impacts Management in Patients with Hepatocellular Carcinoma. J Nucl Med. 2021;62(9):1235-41.

PART 1

PSMA PET/CT in prostate cancer



CHAPTER 2

Stage migration on PSMA PET/CT in comparison to conventional imaging in patients with high-risk prostate cancer referred for radiation therapy: results from the phase 2/3 THUNDER trial

Authors
Fleur Kleiburg, Piet Dirix, Valérie Fonteyne, Samuel Bral, Bart De Troyer, Brieuc Sautois, Maréva Lamande, Nick Liefhooghe, Guillaume Grisay, Sabine Meersschout, Ad Vandermeulen, Nicolas Jullian, Lorenzo Staelens, Filip Poelaert, Michiel Strijbos, Jolien Verschueren, Karolien Goffin, Nadia Withofs, Piet Ost

Published
European Urology Oncology
2025 Oct;8(5):1333-1339
DOI: 10.1016/j.euo.2025.08.005

Supplementary materials
https://doi.org/10.1016/j.euo.2025.08.005

ABSTRACT

Background

In high-risk prostate cancer, the proPSMA trial showed upstaging with prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/computed tomography (CT) in 14% of patients. We hypothesised that the probability of stage migration in a patient population referred for curative-intent radiotherapy would be higher. Here we report stage migration results according to PSMA PET/CT in the first year of inclusion in the phase 2/3 THUNDER trial (NCT06282588).

Methods

Patients with high-risk prostate cancer screened between December 2023 and December 2024 in the THUNDER trial with both conventional imaging (CT, bone scintigraphy) and PSMA PET/CT within 16 weeks before screening were included (n = 142). Stage migration according to the TNM classification versus the molecular imaging (miTNM) classification (PROMISE v2 criteria) was assessed using descriptive statistics.

Results

PSMA PET/CT led to stage migration in 43 patients, of whom 42 (30%) were upstaged and one (1%) was downstaged. Upstaging to miN1-2 disease occurred in 32 patients (23%), and to miM1a-c disease in 19 patients (13%). The probability of upstaging increased with the number of high-risk features. In the subgroup meeting the STAMPEDE M0 high-risk criteria (n = 73), PSMA PET/CT upstaged 27 patients (37%), including upstaging to miM1a-c disease in 14 (19%). Limitations include the absence of central review of the imaging procedures.

Conclusions

One-third of patients with high-risk prostate cancer referred for curative-intent radiotherapy were upstaged on PSMA PET/CT. This finding supports the use of PSMA PET/CT for staging, especially in patients with multiple high-risk features, and suggests a need for treatment adaptations accordingly, which will be further investigated in the THUNDER trial.

INTRODUCTION

Patients with prostate cancer presenting with one or more high-risk features at diagnosis have a higher likelihood of harbouring metastatic disease, so accurate imaging is essential for disease staging and selecting the optimal treatment strategy. Conventional imaging modalities such as computed tomography (CT) and bone scintigraphy have traditionally been used for staging; however, their sensitivity and specificity are limited [1,2]. Prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/CT has emerged as a more accurate imaging modality for prostate cancer staging [3,4]. PSMA PET/CT uses radiolabelled PSMA ligands that selectively bind to the extracellular domain of PSMA, a transmembrane protein overexpressed in prostate cancer cells, which allows visualisation of metastatic lesions as small as 3–5 mm [5,6]. The multicentre randomised phase 3 proPSMA trial demonstrated that PSMA PET/CT had 27% greater accuracy in comparison to conventional imaging in detecting nodal and distant metastases in high-risk prostate cancer. This led to nodal or distant metastasis upstaging in 14% of patients, which can have a significant impact on treatment strategies [3]. The proPSMA trial included patients who underwent either curative-intent surgery or radiotherapy. However, patients referred for radiotherapy typically present with a greater number of high-risk features than patients for whom surgery is recommended [7]. Thus, we hypothesised that the rate of upstaging with PSMA PET/CT is higher in the former group, which has not previously been tested.

The aim of this preplanned substudy of the THUNDER trial (ClinicalTrials.gov NCT06282588) was to investigate stage migration in patients with high-risk prostate cancer referred for curative-intent radiotherapy using data from the first year of trial inclusion.

METHODS

Study design and participants

Patients who provided written informed consent to participate in the two-part phase 2/3 THUNDER trial between December 13, 2023 and December 12, 2024 were included in this study. In the THUNDER trial, patients with histopathologically proven high-risk local or locally advanced prostate cancer (defined as any of the following: prostate-specific antigen (PSA) >20 ng/ml, stage T3–4, International

Society of Urological Pathology (ISUP) grade group ≥4, or cN1 disease) were screened for study inclusion. Both conventional imaging and PSMA PET/CT had to be performed within 16 weeks before the screening visit. A complete list of the inclusion and exclusion criteria is provided in the Supplementary material. Patients with metastatic lesions on conventional imaging (cM1) at screening were excluded from further participation in the interventional part of THUNDER, but were included in this study on the screening phase of the trial. To reduce the patient burden, from March 2024 it was decided to no longer require additional bone scintigraphy in cases in which PSMA PET/CT showed no bone lesions, as the proPSMA trial showed that the probability of a true-positive bone scintigraphy result in cases with negative PSMA PET/CT findings is <2% [3]. This amendment was discussed with the steering committee and the independent data monitoring committee, and was approved by the ethics committee and governing bodies.

Patients included in the trial enter either a phase 2 treatment de-intensification or a phase 3 treatment intensification trial. At inclusion, patients undergo conventional imaging, PSMA PET/CT imaging and a genomic classifier (GC) test (Decipher Biosciences, San Diego, CA, USA). Patients without PSMA-positive lesions outside the prostate [8] and a GC score <0.60 enter the de-intensification phase 2 trial, receiving radiotherapy to the prostate and/or pelvic lymph nodes (PLNs) with 24 months of darolutamide, for which quality of life is the primary endpoint. All other patients enter the randomised phase 3 trial comparing radiotherapy to the prostate and/or PLNs plus 24 months of androgen deprivation therapy (ADT) with darolutamide or placebo, for which metastasis-free survival is the primary endpoint.

Imaging procedures

Contrast-enhanced CT, bone scintigraphy, and PSMA PET/CT scans were performed according to local procedures at each site and were assessed locally. All PSMA PET tracers were allowed. Results from the CT scan for the combined PSMA PET/CT imaging at screening could be used for conventional imaging. The TNM classification was used for reporting of positive lesions on CT and bone scintigraphy. Assessment of N stage was based on findings from CT or magnetic resonance imaging. The PROMISE v2 framework was used for reporting of PSMA-positive lesions on PSMA PET/CT [8]. Equivocal lesions on both conventional and PSMA PET/CT imaging were not counted.

Study outcomes

The primary aim of this THUNDER substudy was to report any migration in N and/or M stage on PSMA PET/CT in comparison to conventional imaging. Secondary objectives were to evaluate N stage and M stage migration separately, and to assess the effect of the number of high-risk features on the likelihood of stage migration. An additional subgroup analysis was conducted for patients who met the high-risk M0 criteria from the STAMPEDE trial [9].

Statistical analysis

Descriptive statistics were used to assess rates of stage migration. Continuous variables were reported as the median and interquartile range (IQR). Results for categorical variables were reported as the number and proportion of patients. Logistic regression analysis was conducted to identify significant predictors of upstaging to miM1 disease.

RESULTS

Patient characteristics

Between December 13, 2023 and December 12, 2024, 153 patients were screened for inclusion in the THUNDER trial, of whom 11 were excluded (Figure 1). The baseline characteristics of the study cohort of 142 patients are shown in Table 1. Some 59% of these men had at least two high-risk features (PSA >20 ng/ml, stage T3–4, ISUP grade group ≥4, or cN1 disease). Conventional imaging showed regional metastases (cN1) in 27 men (19.0%), of whom five (3.5%) also had distant metastases (cM1). The PSMA radiotracers used for PSMA PET/CT imaging are listed in Supplementary Table 1.

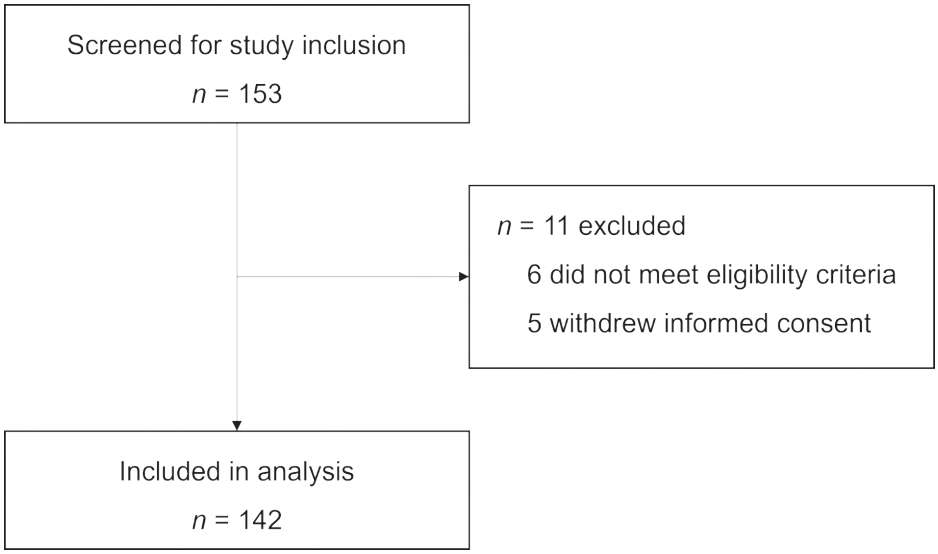


Figure 1. Flow diagram for the study

Table 1. Baseline characteristics for the cohort of 142 patients.

Parameter	Result	
Median age, yr (IQR)	75	(69 – 78)
Median PSA, ng/mL (IQR)	14.3	(8.0 – 32.7)
PSA > 20 ng/mL, n (%)	54	(38%)
Stage ≥ T3, n (%)	87	(61%)
ISUP grade group, n (%)		
1	4	(3%)
2	11	(8%)
3	23	(16%)
4	35	(25%)
5	69	(49%)
ISUP grade group ≥ 4, n (%)	104	(73%)
Stage cN1, n (%)	27	(19%)
Stage cM1, n (%)	5	(4%)

Table 1. *Continued.*

Parameter	Result	
Number of high-risk features, n (%) [a]		
1	58	(41%)
2	48	(34%)
3	26	(18%)
4	10	(7%)
Imaging performed, n (%)		
CT-scan	142	(100%)
Bone scintigraphy [b]	113	(80%)
PSMA PET/CT	142	(100%)

BS = bone scintigraphy; CT = computed tomography; IQR = interquartile range; ISUP = International Society of Urological Pathology; PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen.

[a] High-risk features: PSA >20 ng/ml, stage T3–4, ISUP grade group ≥4, or cN1 disease.

[b] In 29 patients with no bone lesions on PSMA PET/CT, bone scintigraphy was not performed because of the low probability of true positive findings [3], with the aim of reducing the patient burden.

Stage migration

PSMA PET/CT detected positive PLNs in 40.8% of patients (vs 19.0% on CT), positive abdominal distant lymph nodes in 12.0% (vs 0.7% on CT), visceral lesions in 1.4% (vs 0.7% on CT), and bone lesions in 7.7% (vs 1.4% on CT and 2.7% on bone scintigraphy; Table 2). No positive lymph nodes above the diaphragm were observed in the study cohort.

Table 2. Findings on conventional imaging and PSMA PET/CT

Location	CT n = 142	BS n = 113	PSMA PET/CT n = 142
Positive pelvic LNs (N1-2)	19.0%	-	40.8%
Positive extrapelvic LNs (M1a) [a]	0.7%	-	12.0%
Positive bone lesions (M1b)	1.4%	2.7%	7.7%
Positive visceral lesions (M1c)	0.7%	-	1.4%

BS = bone scintigraphy; CT = computed tomography; LNs = lymph nodes; PET = positron emission tomography; PSMA = prostate-specific membrane antigen.

[a] All M1a lesions detected were below the diaphragm.

Figure 2 shows the differences in staging between PSMA PET/CT and conventional imaging. Stage migration was observed in 43 patients, of whom 42 (30%) were

upstaged and one (1%) was downstaged. Specifically, 23 patients with cN0M0 had miN1–2M0 disease, 13 with cN0M0 had miM1 disease, and six with cN1M0 had miM1 disease. The patient with downstaging had cN1M0 and miN0M0 disease.

Regarding N stage, PSMA PET/CT detected positive PLNs that were not detected on conventional imaging in 32 patients (23%). Among these patients, PSMA PET/CT revealed one positive PLN in 14 patients, and multiple positive PLNs in 18 patients. In terms of M stage, PSMA PET/CT revealed distant metastases that were not detected on conventional imaging in 19 patients (13%). Of these, ten had miM1a disease (nine with one to three extrapelvic lymph nodes, one with more than five extrapelvic lymph nodes), eight had miM1b disease (seven with one to three bone lesions and one with five bone lesions), and one had miM1c disease (penile metastasis). In the five patients with cM1 disease on conventional imaging, PSMA PET/CT confirmed this finding. In one of these patients, PSMA PET/CT identified three additional bone lesions (conventional imaging identified one bone lesion). PSMA PET/CT did not reveal any additional lesions in the remaining four patients; one had M1a disease (more than five extrapelvic lymph nodes), two had M1b disease (both had one to three bone lesions), and one had M1c disease (two liver lesions).

Furthermore, the PSMA PET/CT upstaging rate was higher for patients with a greater number of high-risk features (Figure 3). For example, among the patients with four high-risk features, 30% were upstaged to miM1 disease, whereas the upstaging rate to miM1 was 7% in the group with one high-risk feature. Univariate logistic regression analysis revealed that the number of high-risk features was significantly associated with upstaging to miM1 disease (odds ratio per additional high-risk feature: 1.86, 95% confidence interval 1.13–3.07; $p = 0.014$). The individual high-risk features (PSA >20 ng/ml, stage ≥T3, ISUP grade group ≥4, or cN1 disease) taken separately had no predictive value.

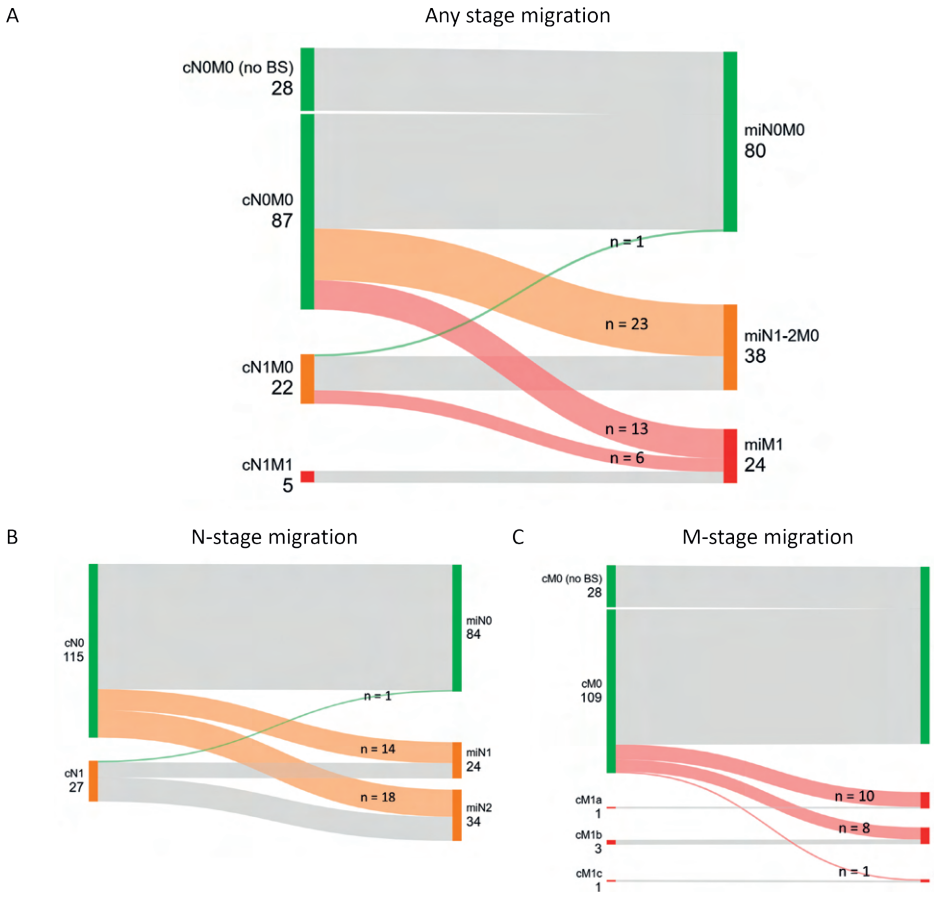


Figure 2. Prostate-specific membrane antigen positron emission tomography/computed tomography findings in comparison to conventional imaging. (A) Any stage migration. (B) N stage migration. (C) M stage migration. BS = bone scintigraphy.

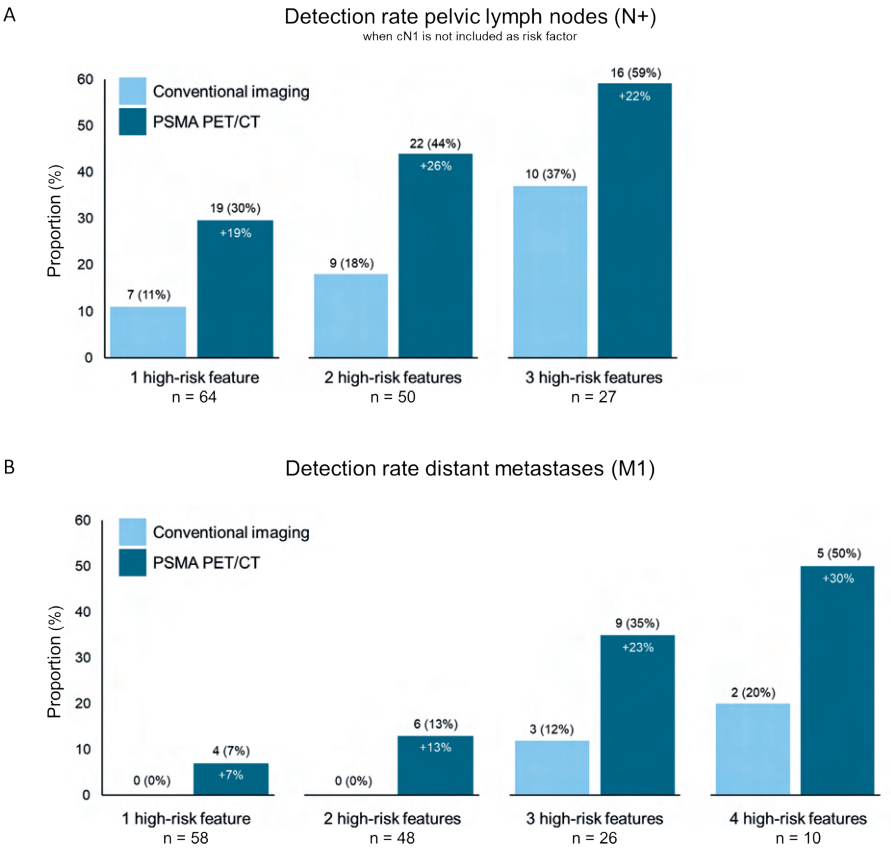


Figure 3. Detection rate on PSMA PET/CT according to the number of high-risk features for (A) pelvic lymph nodes (high-risk features: PSA > 20 ng/ml, T stage ≥3, ISUP grade group ≥4) and (B) distant metastases (high-risk features: PSA >20 ng/ml, T stage ≥3, ISUP grade group ≥4, cN1 disease). CT = computed tomography; ISUP = International Society of Urological Pathology; PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen

Subgroup analysis for patients meeting the STAMPEDE M0 high-risk criteria

The STAMPEDE M0 high-risk criteria (either cN1M0 disease or two of the following: stage ≥T3, PSA >40 ng/ml, ISUP grade group ≥ 4) [9] were met by 73 of the 142 men in the study cohort (51%). Upstaging was observed in 27 patients (37%), with distant metastases in 14 patients (19%). Some 21 patients with localised disease on conventional imaging were upstaged to either pelvic nodal disease (n = 13), miM1a disease (n = 5), miM1b disease (n = 2), or miM1c disease (n = 1), while six patients with pelvic nodal disease on conventional imaging were upstaged to either miM1a (n = 3) or miM1b disease (n = 3; Supplementary Figure 1).

DISCUSSION

Our prospective study showed that PSMA PET/CT led to upstaging in 30% of patients with high-risk prostate cancer referred for curative-intent radiotherapy, including upstaging to miN1–2 disease in 23% and to miM1 disease in 13% of all patients. In addition, the risk of upstaging to miM1 disease increased from 7% for patients with one high-risk feature to 30% for patients with four high-risk features. These findings support the use of PSMA PET/CT for staging, and provide a rationale for prioritising the use of PSMA PET/CT in patients presenting with multiple high-risk features.

The proPSMA trial [3] reported nodal or distant metastatic upstaging in 14% of high-risk prostate cancer patients intended for surgery or radiotherapy, which is half the rate observed in our study. This can likely be attributed to the fact that our patient cohort included only high-risk prostate cancer patients referred for radiotherapy with more high-risk features. In comparison to the proPSMA trial, our cohort had higher proportions of patients with PSA >20 ng/ml (38% vs 22%), stage ≥T3 (61% vs 27%), ISUP grade group ≥4 (73% vs 64%), and cN1 disease (19% vs 9%). A similar trend was observed when comparing our findings to those reported by Hruby et al [10], whose analysis for a cohort with intermediate- or high-risk prostate cancer before definitive radiotherapy revealed upstaging in 21% and miM1 upstaging in 6% of patients. Interestingly, Ravi et al [11] found that patients with multiple risk factors or cN1 disease had worse survival than patients with one risk factor. Together, these results suggest that patients with multiple high-risk features have more aggressive disease with a higher risk of upstaging on PSMA PET/CT and worse survival outcomes. A recent multi-institutional study involving more than 6000 patients staged with PSMA PET/CT revealed that the PET/CT result is at least prognostic [12]. The authors conclude that despite the prognostic value of PSMA PET/CT, they would only recommend this imaging modality as standard practice if a benefit or at least lack of harm is proven when treatment is adapted to the PET/CT result. Although our report focuses on the upstaging potential of PSMA PET/CT in a high-risk population, the primary goal of the trial is to address this fundamental question of whether treatment tailored according to PSMA PET/CT findings improves clinical outcomes.

In the THUNDER trial, patients with extraprostatic disease on PSMA PET/CT or a high Decipher GC score will be randomised to standard-of-care radiotherapy and 2 years of ADT plus either 2 years of placebo or 2 years of darolutamide. In the

STAMPEDE M0 trial, addition of abiraterone to radiotherapy and ADT significantly improved metastasis-free survival and overall survival for men with high-risk cM0 prostate cancer [9]. In our study, 37% of patients meeting the STAMPEDE M0 criteria were upstaged, and 19% had distant metastatic disease on PSMA PET/CT at prostate cancer diagnosis. In the STAMPEDE trial, the 6-year metastasis-free survival was 82% in the combination therapy group and 69% in the control group. The fact that almost one in five of these patients could have had distant metastases on PSMA PET/CT raises the question of whether the benefit of abiraterone observed was not mainly driven by this subgroup of patients.

Strengths of our study include the prospective design, large patient cohort, and standardised reporting of PSMA PET/CT scans. Limitations include the fact that patients with multiple unequivocal metastases on conventional imaging were not screened for inclusion in the THUNDER trial and therefore were potentially missed. Some patients with localised disease on PSMA PET/CT did not undergo bone scintigraphy to minimise the patient burden, which may have resulted in underestimation of downstaging. In addition, PSMA PET/CT may yield false-positive findings for regional lymph node metastases, although this risk is relatively low, with specificity of ~94% reported [13]. Furthermore, scans were interpreted by a single reader, without blinding to prior imaging, and different PSMA tracers were used. However, interobserver agreement for PSMA PET/CT is substantial to excellent. Agreement is highest for regional lymph node and bone metastases, and somewhat lower for soft-tissue metastases and local tumour assessment [14,15]. A structured approach based on the PROMISE criteria was used to minimise interobserver variability in the study, as it has been demonstrated that this yields excellent compartment-based scores indicating excellent agreement, even among readers with varying experience [13].

CONCLUSIONS

In patients with high-risk prostate cancer referred for radiotherapy, who present with more high-risk features than the overall high-risk prostate cancer population, PSMA PET/CT led to upstaging in 30% of cases, and upstaging to miM1 disease in 13%. The risk of upstaging increased with the number of high-risk features. The THUNDER trial will investigate if adapting treatment strategies accordingly can improve metastasis-free survival.

REFERENCES

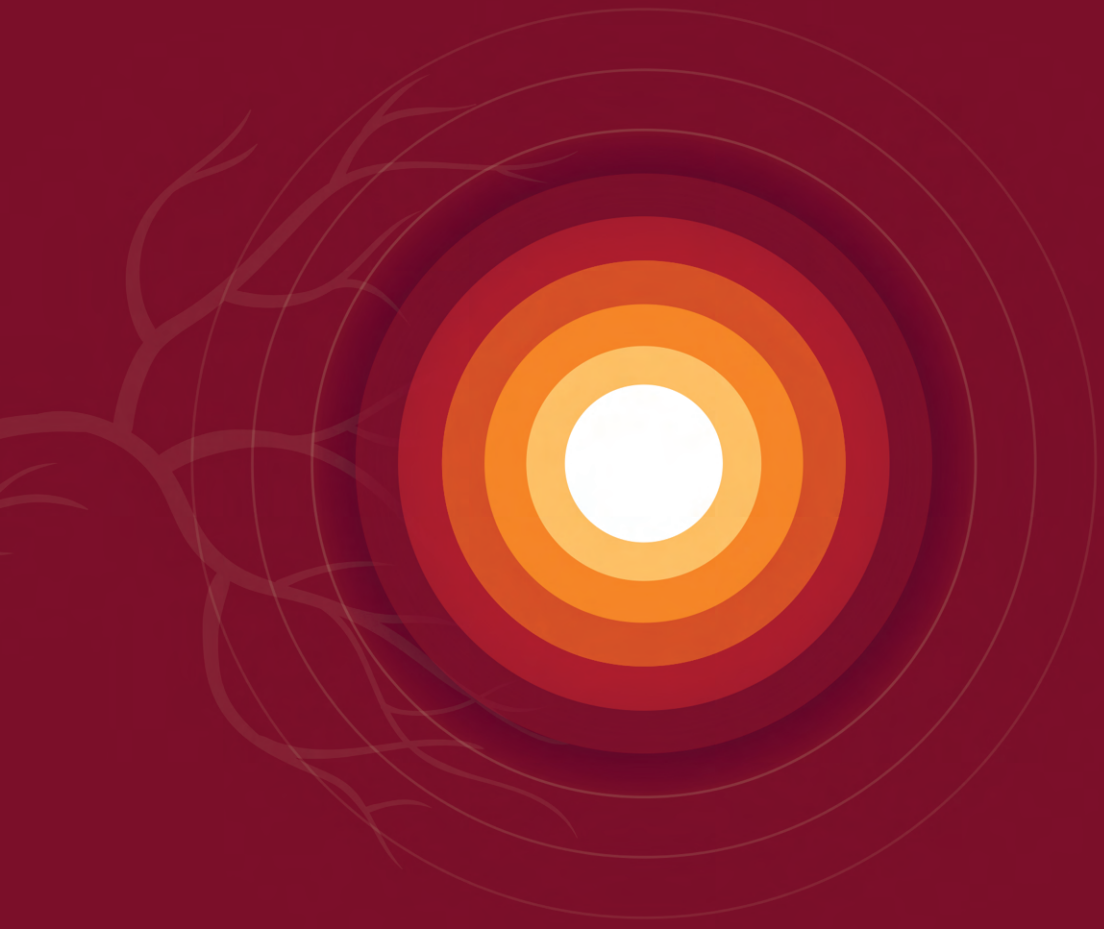
1. Briganti A, Abdollah F, Nini A, et al. Performance characteristics of computed tomography in detecting lymph node metastases in contemporary patients with prostate cancer treated with extended pelvic lymph node dissection. Eur Urol 2012;61:1132–8.
2. Shen G, Deng H, Hu S, Jia Z. Comparison of choline-PET/CT,MRI, SPECT, and bone scintigraphy in the diagnosis of bone metastases in patients with prostate cancer: a meta-analysis. Skeletal Radiol2014;43:1503–13.
3. Hofman MS, Lawrentschuk N, Francis RJ, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA):a prospective, randomised, multicentre study. Lancet 2020;395:1208–16.
4. Chow KM, So WZ, Lee HJ, et al. Head-to-head Comparison of the diagnostic accuracy of prostate-specific membrane antigen positron emission tomography and conventional imaging modalities for initial staging of intermediate-to high-risk prostate cancer: a systematic review and meta-analysis. Eur Urol 2023;84:36 –48.
5. Incesu RB, Preisser F, Pompe RS, et al. Diagnostic accuracy of prostate-specific membrane antigen positron emission tomography/computed tomography for primary lymph node staging before radical prostatectomy. Eur Urol Focus. In press. https://doi.org/10.1016/j.euf.2025.01.015.
6. Giesel FL, Fiedler H, Stefanova M, et al. PSMA PET/CT with Glu-urea-Lys-(Ahx)-[68 Ga(HBED-CC)] versus 3D CT volumetric lymph node assessment in recurrent prostate cancer. Eur J Nucl Med Mol Imaging 2015;42:1794–800.
7. Beesley LJ, Morgan TM, Spratt DE, et al. Individual and population comparisons of surgery and radiotherapy out comes in prostate cancer using Bayesian multistate models. JAMA Netw Open 2019;2 :e187765.
8. Seifert R, Emmett L, Rowe SP, et al. Second version of the Prostate Cancer Molecular Imaging Standardized Evaluation framework including response evaluation for clinical trials (PROMISE v2). Eur Urol 2023;83:405– 12.
9. Attard G, Murphy L, Clarke NW, et al. Abiraterone acetate and prednisolone with or without enzalutamide for high-risk non-metastatic prostate cancer: a meta-analysis of primary results from two randomised controlled phase 3 trials of the STAMPEDE platform protocol. Lancet 2022;399:447–60.
10. Hruby G, Eade T, Emmett L, et al.68 Ga-PSMA-PET/CT staging prior to definitive radiation treatment for prostate cancer. Asia Pac J Clin Oncol 2018;14:34 3–6.
11. Ravi P, Xie W, Buyse M, et al. Refining risk stratification of high-risk and locoregional prostate cancer: a pooled analysis of randomized trials. Eur Urol 2025;87:217–24.

12. Karpinski MJ, Rahbar K, Bogemann M, et al. Updated prostate cancer risk groups by prostate-specific membrane antigen positron emission tomography prostate cancer molecular imaging standardized evaluation (PPP2): results from an international multicentre registry study. Eur Urol. In press. https://doi.org/10. 1016/j. eururo.2025.04.017.

13. Mazzone E, Cannoletta D, Quarta L, et al. A comprehensive systematic review and meta-analysis of the role of prostate-specific membrane antigen positron emission tomography for prostate cancer diagnosis and primary staging before definitive treatment. Eur Urol 2025;87:654–71.

14. Fendler WP, Calais J, Allen-Auerbach M, et al.68 Ga-PSMA-11 PET/ CT interobserver agreement for prostate cancer assessments: an international multicenter prospective study. J Nucl Med2017;58:1617–23.

15. Chavoshi M, Mirshahvalad SA, Metser U, Veit-HaibachP.68 Ga-PSMA PET in prostate cancer: a systematic review and meta-analysis of the observer agreement. Eur J Nucl Med Mol Imaging2022;49:1021–9.



CHAPTER 3

Prognostic value of baseline PSMA PET/CT for survival in synchronous metastatic hormone-sensitive prostate cancer: towards redefining low- and high-volume disease

Authors
Fleur Kleiburg, Linda Heijmen, Annemieke Witteveen, Kiki van Duuren, Paul Zuidgeest, Frits Smit, Lioe-Fee de Geus-Oei, Tom van der Hulle

Submitted
To European Journal of Nuclear Medicine and Molecular Imaging on 16-10-2025.

ABSTRACT

Background

Traditional metastatic burden classifications in metastatic hormone-sensitive prostate cancer (mHSPC) rely on conventional imaging using CHAARTED criteria. As PSMA PET/CT is increasingly used for staging, revised definitions of low-volume (LVD) and high-volume disease (HVD) are needed to guide treatment decisions.

Methods

This study included 204 patients with synchronous mHSPC staged with PSMA PET/CT between 2017-2023. Patients were classified as LVD or HVD by multidisciplinary team (MDT) consensus. Retrospective analysis collected PSMA-derived total tumour volume (PSMA-TV), total lesion uptake (TL-PSMA), maximum distance between voxels (DmaxVox), SUV_{max}, SUV_{mean} and metastatic status. Additionally, age, WHO performance status (WHO-PS), Gleason score and laboratory values were retrieved. Cox regression identified predictors of overall survival (OS). Model performance was assessed using Harrell's C-index with internal validation via bootstrapping.

Results

By MDT consensus, 75 patients (37%) had LVD and 129 patients (63%) had HVD, differing significantly in PSMA-TV (median: 60 vs. 440 mL) and OS (median: 69 vs. 41 months). PSMA-TV was a robust predictor of OS (HR = 1.39 per doubling, $p < 0.001$), independent of WHO-PS (HR = 1.69 per score increase, $p < 0.001$) and treatment. A model combining PSMA-TV and WHO-PS achieved a C-index of 0.71 (95% C.I. 0.66 – 0.77) after bootstrapping. The optimal threshold for PSMA-TV-based high-volume disease was ≥150 mL or any visceral metastasis, which slightly outperformed MDT-based classification (HR = 3.01 vs. HR = 2.68).

Conclusions

While MDT-based stratification of mHSPC patients was adequate, incorporating PSMA-TV can further improve risk assessment. Instead of a dichotomous LVD/HVD approach, we propose using a model with PSMA-TV as a continuous variable to guide treatment decisions. Multicentre validation is needed before clinical implementation.

INTRODUCTION

For patients with metastatic hormone-sensitive prostate cancer (mHSPC), androgen deprivation therapy (ADT) has been the backbone of treatment for decades. There have been significant advancements in treatment intensification that have improved survival, including the addition of radiotherapy, docetaxel, androgen receptor targeting agents (ARTAs), and even triple therapy combining ADT with docetaxel and an ARTA [1-4]. The CHAARTED trial introduced the stratification of mHSPC patients into low-volume disease (LVD) and high-volume disease (HVD) to guide treatment decisions, with HVD defined as the presence of visceral metastases or ≥ 4 bone metastases with ≥ 1 beyond the vertebral bodies and pelvis [1, 2]. This dichotomous LVD/HVD distinction based on bone scintigraphy and CT-scan has been used in virtually all phase 3 studies since then and still plays an important role in the choice of therapy. In terms of survival, patients with LVD benefitted from radiotherapy to the prostate and patients with HVD benefitted from docetaxel chemotherapy, both in addition to ADT. However, the definitions of LVD and HVD offer a rather simplistic representation of tumour burden, which we now can be quantified far more accurately with new imaging techniques. In current clinical practice, prostate-specific membrane antigen (PSMA) PET/CT has replaced conventional imaging in many clinical centres due to its improved sensitivity, specificity and accuracy [5]. This led change in staging in approximately 40% of mHSPC patients, by identifying lesions missed on conventional imaging, while reducing false positives [6, 7]. Since the use of PSMA PET/CT for initial staging, it has been unclear which patients benefit from which treatment intensification strategy, highlighting the need to reclassify LVD and HVD. PSMA PET/CT provides a more accurate assessment of small metastases [8] and enables quantification of volumetric parameters, which is a valuable factor not captured by existing criteria. Furthermore, other well-known prognostic clinical parameters such as WHO performance status, Gleason score and initial prostate-specific antigen level (iPSA) [9] are not taken into account in the current treatment decision-making. Translating existing CHAARTED criteria to PSMA PET/CT is complex because there are no defined thresholds, and developing new criteria followed by reconducting clinical trials is not feasible. Furthermore, simplifying the results on the PSMA PET/CT to distinguishing between LVD and HVD ignores the much more accurate parameters that the PSMA scan has to offer. Therefore, for both daily practice and future clinical trials, there is a need to explore structured ways of using PSMA PET/CT findings as prognostic factors to

guide optimal treatment selection and to intensify treatment for those who need it, with the aim of improving patient outcomes.

With this study, we aim to provide a basis for redefining risk stratification in synchronous mHSPC patients. For this aim, we describe the PSMA PET/CT-based classification of patients as LVD or HVD from two centres that use PSMA PET/CT as the standard imaging modality for staging high-risk prostate cancer patients. By identifying prognostic imaging biomarkers from baseline PSMA PET/CT in combination with clinical parameters, we seek to lay the groundwork for developing a prediction tool that can support personalised treatment decisions in mHSPC.

METHODS

Patient population

Patients who were diagnosed with mHSPC at the Leiden University Medical Centre (Leiden, The Netherlands) and the Alrijne Hospital (Leiderdorp, The Netherlands) between April 2017 and August 2023 after staging with PSMA PET/CT, which replaced conventional imaging in April 2017, and who started ADT were included in this bicentric retrospective study. Patients were excluded if the PSMA PET/CT was made > 4 months before start of ADT or if the PSMA PET/CT was performed in another hospital. Informed consent for the use of their data for scientific research was obtained from all included patients. The local institutional ethics committee approved the study protocol on 29-03-2023.

PSMA PET/CT imaging and analysis

During the inclusion period, all PSMA PET/CT scans were made at the Alrijne Hospital. Before April 2019, patients were scanned after injection of [^{18}F]DCFPyL with the Gemini TF PET/CT (Philips, Amsterdam, The Netherlands). From April 2019, patients were scanned after injection of [^{18}F]PSMA-1007 with the Discovery MI 5-Ring PET/CT (GE Healthcare, Chicago, Illinois, USA). Scan details are provided in the Supplemental Materials [10]. After the evaluation of PSMA PET/CT images during multidisciplinary team (MDT) meetings, consisting of urologists, medical oncologists, radiation oncologists and a nuclear medicine physician, patients were visually categorised as having low-volume disease (hereafter: LVD_{MDT}) or high-volume disease (hereafter: HVD_{MDT}).

The quantitative analysis of baseline PSMA PET/CT scans was performed retrospectively, to retrieve the following parameters: SUV_{max}, SUV_{mean}, PSMA-TV (total tumour volume in mL), TL-PSMA (total lesion uptake: PSMA-TV × SUV_{mean}), DmaxVox (distance between the two outermost voxels as a parameter for dissemination [11]) and metastatic status (M1a, M1b or M1c disease [12]). Details of the delineation methods are provided in the Supplemental Materials [13-15].

Clinical parameters

In addition to the previously described PSMA PET/CT parameters, multiple clinical parameters were collected for inclusion in the analysis due to their known prognostic effect in metastatic prostate cancer patients [9, 16, 17]: age, WHO performance status, iPSA level, Gleason Score, alkaline phosphatase (ALP) level and haemoglobin (Hb) level. Missing data was handled using complete case analysis.

Study endpoints

The primary endpoint of this study was overall survival (OS), defined as the time from start of ADT to time of death in months. The secondary endpoint was PSA progression-free survival (PSA-PFS), defined as time from start of ADT to time of PSA progression (according to EAU guidelines [18]) in months. In case of censored data, the time to last hospital visit was used.

Statistical analysis

SPSS version 29 (IBM Corp, Armonk, New York, USA) and RStudio version 2024.04.2 (RStudio, Boston, Massachusetts, USA) were used for statistical analyses. Mann-Whitney U and Chi-square tests compared continuous and categorical parameters, respectively, between LVD_{MDT} and HVD_{MDT} patients. Spearman's rank tests assessed correlations between continuous variables. Cox regression analyses identified significant predictors of survival. Significant univariable predictors were included in a multivariable model. For the final multivariable model, insignificant predictors were excluded. For continuous input variables with skewness > 1, logarithmic (log_2) transformation was done to provide more normally distributed data as input for Cox regression analysis. Statistical significance was set at $p < 0.05$. Performance of the multivariable model was assessed using Harrell's C-index and internal validation was done using bootstrapping with 1000 samples. In post-hoc analysis, the optimal PSMA-TV cut-off for distinguishing between $LVD_{PSMA-TV}$ and $HVD_{PSMA-TV}$ was identified by testing 25 mL increments and selecting the threshold that yielded the highest hazard ratio for OS. In this analysis, all patients

with visceral metastases were classified as having $HVD_{PSMA-TV}$, regardless of their PSMA-TV, due to the known negative impact of visceral disease on survival [19].

RESULTS

Patient characteristics

A total of 204 patients with synchronous mHSPC were included in this study. Table 1 shows the baseline patient characteristics. A [^{18}F]DCFPyL PET/CT was made in 40 patients and a [^{18}F]PSMA-1007 PET/CT was made in 164 patients. Median time between PSMA PET/CT and start of ADT was 10 days (IQR 7 – 16 days). At the time of analysis, 96 patients (47%) had died and 141 (69%) patients showed PSA progression. Median OS was 53 months and median PSA-PFS was 23 months. Median follow-up was 50 months (IQR 39 – 58 months) at the time of analysis. Five patients were censored due to transfer to another hospital.

High-volume versus low-volume disease

After evaluation of PSMA PET/CT images during MDT meetings, 37% of patients were classified as LVD_{MDT} and 63% as HVD_{MDT}. Among patients treated with ADT alone, the most common reported reasons for not starting additional treatment were comorbidities (n = 18), patient preference (n = 6), and pre-existing lower urinary tract symptoms that that could have been exacerbated by prostate radiotherapy (n = 19). Between LVD_{MDT} and HVD_{MDT} patients, there were significant differences in iPSA and ALP level (both $p < 0.001$), but not in age, WHO performance status, Gleason score at diagnosis and Hb level. Table 2 describes the differences in baseline PSMA PET/CT parameters and survival outcomes between LVD_{MDT} and HVD_{MDT} patients. LVD_{MDT} patients had a median PSMA-TV of 60 mL (IQR 30 – 125 mL) and, HDV_{MDT} patients had a median PSMA-TV of 471 mL (IQR 176 – 1304 mL). In 2 HVD_{MDT} patients, PSMA PET/CT showed no tracer uptake, so no PET parameters could be extracted. Median OS was 69 months for LVD_{MDT} and 41 months for HVD_{MDT}, with a 3-year OS of 78% and 57%, respectively. Median PSA-PFS was 38 months for LVD_{MDT} and 19 months for HVD_{MDT}, with a 3-year PSA-PFS of 52% and 19%, respectively.

Table 1. Patient characteristics (n = 204), grouped by low-volume disease (LVD) and high-volume disease (HVD) according to multidisciplinary team (MDT) consensus.

Characteristic	LVD_{MDT} (n = 75)		HVD_{MDT} (n = 129)	
Age at diagnosis in years, median (IQR)	74	(69 – 78)	73	(67 – 79)
WHO performance status score, n (%)				
0	47	(63%)	84	(65%)
1	22	(29%)	33	(26%)
2	5	(7%)	9	(7%)
3			3	(2%)
Gleason score at diagnosis, n (%)				
6	2	(3%)	2	(2%)
7	13	(17%)	9	(7%)
8	26	(35%)	30	(23%)
9	21	(28%)	50	(39%)
10	2	(3%)	5	(4%)
Unknown	11	(15%)	33	(26%)
iPSA level in ng/ml, median (IQR)	36	(20 – 100)	286	(104 – 735)
ALP level in U/L, median (IQR)*	70	(61 – 92)	181	(106 – 425)
Hb level in mmol/L, median (IQR)**	8.9	(8.0 – 9.6)	8.6	(8.0 – 9.4)
Treatment for synchronous mHSPC, n (%)				
ADT only	53	(71%)	41	(32%)
ADT + radiotherapy to the prostate	22	(29%)		
ADT + docetaxel			80	(62%)
ADT + enzalutamide			2	(2%)
ADT + abiraterone			4	(3%)
ADT + docetaxel + abiraterone			2	(2%)

IQR = interquartile range. iPSA = initial prostate-specific antigen. ALP = alkaline phosphatase. Hb = haemoglobin. mHSPC = metastatic hormone-sensitive prostate cancer. ADT = androgen deprivation therapy. * 59 missing ALP level values (29%). ** 53 missing Hb level values (26%).

Table 2. Differences in baseline PSMA PET/CT parameters and survival outcomes between patients with low-volume disease (LVD) and high-volume disease (HVD), according to multidisciplinary team (MDT) consensus.

	LVD_{MDT} (n = 75)		HVD_{MDT} (n = 129)		p-value
Baseline PSMA PET/CT parameters					
PSMA-TV in mL, median (IQR)	60	(30 – 125)	483	(184 – 1313)	<0.001
TL-PSMA in SUV_{mean} × mL, median (IQR)	536	(216 – 1187)	4180	(1672 – 1131)	<0.001
DmaxVox in cm, median (IQR)	47	(22 – 58)	77	(64 – 85)	<0.001
Metastatic status, n (%)					<0.001
Lymph node only (M1a)	19	(25%)	1	(1%)	
Bone +/- lymph node (M1b)	56	(75%)	101	(78%)	
Any visceral (M1c)			27	(21%)	
Survival outcomes					
OS in months, median (Q1 – Q3)	69	(44 – 83)	41	(23 – 74)	<0.001
OS at 3 years	78%		57%		
PSA-PFS in months, median (Q1 – Q3)	38	(21 – 56)	19	(11 – 30)	<0.001
PSA-PFS at 3 years	52%		19%		

PSMA-TV = PSMA PET/CT-derived tumour volume. TL-PSMA = total lesion uptake (PSMA-TV × SUV_{mean}). SUV = standardised uptake value. DmaxVox = maximum distance between outermost voxels. OS = overall survival. PSA-PFS = PSA progression-free survival.

Table 3. Multivariable Cox regression analysis for OS, stratified for received treatment (n = 204 patients).

Variable name (unit)	HR*	95% C.I.	p-value
Log_2(PSMA-TV (mL))	1.387	1.224-1.572	**<0.001**
WHO performance status (score)	1.690	1.263-2.261	**<0.001**

HR = hazard ratio. * displayed per unit increase or, in case of log_2 transformation, per doubling.

Survival prediction

The parameters PSMA-TV, TL-PSMA, SUV_{max}, iPSA level and ALP level were highly right-skewed and log_2-transformed before survival analysis. In univariable analysis for OS and PSA-PFS, significant predictors were PSMA-TV ($p < 0.001$), TL-PSMA ($p < 0.001$), DmaxVox ($p < 0.001$) and ALP level ($p < 0.001$), see Supplemental Table S1. Additional predictors of OS were age ($p = 0.030$), WHO performance status ($p < 0.001$) and Hb level ($p = 0.020$), and additional predictors of PSA-PFS were metastatic status ($p < 0.001$), iPSA ($p < 0.001$) and Gleason score ($p = 0.001$). Supplemental Figure S1 shows the correlation matrix of all input variables. PSMA-TV strongly correlated with TL-PSMA ($r_s = 0.98$, $p < 0.001$) and DmaxVox ($r_s = 0.80$, $p < 0.001$). To avoid multicollinearity, only PSMA-TV was selected for multivariable analysis, as it demonstrated the highest HR for OS, with confidence interval widths similar to TL-PSMA and DmaxVox.

Multivariable Cox regression results for OS are shown in Table 3. After stratification for received treatment, PSMA-TV (HR = 1.387 per doubling, $p < 0.001$) and WHO performance status (HR = 1.690 per score increase, $p < 0.001$) remained independent predictors. Age, ALP level and Hb level lost significance and were excluded from the final model. A prediction model for OS based on PSMA-TV and WHO performance status achieved a C-index of 0.72 (95% C.I. 0.67 – 0.78), which remained 0.71 (95% C.I. 0.66 – 0.77) after internal validation. Multivariable Cox regression results for PSA-PFS are shown in Supplemental Table S2. PSMA-TV (HR = 1.530 per doubling, $p < 0.001$), metastatic status (M1c versus M1a: HR = 3.109, $p = 0.019$) and iPSA (HR = 0.868 per doubling, $p = 0.008$) remained independent predictors, while Gleason score and ALP level lost significance.

When using DmaxVox as PET parameter instead of PSMA-TV in multivariable analysis, DmaxVox was also an independent predictor of OS (HR = 1.234 per 10 cm increase, $p < 0.001$) and PSA-PFS (HR = 1.151 per 10 cm increase, $p = 0.013$, see Supplemental Table S3 and Figure S2).

Patient stratification based on PSMA-TV

Figure 1 illustrates the distribution of PSMA-TV among LVD_{MDT} and HVD_{MDT} patients. In this cohort, the optimal PSMA-TV cut-off for patient stratification based on OS was 150 mL, where all patients with visceral metastases were classified as $HVD_{PSMA-TV}$. PSMA-TV-based stratification performed similarly to MDT-based stratification, and had a higher HR (HR = 3.01 versus HR = 2.68; Figure 2). Overall, 30 patients (15%) were reclassified: 10 from LVD_{MDT} to $HVD_{PSMA-TV}$ and 20 from HVD_{MDT} to $LVD_{PSMA-TV}$.

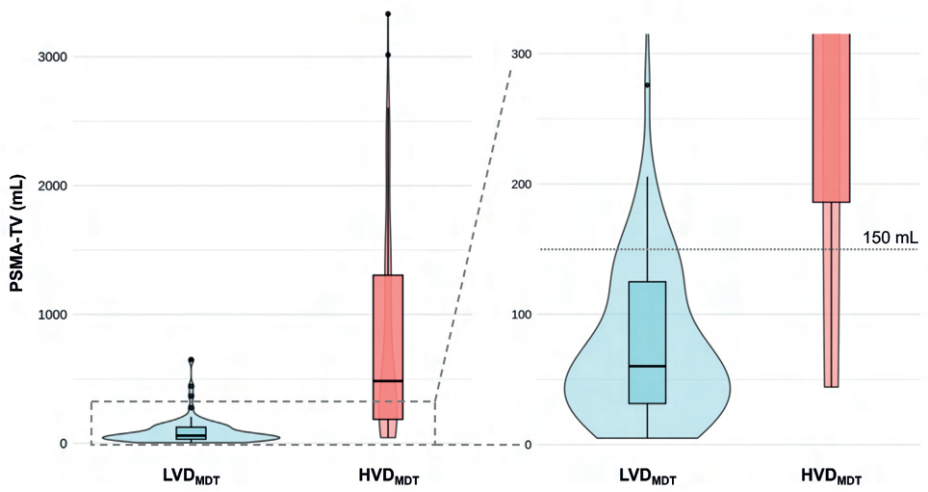


Figure 1. Violin plot depicting the distribution of PSMA-TV (in mL) in patients with low-volume disease (LVD) and high-volume disease (HVD), according to multidisciplinary team (MDT) consensus.

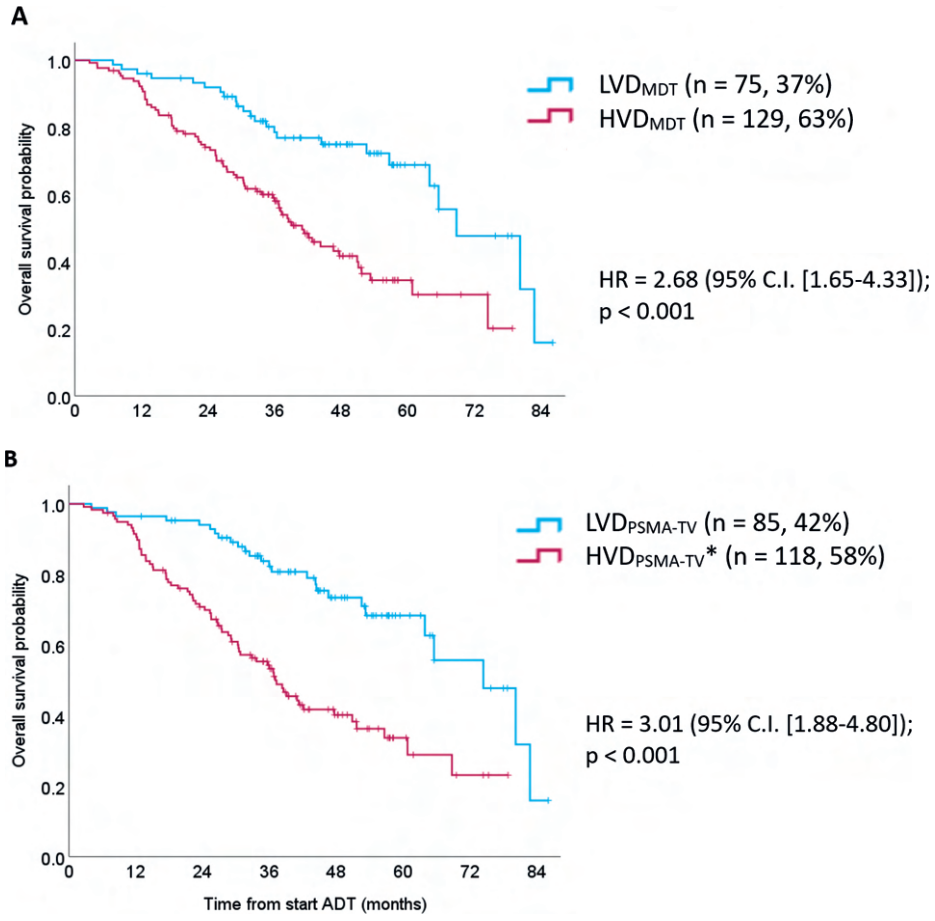


Figure 2. Overall survival probability of LVD patients (blue) versus HVD patients (red), with LVD/HVD stratification based on either MDT (A), or PSMA-TV (B). * Defined as PSMA-TV ≥ 150 mL or any visceral metastases; all other patients were classified as $LVD_{PSMA\text{-}TV^*}$.

DISCUSSION

For patients with synchronous mHSPC, the CHAARTED and STAMPEDE trials have demonstrated the importance of risk stratification based on volume of disease for guiding treatment decisions and improving patient survival [1, 2]. As PSMA PET/CT is increasingly replacing conventional imaging worldwide for disease staging, there is a high need for strategies that use PSMA PET/CT findings for making treatment decisions in synchronous mHSPC patients. Prior research has established the prognostic value of PSMA-TV for OS across various disease stages [20-22]. However, to the best of our knowledge, our study is the first to specifically

analyse synchronous mHSPC patients. In this study, we showed that PSMA-TV was a robust predictor of survival (HR = 1.39 per doubling, 95% C.I. [1.22 – 1.57], p < 0.001), independent of WHO performance status and treatment. Therefore, we recommend PSMA-TV-based risk stratification to guide treatment decisions in synchronous mHSPC.

Patients in this study were classified as either LVD_{MDT} (n = 75, 37%; 3-year OS: 78%) or HVD_{MDT} (n = 129, 63%; median OS: 41 months) based on visual assessment of PSMA PET/CT images during MDT meetings. These proportions and survival outcomes are consistent with those reported in the CHAARTED and STAMPEDE trials [1, 2]. When reclassifying patients using PSMA-TV, the optimal definition of $HVD_{PSMA\text{-}TV}$ was a PSMA-TV ≥ 150 mL or the presence of any visceral metastases. Notably, PSMA-TV-based patient classification slightly outperformed MDT-based patient classification in predicting survival outcomes. While PSMA-TV-based stratification has been proposed previously by Unterrainer et al. who identified a 107 mL threshold in 67 mHSPC patients, their study did not report survival outcomes [6]. Major limitations of dichotomising PSMA-TV include its susceptibility to overfitting and the loss of prognostic accuracy, as PSMA-TV is a continuous variable where higher values correlate with higher mortality risk. Instead of relying on a fixed cut-off, we suggest developing a predictive model that incorporates PSMA-TV as a continuous variable. This would enable survival predictions to be more accurately tailored to patient characteristics and would help to inform treatment decisions regarding both intensification and de-escalation.

Although PSMA-TV was identified as the most robust predictor of OS in our study, model performance was acceptable but not optimal. No external dataset was available; however, the C-index remained virtually unchanged after internal validation, suggestion the model is likely generalisable to other populations. To further improve predictive accuracy and evaluate clinical impact, larger patient cohorts and net benefit analysis are needed. For eventual clinical implementation of PSMA-TV calculations, particularly in time-constrained settings, automated segmentation software will be essential. In centres where such tools are unavailable, DmaxVox, which was also predictive of OS (HR = 1.23 per 10 cm, 95% C.I. [1.10 – 1.38], p < 0.001), may offer a simpler, more practical alternative.

Among the clinical parameters investigated in this study, WHO performance status, an established prognostic parameter [23], was the only independent predictor of OS. As a marker of lower physical functioning, it often indicates treatment de-

escalation, whereas PSMA-TV would indicate treatment intensification. Therefore, both should be considered in clinical decision-making. In contrast, neither iPSA nor Gleason score had predictive value in this study, suggesting that imaging-based parameters outperform clinical parameters as markers of tumour aggressiveness and patient outcomes.

Limitations of this study include its retrospective design, so potential biases regarding patient selection and survival cannot be ruled out. Furthermore, the use of two different scanners and scanning protocols during the inclusion period may have contributed to variability in quantitative PET parameters. To improve reliability and generalisability of predictive models, we recommend future research to incorporate data from multiple centres and to use data from different scanners and tracers.

CONCLUSIONS

This study demonstrated that PSMA-TV slightly outperformed MDT-based risk stratification of synchronous mHSPC patients staged with PSMA PET/CT. PSMA-TV was a robust and independent predictor of OS and therefore, PSMA-TV should be integrated into clinical decision-making. We recommend further research to focus on developing and validating predictive models that incorporate PSMA-TV and other relevant clinical parameters, to support personalised treatment intensification and de-escalation strategies in routine clinical practice and future phase 3 clinical trials.

REFERENCES

1. Parker CC, James ND, Brawley CD, Clarke NW, Hoyle AP, Ali A, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. Lancet. 2018;392:2353–66. doi:10.1016/S0140-6736(18)32486-3.
2. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373:737–46. doi:10.1056/NEJMoa1503747.
3. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double-blind, phase 3 trial. Lancet Oncol. 2019;20:686–700. doi:10.1016/S1470-2045(19)30082-8.
4. Fizazi K, Foulon S, Carles J, Roubaud G, McDermott R, Flechon A, et al. Abiraterone plus prednisone added to androgen deprivation therapy and docetaxel in de novo metastatic castration-sensitive prostate cancer (PEACE-1): a multicentre, open-label, randomised, phase 3 study with a 2 x 2 factorial design. Lancet. 2022;399:1695–707. doi:10.1016/S0140-6736(22)00367-1.
5. Hofman MS, Lawrentschuk N, Francis RJ, Tang C, Vela I, Thomas P, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. Lancet. 2020;395:1208–16. doi:10.1016/S0140-6736(20)30314-7.
6. Unterrainer LM, Hope TA, Fendler WP, Grogan T, Ndlovu H, Armstrong W, et al. Low- and High-Volume Disease in Metastatic Hormone-Sensitive Prostate Cancer: From CHAARTED to PSMA PET-An International Multicenter Retrospective Study. J Nucl Med. 2025;66:54–60. doi:10.2967/jnumed.124.268441.
7. Hope TA, Benz M, Jiang F, Thompson D, Barbato F, Juarez R, et al. Do Bone Scans Overstage Disease Compared with PSMA PET at Initial Staging? An International Multicenter Retrospective Study with Masked Independent Readers. J Nucl Med. 2023;64:1744–7. doi:10.2967/jnumed.123.265916.
8. Chow KM, So WZ, Lee HJ, Lee A, Yap DWT, Takwoingi Y, et al. Head-to-head Comparison of the Diagnostic Accuracy of Prostate-specific Membrane Antigen Positron Emission Tomography and Conventional Imaging Modalities for Initial Staging of Intermediate- to High-risk Prostate Cancer: A Systematic Review and Meta-analysis. Eur Urol. 2023;84:36–48. doi:10.1016/j.eururo.2023.03.001.
9. Glass TR, Tangen CM, Crawford ED, Thompson I. Metastatic carcinoma of the prostate: identifying prognostic groups using recursive partitioning. J Urol. 2003;169:164–9. doi:10.1016/S0022-5347(05)64059-1.

10. Sanchez-Jurado R, Devis M, Sanz R, Aguilar JE, del Puig Cozar M, Ferrer-Rebolleda J. Whole-body PET/CT studies with lowered (1)(8)F-FDG doses: the influence of body mass index in dose reduction. J Nucl Med Technol. 2014;42:62–7. doi:10.2967/jnmt.113.130393.

11. Aksu A, Kucuker KA, Solmaz S, Turgut B. A different perspective on PET/CT before treatment in patients with Hodgkin lymphoma: importance of volumetric and dissemination parameters. Ann Hematol. 2024;103:813–22. doi:10.1007/s00277-023-05547-1.

12. EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer. European Association of Urology 2022.

13. Nioche C, Orlhac F, Boughdad S, Reuze S, Goya-Outi J, Robert C, et al. LIFEx: A Freeware for Radiomic Feature Calculation in Multimodality Imaging to Accelerate Advances in the Characterization of Tumor Heterogeneity. Cancer Res. 2018;78:4786–9. doi:10.1158/0008-5472.CAN-18-0125.

14. Mittlmeier LM, Brendel M, Beyer L, Albert NL, Todica A, Zacherl MJ, et al. Feasibility of Different Tumor Delineation Approaches for (18)F-PSMA-1007 PET/CT Imaging in Prostate Cancer Patients. Front Oncol. 2021;11:663631. doi:10.3389/fonc.2021.663631.

15. O JH, Lodge MA, Wahl RL. Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0. Radiology. 2016;280:576–84. doi:10.1148/radiol.2016142043.

16. Gravis G, Boher JM, Fizazi K, Joly F, Priou F, Marino P, et al. Prognostic Factors for Survival in Noncastrate Metastatic Prostate Cancer: Validation of the Glass Model and Development of a Novel Simplified Prognostic Model. Eur Urol. 2015;68:196–204. doi:10.1016/j.eururo.2014.09.022.

17. James ND, Spears MR, Clarke NW, Dearnaley DP, De Bono JS, Gale J, et al. Survival with Newly Diagnosed Metastatic Prostate Cancer in the "Docetaxel Era": Data from 917 Patients in the Control Arm of the STAMPEDE Trial (MRC PR08, CRUK/06/019). Eur Urol. 2015;67:1028–38. doi:10.1016/j.eururo.2014.09.032.

18. EAU Guidelines. Edn. presented at the EAU Annual Congress Madrid 2025. ISBN 978-94-92671-29-5.

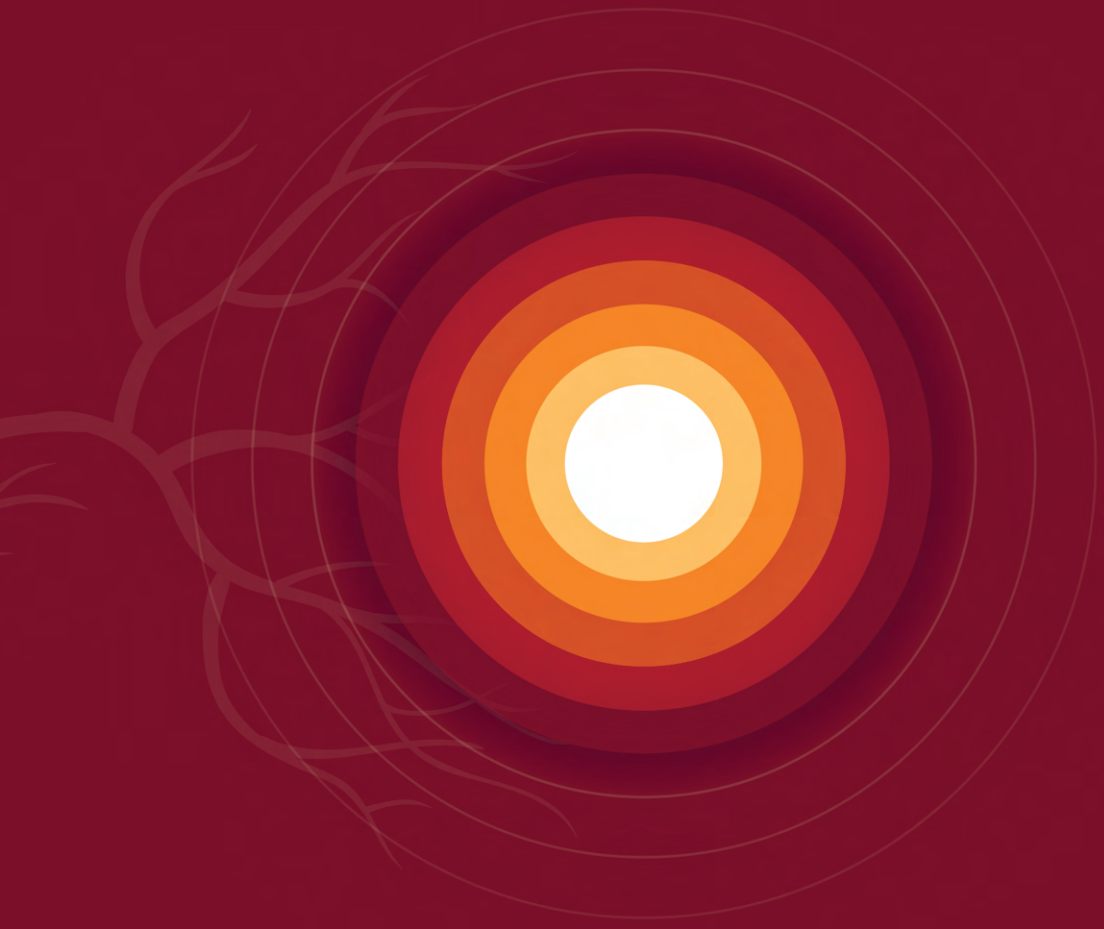
19. Halabi S, Kelly WK, Ma H, Zhou H, Solomon NC, Fizazi K, et al. Meta-Analysis Evaluating the Impact of Site of Metastasis on Overall Survival in Men With Castration-Resistant Prostate Cancer. J Clin Oncol. 2016;34:1652–9. doi:10.1200/JCO.2015.65.7270.

20. Karpinski MJ, Husing J, Claassen K, Moller L, Kajuter H, Oesterling F, et al. Combining PSMA-PET and PROMISE to re-define disease stage and risk in patients with prostate cancer: a multicentre retrospective study. Lancet Oncol. 2024;25:1188–201. doi:10.1016/S1470-2045(24)00326-7.

21. Seifert R, Rasul S, Seitzer K, Eveslage M, Rahbar Nikoukar L, Kessel K, et al. A Prognostic Risk Score for Prostate Cancer Based on PSMA PET-derived Organ-specific Tumor Volumes. Radiology. 2023;307:e222010. doi:10.1148/radiol.222010.

22. Kleiburg F, de Geus-Oei LF, Spijkerman R, Noortman WA, van Velden FHP, Manohar S, et al. Baseline PSMA PET/CT parameters predict overall survival and treatment response in metastatic castration-resistant prostate cancer patients. Eur Radiol. 2025. doi:10.1007/s00330-025-11360-3.

23. Assayag J, Kim C, Chu H, Webster J. The prognostic value of Eastern Cooperative Oncology Group performance status on overall survival among patients with metastatic prostate cancer: a systematic review and meta-analysis. Front Oncol. 2023;13:1194718. doi:10.3389/fonc.2023.1194718.



CHAPTER 4

Baseline PSMA PET/CT parameters predict overall survival and treatment response in metastatic castration-resistant prostate cancer patients

Authors
Fleur Kleiburg, Lioe-Fee de Geus-Oei, Romy Spijkerman, Wyanne Noortman, Floris van Velden, Srirang Manohar, Frits Smit, Frank Toonen, Saskia Luelmo, Tom van der Hulle, Linda Heijmen.

Published
European Radiology
2025 Jul;35(7):4223-4232
DOI: 10.1007/s00330-025-11360-3

Supplementary materials
https://doi.org/10.1007/s00330-025-11360-3

ABSTRACT

Background

Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease with varying survival outcomes. This study investigated whether baseline PSMA PET/CT parameters are associated with survival and treatment response.

Methods

Sixty mCRPC patients underwent [^{18}F]PSMA-1007 PET/CT before treatment with androgen receptor-targeted agents (ARTAs) or chemotherapy. Intensity-based parameters, volumetric parameters, metastatic sites and DmaxVox (distance between the two outermost voxels) from baseline PSMA PET/CT were collected, as well as age, Gleason score and laboratory parameters. Cox regression analysis evaluated their prognostic value for overall survival (OS). Additionally, a preliminary lesion-level analysis was done (n = 241 lesions) with lesion location and twelve radiomic features selected from previous literature. Logistic regression evaluated their association with PSMA PET/CT-based lesion progression after 3-4 months of treatment.

Results

Total tumour volume (PSMA-TV) (HR = 1.41 per doubling [1.17-1.70]), total lesion uptake (TL-PSMA) (HR = 1.40 per doubling [1.16-1.69]) and DmaxVox (HR = 1.31 per 10 cm increase [1.07-1.62]) were prognostic for OS, each independent of baseline PSA level (HR = 0.82 per doubling [0.68-0.98]), haemoglobin level (HR = 0.68 per mmol/L increase [0.49-0.95]) and line of treatment. On lesion-level, location (prostate vs bone OR = 0.23 [0.06-0.83]) and SUV_{mean} (OR = 1.72 per doubling [-1.08 2.75]) were independent prognostic markers for lesion progression, morphological and texture-based radiomic features were not.

Conclusions

Baseline PSMA PET/CT scans have prognostic value in mCRPC patients and can potentially aid in treatment decision-making. DmaxVox can serve as a simpler alternative to PSMA-TV when automated segmentation software is not available. When combined with PSMA-TV, lower PSA levels indicated worse OS, which may be a marker of tumour dedifferentiation. Further research is needed to validate these models in larger patient cohorts.

INTRODUCTION

Metastatic castration-resistant prostate cancer (mCRPC) represents an advanced stage of prostate cancer, characterised by resistance to androgen deprivation therapy and the development of distant metastases [1]. Despite the availability of several novel therapies, all therapies are palliative and the prognosis remains poor with a median overall survival (OS) of 2–3 years [2]. The challenge in managing mCRPC patients is the heterogeneity of the clinical course, with some patients having indolent disease and long periods of disease control, while others have aggressive and rapidly progressing diseases with poor survival [2]. Therefore, the ability to accurately predict patient prognosis, including the likelihood of treatment response and survival, is important so that the expected treatment effects can be weighed against toxicity, patient burden, and associated costs for each individual patient. While several clinical parameters, such as Gleason score at initial biopsy [3] and prostate-specific antigen (PSA) [4], have been shown to have prognostic value in mCRPC patients, they do not capture the full heterogeneity of the disease.

A molecular imaging technique that may provide more accurate prognostic information is prostate-specific membrane antigen (PSMA) PET/CT, which is increasingly being used in clinical practice [1]. PSMA PET/CT imaging provides molecular characteristics of prostate cancer that reflect tumour biology [5, 6] and allows for the quantification and analysis of, for example, the intensity, distribution and heterogeneity of PSMA uptake. We hypothesised that disease characteristics on PSMA PET/CT can be used as an imaging biomarker to improve the prognostication of mCRPC patients and optimise treatment decision-making.

The primary objective of this study was to determine upfront the predictive and prognostic value of baseline PSMA PET/CT parameters in mCRPC patients, receiving either androgen receptor-targeted agents (ARTAs) or chemotherapy. Analysis was performed both at patient level and, as a preliminary study, at lesion level using a selection of hypothesis-driven parameters, including radiomic features.

METHODS

Patient population

Patients with mCRPC treated with either an ARTA (enzalutamide or abiraterone) or chemotherapy (docetaxel or cabazitaxel) as first- or second-line treatment between 01-07-2019 and 31-06-2023 at the Leiden University Medical Center (Leiden, The Netherlands) and the Alrijne Hospital (Leiderdorp, The Netherlands) were included in this retrospective study. Administered treatment dosages were according to the European Association of Urology guidelines [1]. Clinical data such as age, medical history, pathology reports, radiology reports, laboratory results and survival data were retrieved from electronic patient records. All patients gave written informed consent for the use of their data for scientific research. The study protocol was approved on 03-03-2022 by the local institutional ethics committee.

PSMA PET/CT imaging

In line with our local protocols, all patients underwent a [^{18}F]PSMA-1007 PET/CT (in short: PSMA PET/CT) within 8 weeks before the start of treatment. PSMA PET/CT acquisitions and reconstructions were performed at the Alrijne Hospital using the 5-Ring Discovery MI PET/CT (GE Healthcare) in all included patients. According to the clinical scan protocol, injection-to-scan times were 60–120 min (depending on PSA, < 4 ng/mL: 120 min, 4–40 ng/mL: 80 min, > 40 ng/mL: 60 min) and injected doses of [^{18}F]PSMA-1007 were 1.5–2.1 MBq/kg body weight (depending on BMI, < 25: 1.5 MBq/kg, 25–30: 1.8 MBq/kg, > 30: 2.1 MBq/kg [7]). First, a low-dose CT scan (15–550 mA, 120 kV) was performed from skull to mid-thigh for localisation and attenuation correction purposes, followed by a PET scan with 120 s per bed position. CT images were reconstructed at a 512 × 512 matrix with a slice thickness of 2.5 mm. PET images were reconstructed at a 256 × 256 matrix with a slice thickness of 2.78 mm. A Bayesian penalised-likelihood iterative image algorithm (Q.Clear with a beta value of 900) was used.

In line with our local protocols, all patients also received a PSMA PET/CT for imaging-based treatment response evaluation. In the case of ARTA treatment, PSMA PET/CT was performed after three months. In the case of chemotherapy, PSMA PET/CT was performed 4–6 weeks (absolute maximum: 8 weeks) after the last administered dose. When PSA progression (+25% and at least 2 ng/mL after at least three cycles [8]) or clinical deterioration (such as new-onset pain) was observed, a PSMA PET/CT was requested at that time.

Image analysis and quantification

Volume of interest (VOI) delineation and feature extraction were done in baseline PSMA PET/CT scans using LIFEx software version 7.2 or higher [9]. Firstly, by applying a fixed absolute threshold of SUV = 4 and a minimum volume of 0.5 cm^3, VOIs were automatically delineated [10]. Secondly, areas of physiological uptake were manually removed, using the low-dose CT as a reference. If liver lesions were present, they were manually delineated using the SUV_{mean} and standard deviation of a VOI of 3 cm in diameter in healthy liver tissue as a fixed threshold; $SUV = 1.5 \times SUV_{mean,healthy\ liver} + 2 \times SD_{healthy\ liver}$ [11]. In each scan, the SUV_{mean}, SUV_{max}, PSMA-TV (total tumour volume), TL-PSMA (total lesion uptake: summed PSMA-TV × SUV_{mean}) and DmaxVox (distance between the two lesions furthest apart using the two outermost voxels) were calculated. Here, DmaxVox represents tumour burden dissemination, a PSMA PET parameter that has been shown to be a prognostic factor in mCRPC patients treated with ^{177}Lu-PSMA radioligand therapy [12]. Furthermore, the involvement of lymph nodes, bone and visceral tissues at sites of metastatic disease was noted [13, 14].

For the lesion-level analysis, only VOIs with at least 64 voxels were included to allow texture features to be included [15]. In each of the following locations, if present, the lesion with the highest SUV_{max} and highest PSMA-TV was selected: prostate, N1 lymph nodes, M1a lymph nodes, locoregional bones (pelvis and lumbar vertebrae), axial non-locoregional bones (cervical and thoracic vertebrae, claviculae, scapulae, ribs and base of the skull), appendicular bones (extremities and other parts of the skull), and visceral tissue. For each lesion, 12 PET parameters were extracted: 2 morphological (PSMA-TV, sphericity), 5 intensity-based (SUV_{mean}, SUV_{max}, SUV-kurtosis, SUV-IQR, and TL-PSMA) and 5 commonly used grey-level co-occurrence matrix (GLCM) texture features (energy, contrast, correlation, entropy, and homogeneity, from Haralick et al [16]). Specifically, sphericity, SUV-kurtosis, SUV-IQR, entropy and homogeneity were chosen, because these PSMA PET parameters have been associated with patient outcomes in mCRPC patients before [17–20]. Supplemental Table S1 describes each included PET parameter in more detail. Features were extracted using LIFEx software and were compliant with the Image Biomarker Standardization Initiative (IBSI) [21]. No feature selection was performed, as a limited number of features relative to the number of lesions [22] were selected from the literature. No voxel resampling was performed since the original voxel spacing was almost isotropic at 2.73 × 2.73 × 2.78 mm^3. A fixed bin size of 0.5 g/mL was applied. To assess lesion response, PSMA-TV and TL-

PSMA were also determined in each included lesion on the PSMA PET/CT acquired for treatment response evaluation.

Clinical parameters

The previously described PSMA PET/CT parameters were compared with baseline PSA levels to assess their independent prognostic value. Age, Gleason score, baseline alkaline phosphatase (ALP) and haemoglobin (Hb) [3, 4] were also included in the analysis. In post-hoc analysis, PSA density was calculated for each patient by dividing baseline PSA level by PSMA-TV (ng/mL^2) [23].

Study endpoints

The primary endpoint of this study was OS, defined as the time from treatment initiation to time of death in months. Censored data used the time to the last hospital visit. At lesion level, the endpoint was PSMA PET/CT-based progression, which was defined as an increase of > 30% in lesion PSMA-TV or lesion TL-PSMA [24].

Statistical analysis

All statistical analyses were performed in SPSS version 29 (IBM Corporation). For the summarising of retrieved data, descriptive statistics were used. Cox regression analysis and binary logistic regression analysis were performed to assess the prognostic value of parameters for OS and lesion progression. For input variables with skewness > 1, logarithmic (log_2) transformation was used to transform skewed data into more normally distributed data required for statistical analyses [25]. A Spearman's rho test was used to test the correlation between continuous variables, and a Chi-squared test was used to compare two categorical values. Kaplan-Meier curves and log-rank tests compared the difference in survival between groups. Statistical significance was reached when the p-value was < 0.05.

RESULTS

In total, 60 mCRPC patients were included in this study (Table 1). Thirty-one patients received ARTAs as either first-line (n = 27) or second-line treatment (n = 4); 29 patients received chemotherapy (median 6 cycles) as either first-line (n = 10) or second-line treatment (n = 19). The median follow-up time was 29 months (range 11–40 months). A total of 38 patients had died. Median OS was 21 months (range 4–31 months). No patients were lost to follow-up. For the baseline PSMA PET/CT

scans, the median administered dose of [^{18}F]PSMA-1007 was 153 MBq (IQR 127–173 MBq) and the median injection-to-scan time was 91 min (IQR 79–127 min).

Table 1. Patient characteristics (*n* = 60)

Characteristic	Value	
Gleason score at diagnosis*		
≤ 7	14	(23%)
≥ 8	40	(67%)
Prior local treatment		
Radical prostatectomy	4	(7%)
Radiotherapy	24	(40%)
Prior treatment for mHSPC		
ADT	60	(100%)
Docetaxel	13	(22%)
Enzalutamide	6	(10%)
Abiraterone	1	(2%)
Time since diagnosis (years)	3.4	(2.1–5.9)
Age at start of treatment (years)	75	(68–78)
Baseline PSA level (ng/mL)	21.1	(8.1–55.6)
Baseline PSMA-TV (mL)	118	(41–328)
Sites of metastatic disease on baseline PSMA PET/CT scan		
Lymph node only	7	(12%)
Bone only	18	(30%)
Lymph node + bone	31	(52%)
Any visceral	4	(7%)
Current treatment		
ARTA	31	(52%)
Enzalutamide	25	
Abiraterone	6	
Chemotherapy	29	(48%)
Docetaxel	24	
Cabazitaxel	5	
Current line of treatment		
1st	37	(62%)
2nd	23	(38%)

Categorical data are presented as numbers (percentage), and continuous data as median (interquartile range). *mHSPC* metastatic hormone-sensitive prostate cancer, *ADT* androgen deprivation therapy, *PSA* prostate-specific antigen, *PSMA-TV* total tumour volume on PSMA PET/CT, *ARTA* androgen receptor-targeted agent. * Gleason score unknown in six patients

Prognostic baseline PSMA PET/CT markers for patient survival

The parameters SUV_{mean}, PSMA-TV, TL-PSMA, PSA level and ALP level were log_2 transformed. Univariate analysis for OS showed that PSMA-TV (HR = 1.395 per doubling), TL-PSMA (HR = 1.368 per doubling), DmaxVox (HR = 1.313 per 10 cm increase), metastatic sites (any visceral vs bone only; HR = 5.381), ALP level (HR = 1.531 per doubling), and Hb level (HR = 0.717 per unit increase) were prognostic markers (Table 2). SUV_{mean}, SUV_{max}, age, Gleason score, and PSA level had no prognostic value in this analysis. PSMA-TV was correlated with TL-PSMA ($r_s(58) = 0.952$, $p < 0.001$) and DmaxVox ($r_s(58) = 0.701$, $p < 0.001$), not with SUV_{mean} and SUV_{max}. There was no difference in OS between patients treated with ARTAs and those treated with chemotherapy within the same line of treatment.

Table 2. Univariate Cox regression analyses OS with PSMA PET/CT and clinical parameters ($n = 60$)

	OS		
Variable name (unit)	**HR***	**95% CI**	***p*-value****
Baseline PSMA PET/CT parameters			
Log_2 (SUV_{mean})	0.895	0.509–1.576	
SUV_{max}	1.005	0.997–1.013	
Log_2 (PSMA-TV (mL))	1.395	1.174–1.657	< 0.001
Log_2 (TL-PSMA (SUV × mL))	1.368	1.149–1.629	< 0.002
DmaxVox (per 10 cm)	1.313	1.085–1.589	0.005
Sites of metastatic disease			0.024
Lymph node only	0.693	0.190–2.531	
Bone only	Ref	Ref	
Lymph node + bone	1.642	0.768–3.510	
Any visceral	5.381	1.624–17.83	0.006
Clinical parameters			
Age (years)	1.016	0.972–1.062	
Gleason score	1.178	0.842–1.648	
Log_2 (baseline PSA (ng/mL))	1.108	0.959–1.281	
Log_2 (baseline ALP (U/L))	1.531	1.093–2.146	0.013
Baseline Hb (mmol/L)	0.717	0.537–0.958	0.024

Ref reference group, *SUV* standardised uptake value, *PSMA-TV* total tumour volume, *TL-PSMA* total lesion uptake, *DmaxVox* distance between outermost voxels, *PSA* prostate-specific antigen, *ALP* alkaline phosphatase, *Hb* haemoglobin, *HR* hazard ratio. * HR, displayed per unit increase or, in case of log_2 transformation, per doubling. ** *p*-values are displayed when < 0.05.

Multivariate Cox regression analysis for OS revealed that PSMA-TV (HR = 1.410 per doubling), PSA level (HR = 0.818 per doubling) and Hb level (HR = 0.680 per mmol/L increase) were all independent prognostic markers of OS, independent of line of treatment (Table 3). TL-PSMA (HR = 1.402 per doubling, $p < 0.001$) and DmaxVox (HR = 1.314 per 10 cm increase, $p = 0.011$) also remained significant prognostic markers of OS, independent of PSA level, Hb level and line of treatment (Supplemental Table S2). Multivariate models had lower −2 log-likelihood values than univariate models, indicating a better fit to the data. ALP levels lost significance in multivariate analysis with any other parameter. For interpretation and visualisation purposes, Table 4 shows the median OS of patients subgrouped by median PSMA-TV or median DmaxVox and Figure 1 shows the survival distribution of patients subgrouped by median PSMA-TV. Figure 2 shows the PSMA PET/CT of two example patients.

Table 3. Multivariate Cox regression analysis for OS (*n* = 60)

	OS		
Variable name (unit)	**HR***	**95% CI**	***p*-value**
Log_2 (PSMA-TV (mL))	1.410	1.168–1.703	< 0.001
Log_2 (baseline PSA (ng/mL))	0.818	0.683–0.978	0.028
Baseline Hb (mmol/L)	0.680	0.489–0.947	0.022
Line of treatment (second- vs first-line)	3.497	1.717–7.123	< 0.001

PSMA-TV total tumour volume, *PSA* prostate-specific antigen, *Hb* haemoglobin, *HR* hazard ratio. * HR displayed per unit increase or, in case of log_2 transformation, per doubling

Table 4. Median OS subgrouped by median PSMA-TV or DmaxVox (distance between outermost voxels) in patients receiving first-line and second-line treatment

	First-line treatment (*n* = 37)		**Second-line treatment (*n* = 23)**	
	< Median	**≥ Median**	**< Median**	**≥ Median**
PSMA-TV range	4.1–88.9 mL	88.9–1095 mL	0.8–220 mL	220–3350 mL
Median OS	*	18.5 months	17.9 months	15.7 months
DmaxVox range	10.1–57.8 cm	57.8–86.1 cm	12.5–74.0 cm	74.0–96.7 cm
Median OS	*	23.5 months	17.9 months	12.5 months

* Not reached, median censoring time = 28.6 months

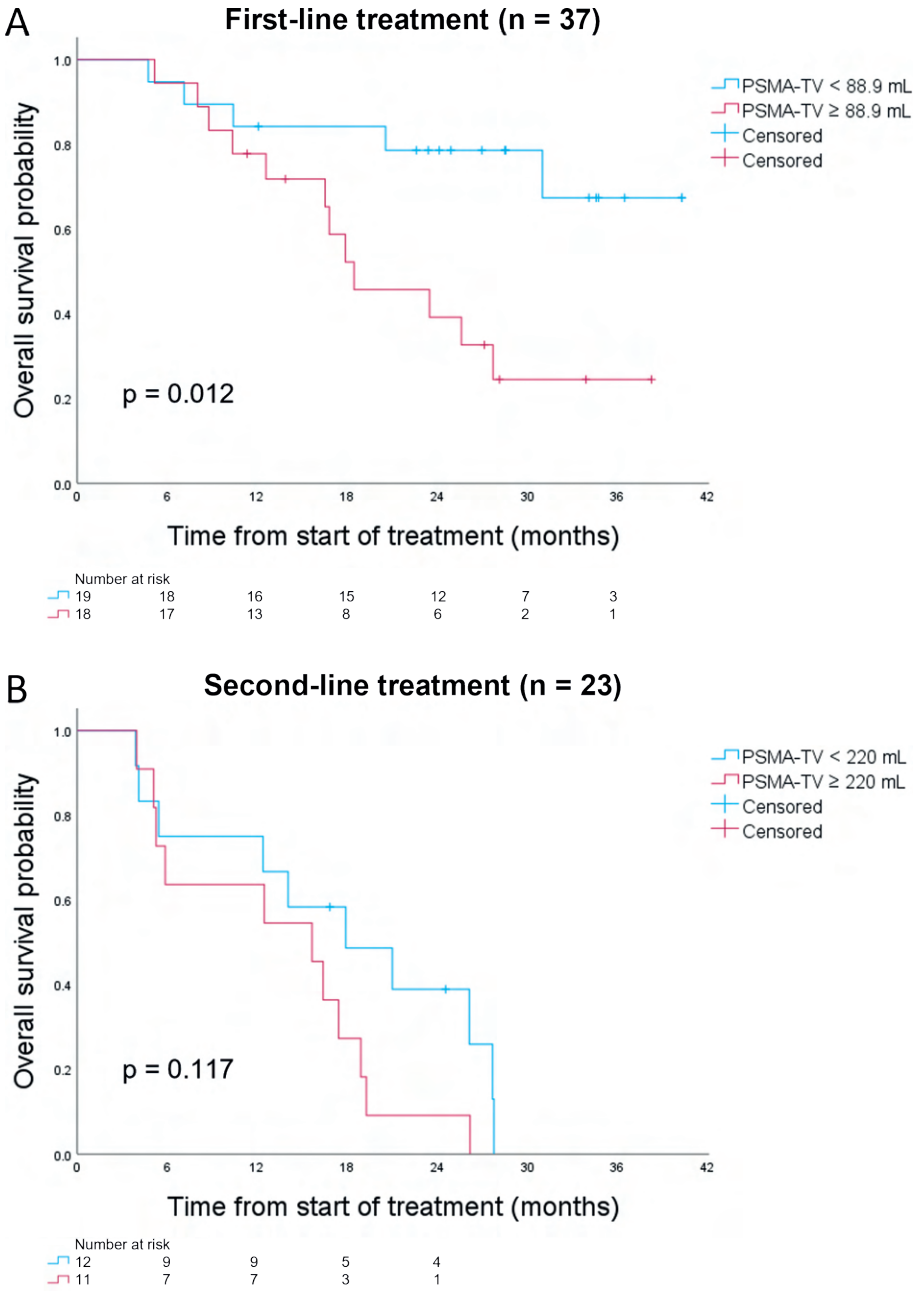


Figure 1. OS of patients receiving first-line (A) or second-line (B) treatment, subgrouped by median PSMA-TV

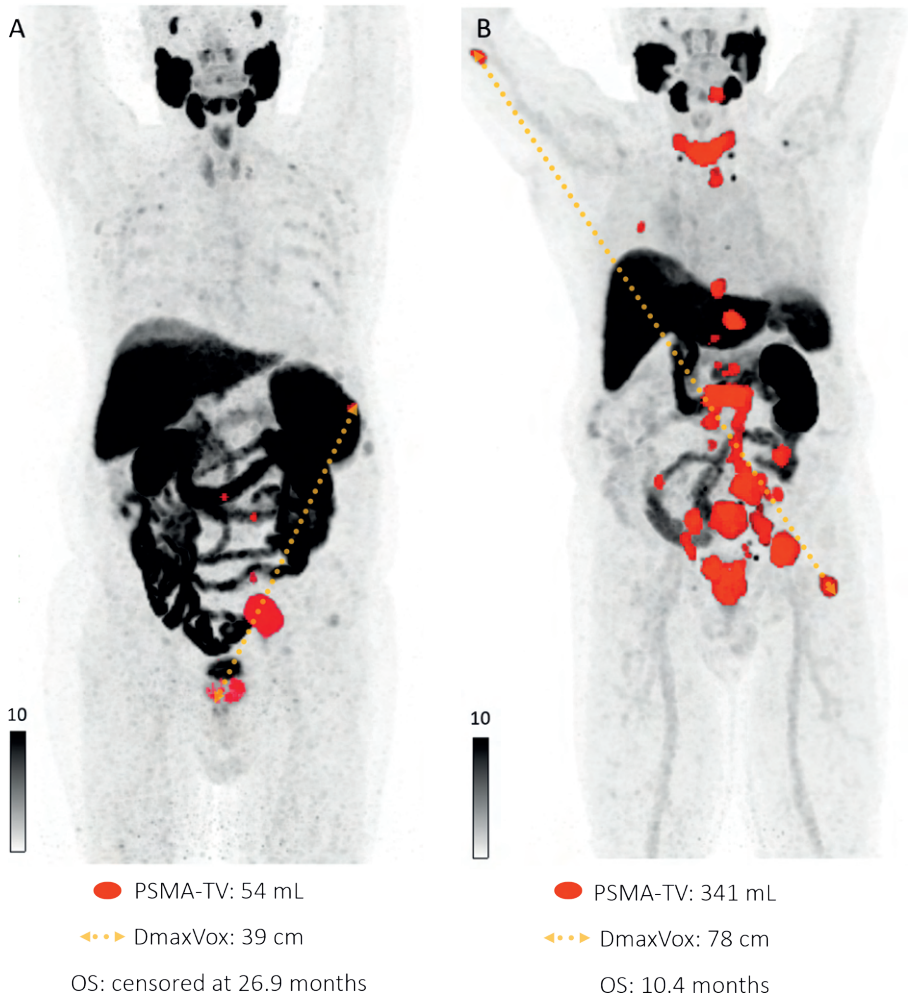


Figure 2. Maximum intensity projections (A, B) of baseline PSMA PET/CT in two different patients, both showing lymph node and bone metastases, before receiving enzalutamide as first-line mCRPC treatment. At diagnosis, both patients had Gleason 9 metastatic prostate cancer. Patient B, with a higher PSMA-TV (segmented in red) and DmaxVox (distance between outermost voxels, see dashed arrow) compared to patient A, had a lower OS

After obtaining these results, the PSA density (ng/mL^2) was calculated for each patient. The median PSA density was 0.25 (IQR 0.10–0.67). Log_2-transformation was done. In univariate analysis, PSA density was significantly associated with OS (HR = 0.803 per doubling, p = 0.006), and remained significant in multivariate analysis (HR = 0.760 per doubling, p < 0.001, Supplemental Table S2).

Association of baseline PSMA PET/CT markers with lesion progression

For the lesion-level analysis, 241 lesions were selected; 113 lesions in patients receiving ARTAs and 128 lesions in patients receiving chemotherapy (Supplemental Table S3). Of 241 lesions, 76 showed progression on PSMA PET/CT; 3 of 32 prostate lesions (9%), 16 of 64 lymph node lesions (25%), 56 of 139 bone lesions (40%), and 1 of 6 visceral lesions (16%). There was no significant difference in the rate of progressive lesions between ARTA treatment (28%) and chemotherapy (34%, $p = 0.313$). Progression was seen in 22% of lesions receiving first-line treatment and 41% of lesions receiving second-line treatment. 57% of patients had a mixed response, with both responding and progressive lesions in the analysed dataset.

PSMA-TV, sphericity, SUV_{mean}, SUV_{max}, SUV-kurtosis, SUV-IQR, TL-PSMA, energy and contrast were log_2 transformed. In logistic regression analysis with correction for line of treatment the lesion location, SUV_{mean}, SUV_{max}, SUV-IQR, energy, contrast, entropy and homogeneity were significantly associated with lesion progression, while PSMA-TV, sphericity, SUV-kurtosis, TL-PSMA and correlation were not (Supplemental Table S4). Specific lymph node location (N1 vs M1a) and bone location (locoregional vs axial non-locoregional vs appendicular) were also not associated with lesion progression. The PET parameters SUV_{mean}, SUV_{max}, SUV-IQR, energy, contrast, entropy and homogeneity were all highly correlated (all $p < 0.003$).

A multivariate logistic regression analysis demonstrated that lesion location (bone vs prostate: OR = 0.23), SUV_{mean} (OR = 1.72 per doubling) and line of treatment (second- vs first-line: OR = 2.16) were independently associated with lesion progression (Table 5).

Table 5. Multivariate logistic regression analysis for imaging-based lesion progression after 3–4 months of treatment

	Lesion progression (*n* = 241 lesions)		
Variable name (unit)	**OR***	**95% CI**	***p*-value****
Location			0.039
Prostate	0.231	0.064–0.825	0.024
Lymph node	0.526	0.265–1.044	
Bone	Ref	Ref	
Visceral	0.277	0.030–2.519	
Log_2 (SUV_{mean})	1.724	1.080–2.753	0.022
Line of treatment (second- vs first-line)	2.158	1.196–3.892	0.011

Ref reference group, *SUV* standardised uptake value, *OR* odds ratio. * OR displayed per unit increase or, in case of log_2 transformation, per doubling. ** *p*-values are displayed when < 0.05.

DISCUSSION

The results of this study showed that baseline PSMA PET/CT scans provide prognostic information for mCRPC patients treated with ARTA or chemotherapy. For each patient, it is important to weigh the expected treatment benefit against the risk of toxicity and associated costs. Prognostic factors currently considered before initiating a new line of treatment include the presence of disease-related symptoms, PSA levels, PSA doubling time and the presence of visceral metastases [1]. However, response to therapy and survival rates among mCRPC patients remain heterogeneous [2] and more accurate predictive and prognostic biomarkers are needed. This study showed that higher PSMA-TV (HR = 1.41 per doubling) was associated with worse OS, independent of line of treatment and PSA level. The same was true for TL-PSMA and DmaxVox, also markers of disease burden. The significant association between TL-PSMA and survival was mainly determined by PSMA-TV, as SUV_{mean} was not associated with survival. Combining PET parameters with the clinical parameters PSA and Hb level in multivariate analysis resulted in improved prognostic models. Interestingly, while PSA levels had no prognostic value in univariate analysis, lower PSA levels (HR = 0.8 per doubling) were associated with worse OS in multivariate analysis when combined with PSMA PET parameters representing disease extensiveness (e.g. PSMA-TV). This was confirmed after calculating PSA density (PSA/PSMA-TV), which was also associated with worse OS and may represent a marker of tumour dedifferentiation.

Aggarwal et al also observed dedifferentiation in low PSA-secreting mCRPC and an association with shorter OS [26].

The prognostic value of PSMA PET/CT parameters, particularly PSMA-TV, in mCRPC patients receiving ARTA treatment or chemotherapy, has also been recognised in previously published literature. In two studies involving 54 mCRPC patients receiving first-line docetaxel or ARTA treatment [27] and 32 mCRPC patients receiving second-line cabazitaxel treatment [28], PSMA-TV was the only independent prognostic marker for OS. In contrast, baseline PSA level, Gleason score, and ECOG performance status had no prognostic value [27]. Has Simsek et al found that PSMA-TV and age were independent prognostic factors of OS in 52 mCRPC patients receiving first-line docetaxel treatment, while PSA level was not [29]. The association between lower Hb levels and worse OS has also been observed previously in mCRPC patients receiving first-line chemotherapy [4]. Until now, the prognostic value of DmaxVox, a dissemination feature representing the metastatic spread of prostate cancer cells, has only been investigated in mCRPC patients receiving ^{177}Lu-PSMA radioligand therapy [12]. In our study, we showed that DmaxVox was also a prognostic marker in mCRPC patients receiving ARTA treatment or chemotherapy, with higher DmaxVox being associated with worse OS. Interestingly, DmaxVox had a similar prognostic value compared to PSMA-TV. Especially since tumour segmentations to obtain volumetric PET parameters can be time-consuming and labour-intensive, DmaxVox may be an easy-to-implement prognostic biomarker in clinical centers where automated segmentation software is not (yet) available.

By performing a preliminary analysis using location and radiomic features at lesion level, we aimed to improve our understanding of CRPC lesion characteristics associated with progression, which in turn could potentially improve our understanding patient level. This study found that lesion location was associated with lesion progression on PSMA PET/CT after 3–4 months, with bone lesions having the highest progression rate. We hypothesise that this is caused by the relatively high density and low vascularisation of bone and by the bone marrow microenvironment, which may protect prostate cancer cells from treatment effects [30]. As for visceral lesions, the included number of lesions (n = 6) was too small to draw any conclusions. Although several GLCM features representing tumour heterogeneity were associated with lesion progression in univariate analysis, they lost significance in multivariate analysis. Furthermore, lesion volume was not associated with progression, in contrast to PSMA-TV at patient level. As this was

a radiomic analysis using twelve selected features from the literature, the potential of a radiomic analysis testing a wide variety of image features was not explored. We suggest that larger numbers of lesions and more advanced methods (e.g. machine learning or deep learning models) are needed to draw robust conclusions and to overcome the challenge of multicollinearity between PET parameters. The use of image filters can also potentially improve textural signal-to-noise ratios and may be considered for future research. Hopefully, improving our understanding of predictive markers for lesion progression, it can be helpful for treatment decision-making, prognostication and therapy response monitoring in the future.

To the best of our knowledge, this is the first study to investigate the prognostic value of baseline PSMA PET/CT scans using a fluorine-18 tracer. Although PET parameters are expected to remain prognostic markers when different tracers are used, the SUV values can differ between PSMA tracers and image reconstruction methods, and volumes can change depending on the segmentation methods. It is important to note that hazard ratios and odds ratios may be affected by these differences. Harmonisation of PET images and parameters, e.g. using image reconstructions compliant with EARL [31], and reaching consensus on the optimal segmentation method will remain key focus points.

Limitations of this study include the retrospective nature, variability in injection-to-scan times, and heterogeneity in terms of prior systemic lines of therapy and administered therapies. However, results were adjusted for prior lines of therapy and no differences were seen between patients treated with ARTA and those treated with chemotherapy. Therefore, we showed that PSMA PET/CT can provide robust prognostic parameters across multiple clinical mCRPC settings. For lesion-level analysis, limitations include the lack of validation data and the inclusion of a specific selection of lesions. The specific selection of lesions was done because texture features could not be extracted from lesions < 64 voxels [15], which with the voxel size in this study corresponds with a lesion volume of 11 × 11 × 11 mm. With the development of higher-resolution PET cameras and advanced image reconstruction algorithms, the extraction of texture features from smaller lesions may be possible in the future. The specific selection of lesions was also done for practical reasons, as many PSMA PET/CT scans contained multiple lesions merged into a single VOI, and it would be too time-consuming to split all lesions for separate analysis. As more automated segmentation tools become available, the segmentation of all individual lesions will become easier in the future as well.

CONCLUSIONS

In this study of 60 mCRPC patients who received a baseline PSMA PET/CT before treatment with ARTAs or chemotherapy, several prognostic factors associated with worse OS were identified, independent of the line of treatment. These factors include higher baseline PSMA-TV, TL-PSMA and DmaxVox, in combination with lower PSA and Hb levels. DmaxVox can be used as an easier alternative to PSMA-TV when automated segmentation software is not available. In combination with PSMA-TV, lower PSA levels indicated a worse OS, which may be a marker of tumour dedifferentiation. The results of this study can be used as input for the development of a prediction tool for mCRPC patients.

REFERENCES

1. EAU (2022) EAU-EANM-ESTRO-ESUR-ISUP-SIOG guidelines on prostate cancer. European Association of Urology, Arnhem
2. Freedland SJ, Davis M, Epstein AJ, Arondekar B, Ivanova JI (2023) Realworld treatment patterns and overall survival among men with metastatic castration-resistant prostate cancer (mCRPC) in the US Medicare population. Prostate Cancer Prostatic Dis. https://doi.org/10.1038/s41391-023-00725-8
3. van Soest RJ, de Morree ES, Shen L, Tannock IF, Eisenberger MA, de Wit R (2014) Initial biopsy Gleason score as a predictive marker for survival benefit in patients with castration-resistant prostate cancer treated with docetaxel: data from the TAX327 study. Eur Urol 66:330–336
4. Halabi S, Lin CY, Kelly WK et al (2014) Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol 32:671–677
5. Bravaccini S, Puccetti M, Bocchini M et al (2018) PSMA expression: a potential ally for the pathologist in prostate cancer diagnosis. Sci Rep 8:4254
6. Roberts MJ, Morton A, Papa N et al (2022) Primary tumour PSMA intensity is an independent prognostic biomarker for biochemical recurrence-free survival following radical prostatectomy. Eur J Nucl Med Mol Imaging 49:3289–3294
7. Sanchez-Jurado R, Devis M, Sanz R, Aguilar JE, del Puig Cozar M, Ferrer-Rebolleda J (2014) Whole-body PET/CT studies with lowered (1)(8)F-FDG doses: the influence of body mass index in dose reduction. J Nucl Med Technol 42:62–67
8. Scher HI, Morris MJ, Stadler WM et al (2016) Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the Prostate Cancer Clinical Trials Working Group 3. J Clin Oncol 34:1402–1418
9. Nioche C, Orlhac F, Boughdad S et al (2018) LIFEx: a freeware for radiomic feature calculation in multimodality imaging to accelerate advances in the characterization of tumor heterogeneity. Cancer Res 78:4786–4789
10. Mittlmeier LM, Brendel M, Beyer L et al (2021) Feasibility of different tumor delineation approaches for (18)F-PSMA-1007 PET/CT imaging in prostate cancer patients. Front Oncol 11:663631
11. O JH, Lodge MA, Wahl RL (2016) Practical PERCIST: a simplified guide to PET response criteria in solid tumors 1.0. Radiology 280:576–584
12. Aksu A, Vural Topuz O, Yilmaz B, Karahan Sen NP, Acar E, Capa Kaya G (2022) Prediction of early biochemical response after 177Lu-PSMA radioligand therapy with 68Ga-PSMA PET, a different perspective with quantitative parameters. Nucl Med Commun 43:468–474
13. Gandaglia G, Karakiewicz PI, Briganti A et al (2015) Impact of the site of metastases on survival in patients with metastatic prostate cancer. Eur Urol 68:325–334

14. Halabi S, Kelly WK, Ma H et al (2016) Meta-analysis evaluating the impact of site of metastasis on overall survival in men with castration-resistant prostate cancer. J Clin Oncol 34:1652–1659

15. Orlhac F, Nioche C, Klyuzhin I, Rahmim A, Buvat I (2021) Radiomics in PET imaging:: a practical guide for newcomers. PET Clin 16:597–612

16. Haralick RM, Shanmugam K, Dinstein I (1973) Textural features for image classification. IEEE Trans Syst Man Cybern SMC 3:610–621

17. Roll W, Schindler P, Masthoff M et al (2021) Evaluation of (68)Ga-PSMA-11 PET-MRI in patients with advanced prostate cancer receiving (177)Lu-PSMA-617 therapy: a radiomics analysis. Cancers (Basel) 13:3489

18. Moazemi S, Erle A, Lutje S, Gaertner FC, Essler M, Bundschuh RA (2021) Estimating the potential of radiomics features and radiomics signature from pretherapeutic PSMA-PET-CT scans and clinical data for prediction of overall survival when treated with (177)Lu-PSMA. Diagnostics (Basel) 11:186

19. Mattoni S, Farolfi A, Formaggio F et al (2022) PSMA PET for the evaluation of liver metastases in castration-resistant prostate cancer patients: a multicenter retrospective study. Cancers (Basel) 14:5680

20. Khurshid Z, Ahmadzadehfar H, Gaertner FC et al (2018) Role of textural heterogeneity parameters in patient selection for 177Lu-PSMA therapy via response prediction. Oncotarget 9:33312–33321

21. Zwanenburg A, Vallieres M, Abdalah MA et al (2020) The image biomarker standardization initiative: standardized quantitative radiomics for high-throughput image-based phenotyping. Radiology 295:328–338

22. Chalkidou A, O'Doherty MJ, Marsden PK (2015) False discovery rates in PET and CT studies with texture features: a systematic review. PLoS One 10:e0124165

23. Nordstrom T, Akre O, Aly M, Gronberg H, Eklund M (2018) Prostate-specific antigen (PSA) density in the diagnostic algorithm of prostate cancer. Prostate Cancer Prostatic Dis 21:57–63

24. Fanti S, Goffin K, Hadaschik BA et al (2021) Consensus statements on PSMA PET/CT response assessment criteria in prostate cancer. Eur J Nucl Med Mol Imaging 48:469–476

25. West RM (2022) Best practice in statistics: the use of log transformation. Ann Clin Biochem 59:162–165

26. Aggarwal R, Romero GR, Friedl V et al (2021) Clinical and genomic characterization of low PSA secretors: a unique subset of metastatic castration resistant prostate cancer. Prostate Cancer Prostatic Dis 24:81–87

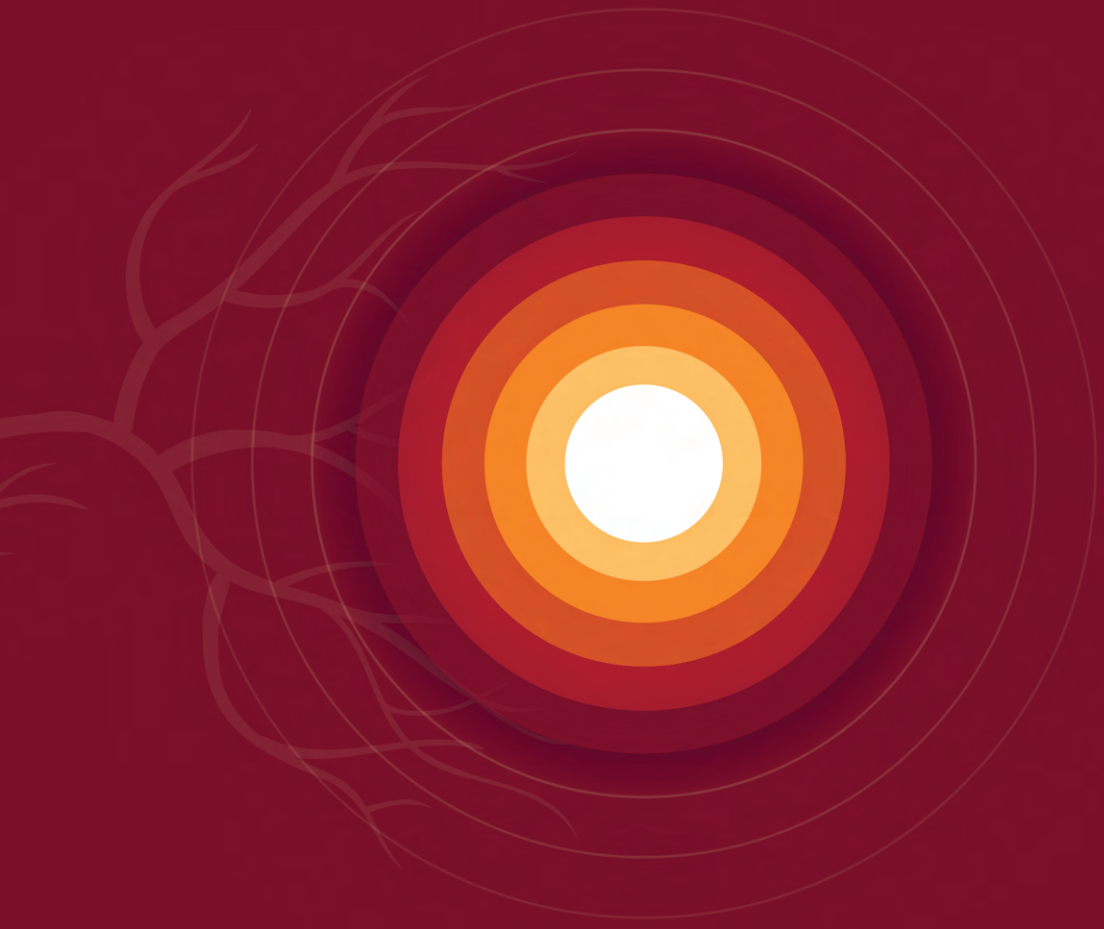
27. Telli TA, Ozguven S, Alan O et al (2022) Role of baseline (68)Ga-PSMA PET/CT-derived whole-body volumetric parameters in predicting survival outcomes of metastatic castration-resistant prostate cancer patients receiving first-line treatment. Ann Nucl Med 36:964–975

28. Shagera QA, Karfis I, Sideris S et al (2023) Tumor volume on PSMA PET as a prognostic biomarker in prostate cancer patients treated with cabazitaxel. Clin Nucl Med 48:775–780

29. Has Simsek D, Kuyumcu S, Karadogan S et al (2021) Can PSMA-based tumor burden predict response to docetaxel treatment in metastatic castration-resistant prostate cancer? Ann Nucl Med 35:680–690

30. Ghiaur G, Valkenburg KC, Esteb C et al (2023) Bone marrow niche chemoprotection of metastatic solid tumors mediated by CYP3A4. Cancer 129:1744–1751

31. Aide N, Lasnon C, Veit-Haibach P, Sera T, Sattler B, Boellaard R (2017) EANM/EARL harmonization strategies in PET quantification: from daily practice to multicentre oncological studies. Eur J Nucl Med Mol Imaging 44:17–31



CHAPTER 5

PSMA PET/CT for treatment response evaluation at predefined time points is superior to PSA response for predicting survival in metastatic castration-resistant prostate cancer patients

Authors
Fleur Kleiburg, Lioe-Fee de Geus-Oei, Saskia Luelmo, Romy Spijkerman, Jelle Goeman, Frank Toonen, Frits Smit, Tom van der Hulle, Linda Heijmen.

Published
European Journal of Radiology
2024 Dec:181:111774.
DOI: 10.1016/j.ejrad.2024.111774

Supplementary materials
https://doi.org/10.1016/j.ejrad.2024.111774

ABSTRACT

Background

In metastatic castration-resistant prostate cancer (mCRPC), using serum prostate-specific antigen (PSA) levels to evaluate treatment response is not always accurate. This study aimed to assess the efficacy of PSMA PET/CT at specific time points for evaluating treatment response and predicting survival in mCRPC patients, compared to PSA.

Methods

Sixty mCRPC patients underwent [^{18}F]PSMA-1007 PET/CT at baseline and for treatment response evaluation of either androgen receptor-targeted agents (after 3 months) or chemotherapy (after completion), and were retrospectively analysed. Visual assessment categorised overall response and response of the worst responding lesion as partial response, stable disease, or progressive disease, using the EAU/EANM criteria. Additionally, percentage changes in SUV_{max}, total tumour volume and total lesion uptake (tumour volume * SUV_{mean}) were calculated. PSA response was defined according to the PCWG3 criteria. Cox regression analysis identified predictors of overall survival.

Results

PSMA PET/CT and PSA response were discordant in 47 % of patients, and PSMA PET/CT response was worse in 89 % of these cases. Overall response on PSMA PET/CT independently predicted overall survival (progression versus non-progression: HR = 4.05, $p < 0.001$), outperforming PSA response (progression versus non-progression: HR = 2.53, $p = 0.010$) and other PSMA PET/CT parameters. Among patients with a PSA decline of > 50 %, 31 % showed progressive disease on PSMA PET/CT, correlating with higher mortality risk (progression versus non-progression: HR = 4.38, $p = 0.008$). No flare in PSMA uptake was observed in this cohort.

Conclusions

PSMA PET/CT for assessing treatment response at predefined time points was superior to PSA-based response for predicting overall survival in mCRPC patients treated with androgen receptor-targeted agents and chemotherapy. PSMA PET/CT showed the ability to detect disease progression earlier than PSA levels, which can affect treatment decisions and has the potential to improve patient outcomes. We recommend further research to validate these findings in larger patient cohorts, to extend the number of treatments, and to evaluate cost-effectiveness and impact on patient outcomes.

INTRODUCTION

Metastatic castration-resistant prostate cancer (mCRPC) is an advanced stage of prostate cancer characterised by resistance to androgen deprivation therapy, with a 5-year survival rate of approximately 30 % [1]. Treatment options for mCRPC mainly involve androgen receptor-targeted agents (ARTAs) and taxane-based chemotherapies [2]. As mCRPC is highly heterogeneous, evaluating treatment response is critical for disease management, with serum prostate-specific antigen (PSA) levels currently being the primary biomarker [2]. However, PSA levels do not always accurately reflect tumour burden. A PSA decrease can be seen in dedifferentiated prostate cancer [3] and a PSA increase can also have benign causes like prostatitis [4]. It is also known that time to PSA progression lacks correlation with overall survival in mCRPC patients and, therefore, is not a valid surrogate endpoint [5]. Additionally, PSA levels do not provide information about the location and biological behaviour of individual lesions, which can be particularly important in case of a mixed response. While in many other solid tumours treatment response is assessed using imaging modalities such as CT scans with RECIST criteria [6], in metastatic prostate cancer no imaging modality up to now has been effective enough to assess treatment response. Conventional imaging, i.e. CT and bone scans, has limited sensitivity in detecting lymph node and bone metastases, often showing no change in early bone metastases and missing more than half of lymph node metastases on CT scans [7]. It can also be complicated to distinguish between progressive and responsive bone lesions [8, 9]. Due to the limitations of PSA and conventional imaging, there is a need to find a reliable alternative method for treatment response evaluation in mCRPC.

Prostate-specific membrane antigen (PSMA) positron emission tomography / computed tomography (PET/CT) may provide a solution. PSMA is a transmembrane protein that is expressed in the epithelial cells of prostatic tissue and is highly overexpressed in prostate cancer [10]. PSMA-targeted PET/CT imaging in prostate cancer offers improved diagnostic sensitivity and specificity compared to conventional imaging, and is currently used either for staging high-risk patients or for restaging patients with biochemical recurrence after primary therapy [2, 11]. To date, however, there is limited data on the use of PSMA PET/CT for treatment response evaluation. It has been reported that PSMA PET/CT- and PSA-response differ in approximately 25 % of patients, but its impact on patient management and outcomes remains unclear [12, 13]. We hypothesize that PSMA PET/CT offers improved treatment response evaluation compared

to PSA, potentially leading to more effective treatment adjustments, improved survival outcomes, and reduced toxicity and costs. This study therefore aims to evaluate the effectiveness of PSMA PET/CT at specific time points in assessing treatment response and predicting survival in mCRPC patients as compared to PSA-based monitoring.

METHODS

Patient population

Since July 2019, [^{18}F]PSMA-1007 PET/CT (in short: PSMA PET/CT) has replaced conventional imaging to assess treatment response in mCRPC patients at Leiden University Medical Centre (Leiden, The Netherlands) and Alrijne Hospital (Leiderdorp, The Netherlands). After approval of the study protocol by the local ethics committee on 03/03/2022, this bicentric study included patients who (1) had been diagnosed with mCRPC, (2) had received treatment with either ARTAs (enzalutamide, abiraterone) or chemotherapy (docetaxel, cabazitaxel) and (3) had undergone PSMA PET/CT at baseline and for treatment response evaluation. Patients with baseline PSMA PET/CT scans from other hospitals, i.e. other PSMA tracers and/or scanners, were excluded. Administered treatment dosages adhered to EAU guidelines [2] and androgen deprivation therapy was continued in all patients. Clinical data, including medical history, laboratory tests, imaging results, overall survival (OS) and progression-free survival (PFS), were collected from electronic clinical records for retrospective analysis. All patients consented to the use of their data.

PSMA PET/CT imaging

Baseline PSMA PET/CT was performed within 8 weeks before treatment initiation. In case of ARTA treatment, the PSMA PET/CT for treatment response evaluation was performed after 3 months of treatment. This time point was chosen to avoid flare in PSMA uptake and/or PSA levels, which can occur in the weeks following ARTA initiation, but is not seen after 3 months [14-17]. In case of chemotherapy, PSMA PET/CT was performed 4–6 weeks (maximum: 8 weeks) after the last administered dose to evaluate treatment response. The PSMA PET/CT was performed earlier if disease progression was suspected after at least 3 chemotherapy cycles, also to avoid flare in PSMA uptake and/or in PSA levels [18, 19]. Disease progression was suspected when PSA levels increased

by > 25 % and > 2 ng/ml [19], or in case of clinical deterioration, such as new-onset pain.

PSMA PET/CT scans were performed 60–120 min after intravenous injection of [^{18}F]PSMA-1007, depending on PSA (<4 ng/ml: 120 min, 4–40 ng/ml: 80 min, >40 ng/ml: 60 min). 1.5–2.1 MBq/kg body weight of [^{18}F]PSMA-1007 was injected, depending on BMI (<25: 1.5 MBq/kg, 25–30: 1.8 MBq/kg, >30: 2.1 MBq/kg). All patients were scanned with the 5-Ring Discovery MI PET/CT (GE Healthcare, Chicago, Illinois, USA [20]) located at Alrijne Hospital (Leiderdorp, The Netherlands), covering the skull vertex to mid-thigh in a supine position. A low-dose CT (15–550 mA, 120 kV) was performed for attenuation correction, followed by a PET scan (120 s per bed position). CT images were reconstructed in 512 x 512 matrices with a slice thickness of 2.5 mm. PET images were reconstructed in 256 x 256 matrices with a slice thickness of 2.78 mm, and a Bayesian penalised-likelihood iterative image algorithm (Q.Clear with a beta value of 900) was applied.

Study endpoints

Overall response to treatment on PSMA PET/CT was assessed visually and reported for clinical use by one of our nuclear medicine physicians, who had 2–4 years of experience with this tracer at the start of this study period, according to the EAU/EANM criteria [21]. Imaging-based progressive disease (iPD) was defined as 2 or more new lesions or a > 30 % increase in uptake or tumour volume; partial response (iPR) as a > 30 % decrease in uptake or tumour volume; stable disease (iSD) as a change in uptake and tumour volume between −30 % and + 30 %. Additionally, our nuclear medicine physicians reported the response of the worst responding lesion using the same criteria (abbreviations: iPD_{worst}, iPR_{worst} and iSD_{worst}). Complete response was not assigned due to the palliative setting. Potential flare in PSMA uptake was assessed by follow-up of PSA levels, subsequent PSMA PET/CT scans and clinical course.

Specifically for this research, additional quantitative PSMA PET/CT analyses were done. In each scan, SUV_{max} (highest of all lesions), PSMA-TV (total tumour volume) and TL-PSMA (total lesion uptake: PSMA-TV * SUV_{mean}) were retrieved using LIFEx software [22] and the percentage changes in PET parameters were calculated as measures for treatment response (ΔSUV_{max}%, ΔPSMA-TV% and ΔTL-PSMA%). A fixed absolute threshold of SUV = 4 was used for semi-automatic scan delineation [23]. Further details on delineation methods are described in the Supplemental Materials [24].

Biochemical response was determined by comparing baseline PSA levels with those at the time of treatment response evaluation (ΔPSA%). According to the PCWG3 recommendations, a > 25 % and > 2 ng/ml increase from the nadir indicated biochemical progressive disease (bPD), a > 50 % decrease indicated partial response (bPR), and a PSA change between −50 % and + 25 % indicated stable disease (bSD) [19].

Statistical analysis

Descriptive statistics were used to summarise the retrieved data. Patients were in follow-up until deceased or until the moment of data analysis. OS was defined as time from PSMA PET/CT for treatment response evaluation to death in months. PFS was defined as time from PSMA PET/CT for treatment response evaluation to either PSA progression or clinical deterioration in months. Censored data used the time to the last hospital visit. For the survival analyses, progressive disease (PD) was compared with non-progressive disease (non-PD: partial response or stable disease), as this guides treatment alteration or continuation in clinical practice [21]. Cox regression analyses were used to test the predictive value of input parameters for OS and PFS, and log-rank tests were used to compare survival distributions (PD versus non-PD). Statistical analyses were performed using SPSS version 29 (IBM Corporation, Armonk, New York, USA). Statistical significance was defined as a p-value < 0.05.

RESULTS

Patient characteristics

From December 2019 to December 2023, 60 mCRPC patients underwent a PSMA PET/CT at baseline and at predefined time points, and were included in this study. Baseline characteristics are shown in Table 1. Thirty-one patients received ARTA treatment and twenty-nine received chemotherapy. The median time between baseline PSMA PET/CT and treatment initiation was 11 days (IQR 8 – 20 days). According to the local protocol, the response PSMA PET/CT was performed either after 3 months of ARTA treatment (median 95 days, IQR 82 – 110 days), or 3 weeks (median 21 days, IQR 16 – 31 days) after completion of 3–8 cycles (median 6 cycles) of chemotherapy. In 8 of 60 patients, the PSMA PET/CT was performed earlier than planned (after 3 or 4 cycles) due to either PSA progression (n = 6) or clinical deterioration (n = 2) during chemotherapy. At the time of analysis, 38 of 60 patients were deceased, and 47 of 60 patients showed either PSA progression

or clinical deterioration. Median OS was 16.5 months and median PFS was 3.8 months. The median follow-up time was 26 months (range 7 – 35 months). One patient was transferred to another hospital during follow-up; no data was available on disease progression, but the date of death was reported. No other patients were lost to follow-up.

Table 1. Patient characteristics (n = 60).

Characteristic	Value	
Gleason score at diagnosis*, n (%)		
6	6	(10 %)
7	8	(13 %)
≥ 8	40	(67 %)
Metastatic status at diagnosis, n (%)		
M0	22	(37 %)
M1	38	(63 %)
Prior treatment of the primary tumour, n (%)		
Radical prostatectomy	4	(7 %)
Local radiotherapy	24	(40 %)
ADT only	18	(30 %)
ADT + docetaxel upfront	13	(22 %)
ADT + abiraterone upfront	1	(2 %)
Enzalutamide upfront**	6	(10 %)
Previous systemic therapy lines for mCRPC, n (%)		
0	37	(62 %)
1	23	(38 %)
Age at treatment initiation in years, median (IQR)	75	(68 – 78)
Serum PSA before treatment initiation in ng/ml, median (IQR)	21.1	(8.1 – 55.6)
PSMA-TV before treatment initiation in mL, median (IQR)	118	(41 – 328)
Site of disease on baseline PSMA PET/CT scan, n (%)		
Lymph nodes only	7	(12 %)
Bone only	18	(30 %)
Lymph nodes + bone	31	(52 %)
Bone + visceral	1	(2 %)
Lymph nodes + bone + visceral	3	(5 %)
Current treatment, n (%)		
Enzalutamide	25	(42 %)
Abiraterone	6	(10 %)
Docetaxel	24	(40 %)
Cabazitaxel	5	(8 %)

* Gleason score unknown in 6 patients. ** As part of a clinical trial (EudraCT 2014–001161-27). ADT = androgen deprivation therapy. IQR = interquartile range. PSMA-TV = total tumour volume on PSMA PET/CT.

PSMA PET/CT response versus PSA response

PSMA PET/CT response and PSA response were discordant in 28 of 60 patients (47 %). In 25 (89 %) of these patients, of which 15 received ARTA treatment and 10 chemotherapy, overall response on PSMA PET/CT was worse than PSA response: 10 had bPR and iPD, 9 had bPR and iSD, and 6 had bSD and iPD. In the other 3 patients, all receiving chemotherapy, PSA response was worse than overall response on PSMA PET/CT: 1 had bSD and iPR, and 2 had bPD and iSD. Strikingly, all three patients had decreasing PSA levels at the time of treatment response evaluation, so PSA flare could not be ruled out [25]. In the 32 patients with concordance between PSMA PET/CT response and PSA response (53 %), 12 patients had PD, 7 had SD and 13 had PR. In the 8 patients who underwent an early PSMA PET/CT for suspected progression, all were confirmed to have iPD. Figure 1 shows the ΔPSA% and overall response on PSMA PET/CT for each patient. Supplemental Table S1 shows a direct comparison of all five PSMA PET/CT response parameters and ΔPSA% in each patient.

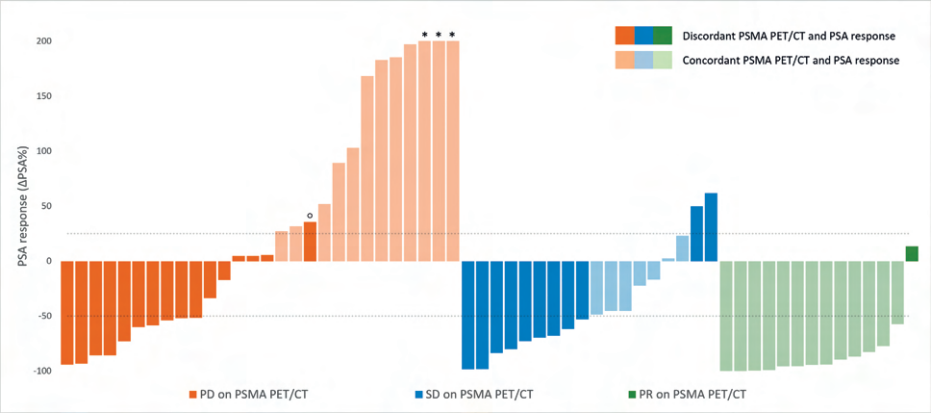


Figure 1. Waterfall plot of the percentage change in PSA level from before treatment initiation to the moment of treatment response evaluation in each included patient, grouped by the overall response on PSMA PET/CT. Discordance between PSMA PET/CT and PSA response was seen in 28 of 60 patients (47 %). Grey dotted reference lines: +25 % and −50 % change in PSA levels. ° The PSA response of this patient was classified as stable disease, because the absolute PSA increase was 0.5 ng/ml (PSA went from 1.4 to 1.9 ng/ml). * ΔPSA% exceeded + 200 %. PD = progressive disease (orange), SD = stable disease (blue), PR = partial response (green).

Survival outcomes

Univariate Cox regression analysis assessed the predictive value of all PSMA PET/CT response parameters, PSA response and age for OS (see Table 2). Overall response on PSMA PET/CT (iPD vs. non-iPD, $p < 0.001$), response of the worst responding lesion (iPD_{wors} vs. non-iPD_{wors}, $p < 0.001$), PSA response (bPD vs. non-bPD; $p = 0.010$), ΔPSA% ($p = 0.027$), ΔPSMA-TV% ($p < 0.001$) and ΔTL-PSMA% ($p = 0.002$) were significant predictors of OS, whereas age and ΔSUV_{max}% were not. There was no significant difference in OS between patients treated with an ARTA and those treated with chemotherapy ($p = 0.374$). In multivariate Cox regression analyses using ΔPSA% as the first variable and a PSMA PET/CT response parameter as the second, overall response (HR = 4.05, $p < 0.001$) and response of the worst responding lesion (HR = 3.95, $p < 0.001$) remained the best predictors of OS, outperforming the quantitative PET response parameters ΔPSMA-TV% ($p = 0.016$) and ΔTL-PSMA% ($p = 0.039$), see Table 2. ΔPSA% became nonsignificant in these analyses (all $p > 0.4$).

Kaplan-Meier curves for OS illustrate the differences between bPD vs. non-bPD (Figure 2A) and between iPD vs. non-iPD (Figure 2B). Notably, overall response on PSMA PET/CT ($X^2(1) = 18.9$, $p < 0.001$, median OS 12.3 versus 26.9 months) differentiated more effectively between short-term and long-term survivors than PSA response ($X^2(1) = 7.2$, $p = 0.006$, median OS 12.3 versus 23.0 months). This was also the case when compared to response of the worst responding lesion on PSMA PET/CT ($X^2(1) = 14.9$, $p < 0.001$).

Table 2. Univariate and multivariate Cox regression analyses for OS.

	OS					
	Univariate			Multivariate (ΔPSA% + PET parameter)		
Categorical variable	**HR***	**95 % CI**	**p-value****	**HR***	**95 % CI**	**p-value****
Overall response on PSMA PET/CT (iPD vs. non-iPD)	4.213	2.104–8.433	< 0.001	4.047	1.926–8.503	< 0.001
Response of the worst responding lesion on PSMA PET/CT (iPD_{worst} vs. non-iPD_{worst})	4.251	1.922–9.401	< 0.001	3.948	1.747–8.920	< 0.001
PSA response (bPD vs. non-bPD)	2.529	1.253–5.103	0.010			
Continuous variable						
Age at treatment initiation (per year)	1.016	0.973–1.062				
ΔPSA% (per %)	1.001	1.000–1.003	0.027			
ΔSUV_{max}% (per %)	1.004	0.999–1.009				
ΔPSMA-TV% (per %)	1.003	1.001–1.004	< 0.001	1.003	1.001–1.006	0.016
ΔTL-PSMA% (per %)	1.002	1.001–1.004	0.002	1.002	1.000–1.004	0.039

* HR = hazard ratio. ** p-values are only displayed when < 0.05.

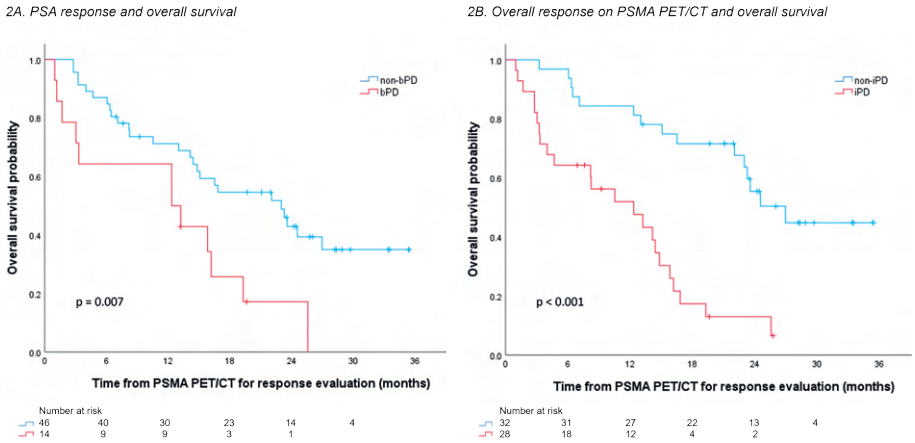


Figure 2. Kaplan-Meier plots showing the difference in overall survival between patients with progression (red) and patients without progression (blue), either based on PSA response (Figure 2A) or on overall response on PSMA PET/CT (Figure 2B). PSMA PET/CT better differentiated between short-term and long-term survivors than PSA. +: censored patients. bPD = PSA progression, non-bPD = no PSA progression. iPD = progressive disease as overall response on PSMA PET/CT, non-iPD = non-progressive disease as overall response on PSMA PET/CT.

Among all patients with a PSA decline of > 50 % (bPR, n = 32), PSMA PET/CT showed 10 with iPD (31 %), 9 with iSD (28 %) and 13 with iPR (41 %). In these 32 patients, overall response on PSMA PET/CT was the best predictor of OS (iPD vs. non-iPD: HR = 4.38 [1.47–13.05], p = 0.008) while ΔPSA% was not a predictor (p = 0.516). No flare on PSMA PET/CT was observed; all identified progression was confirmed as true disease progression, evident through multiple new PSMA-avid lesions, subsequent PSA progression, clinical deterioration, or further progression on a follow-up PSMA PET/CT. This suggests that PSMA PET/CT was able to detect progression earlier than PSA levels. The majority of patients with bPR and iPD were receiving first-line treatment (n = 8). An example patient with bPR and iPD is shown in Figure 3.

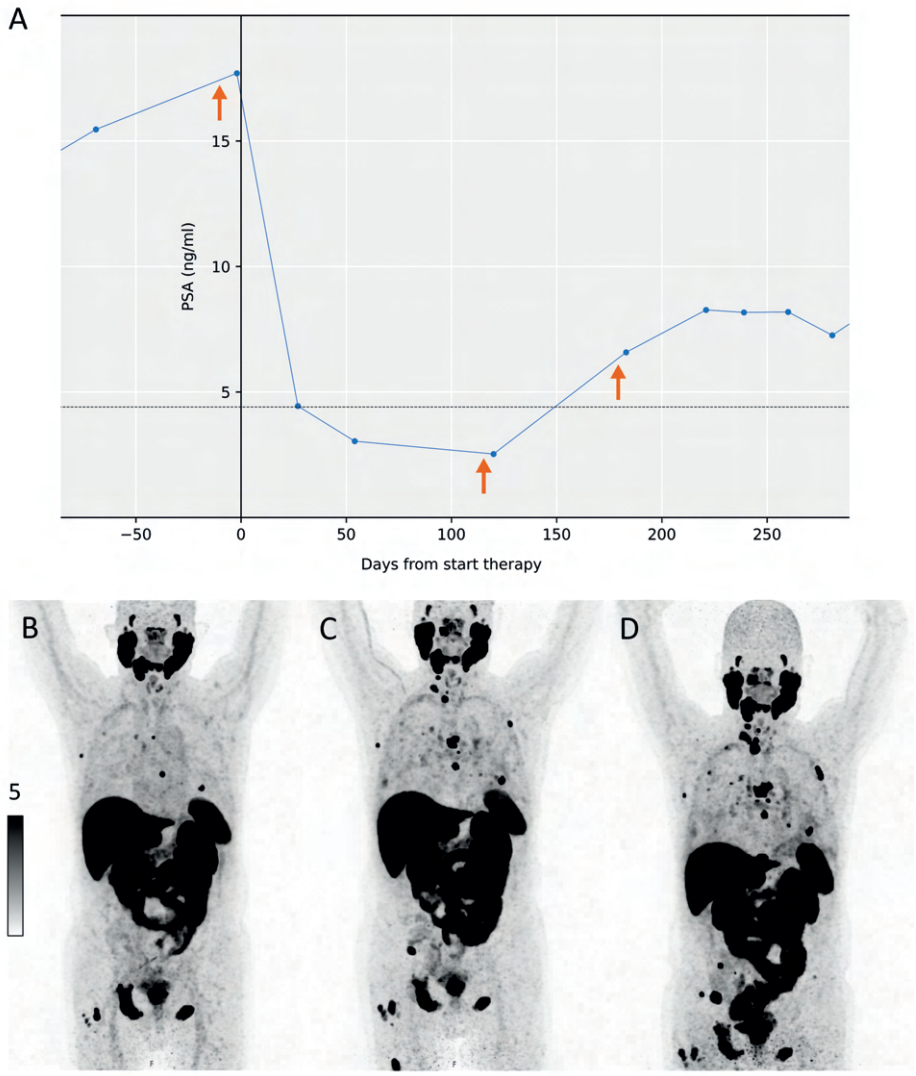


Figure 3. The PSA levels and [^{18}F]PSMA-1007 PET/CT scans of a 72-year-old male with first-line enzalutamide treatment for mCRPC showed a discrepancy after four months: an 86% PSA decline, but disease progression on PSMA PET/CT with multiple new lesions. In a multidisciplinary team meeting it was decided to continue treatment as long as PSA values remained stable. After six months, treatment was stopped due to PSA progression and further progression on PSMA PET/CT. A: a graph of the patient's PSA levels, orange arrows indicate PSMA PET/CT scan times. B: baseline PSMA PET/CT showed a PSMA-avid primary tumour and multiple bone metastases. C: PSMA PET/CT after four months of treatment demonstrated an increase in total tumour volume and tracer uptake and over 6 new lesions. D: PSMA PET/CT after six months of treatment showed a further increase in total tumour volume and tracer uptake, and over 10 new lesions.

45 Patients had no biochemical progression or clinical deterioration when response was evaluated. In these patients, overall response (HR = 6.88, p < 0.001) and response of the worst responding lesion (HR = 8.31, p < 0.001) on PSMA PET/CT were the best imaging-based predictors of PFS, independent of ΔPSA%. ΔPSA% was also a significant independent predictor of PFS. Detailed results are provided in Supplemental Table S2

DISCUSSION

In the management of mCRPC patients, accurate assessment of treatment response is essential to optimise treatment decisions and patient outcomes, and this study found PSMA PET/CT to be superior to PSA levels. We assessed the systematic use of PSMA PET/CT for response evaluation of treatment with ARTAs (after 3 months) or chemotherapy (after completion) in comparison to PSA response in 60 mCRPC patients. Discordance between PSMA PET/CT and PSA response was observed in 47 % of patients, and overall response on PSMA PET/CT was the best independent predictor of OS (iPD vs. non-iPD: HR = 4.05, p < 0.001), outperforming PSA (bPD vs. non-bPD: HR = 2.53, p = 0.010). This means that PSMA PET/CT could better differentiate between short-term and long-term survivors than PSA. Interestingly, among patients with a PSA decline > 50 %, 31 % showed progressive disease on PSMA PET/CT, which was significantly associated with worse OS (HR = 4.4, p = 0.008). These results suggest that the superior predictive ability of PSMA PET/CT for OS is due to its ability to detect progressive disease earlier than PSA in a significant proportion of patients. The systematic use of PSMA PET/CT for treatment response evaluation can allow for earlier discontinuation of ineffective treatments, minimising unnecessary toxicity and costs, and providing the opportunity to initiate potentially effective treatment earlier in these patients. It can also allow for continuation of treatment in those patients with the greatest survival benefit. In addition, PSMA PET/CT has the advantage over PSA of providing information on metastatic sites, so that e.g. metastasis-targeted treatments can be considered.

Previous studies have also reported discordance between PSMA PET/CT response and PSA response in mCRPC patients, with discordance rates ranging from 7-46 % [15, 16, 26–32]. However, most studies included fewer than 30 patients, introducing uncertainty, and variations in PSMA-tracers and PSMA PET/CT response definitions make direct comparisons difficult. Several articles investigated

the predictive value of PSMA PET/CT for survival in mCRPC patients and found the following significant predictors: ΔPSMA-TV% for OS [26], PSMA PET/CT response for OS [16, 27], and PSMA PET/CT response for PFS [28], all outperforming PSA as a parameter for treatment response. Additionally, Küper et al. [33] reported that absolute difference in PSMA-TV significantly predicted OS in 25 mCRPC patients. Our study provided robust and confirmatory results. In contrast to most previous studies, we used predefined fixed time points to assess treatment response in all mCRPC patients. By using PSMA PET/CT for treatment response evaluation regardless of PSA levels, our analysis could demonstrate its ability to detect progressive disease earlier than PSA levels in a proportion of the included patients. This study is also the first to use the radiotracer [^{18}F]PSMA-1007 for treatment response evaluation in this patient population, and its use is expected to increase due to its advantages over ^{68}Ga-labelled tracers, including improved spatial resolution and a longer half-life (110 min vs. 68 min), which allows for wider distribution [34]. However, [^{18}F]PSMA-1007 has an increased risk of unspecific bone uptake, which requires increased awareness and accurate interpretation from nuclear medicine physicians to avoid false positives [35]. In case of a negative PSMA PET/CT at baseline or difficulty assessing liver lesions due to high background uptake, neither of which occurred in this study, other imaging modalities can be considered, e.g. whole body MRI or [^{18}F]FDG PET/CT.

By maintaining a minimum interval of 3 months (ARTA) or 3 cycles (chemotherapy) for treatment response evaluation, the risk of false-positive PSMA PET/CT results due to flare [36] was minimised. Importantly, none of the analysed PSMA PET/CT scans showed flare, and progression on PSMA PET/CT was confirmed to be true disease progression. Physicians should also be aware of PSA flare [25], which was suspected in the 3 patients with worse PSA response than PSMA PET/CT response. PSMA PET/CT imaging could potentially distinguish between PSA flare and genuine progression, but further research is necessary to validate this capability. Of course, in case of discordant results, it always remains important to consider clinical signs of deterioration, especially in case of tumour dedifferentiation [37].

To the best of our knowledge, our study is the first to compare different qualitative and quantitative PSMA PET/CT response parameters for predicting survival outcomes in mCRPC patients. Notably, the qualitative parameters (visual assessment of overall response and response of the worst responding lesion) clearly outperformed the quantitative parameters in predicting OS. We hypothesise that this can be explained by the heterogeneity of CRPC within patients in terms of genotype and response

to treatment [38], and that a patient's prognosis primarily depends on the least treatment-sensitive cancer cells. Qualitative parameters took into account non-responding or new lesions, which appeared to be prognostically most unfavourable, whereas quantitative parameters assessed changes in total tumour burden. We recommend using the EAU/EANM criteria for response assessment [21] for further research and in clinical practice, as these criteria were easily usable and revealed to be the best predictor of OS. This study did not make a direct comparison with RECIP 1.0 [39] and did not assess interobserver variability. However, Shagera et al. [16] found that the EAU/EANM and RECIP 1.0 criteria performed equally well and were both good predictors of OS in mCRPC patients receiving ARTAs.

Limitations of this study include the relatively small bicentric cohort of 60 patients, its retrospective nature and the fact that no diagnostic CT or bone scans were performed, so PSMA PET/CT results could not be directly compared with conventional imaging. Furthermore, only mCRPC patients who were treated with an ARTA or chemotherapy were included. We recommend future research to include more types of mCRPC treatment, such as radium-223 therapy and PARP inhibitors, and to prospectively validate these findings in larger patient cohorts. In addition, cost-effectiveness and impact on treatment decisions and subsequent patient outcomes need to be assessed.

CONCLUSIONS

PSMA PET/CT response was superior to PSA-based response for predicting OS in mCRPC patients, likely due to its ability to detect disease progression earlier than PSA levels. Based on the results of this study, the systematic use of PSMA PET/CT for evaluating treatment response to an ARTA (after 3 months) or chemotherapy (after completion) should be considered, to provide better guidance for treatment decision-making and potentially improve patient outcomes and cost-effectiveness. In predicting OS, qualitative PSMA PET/CT criteria outperformed quantitative PSMA PET/CT criteria, and the EAU/EANM criteria for response assessment [21] had the best prognostic value in this study. Performing PSMA PET/CT after suspected disease progression was considered reliable. No flare on PSMA PET/CT was observed in this study where minimum intervals (3 months of ARTA or 3 chemotherapy cycles) were maintained. We recommend further research to focus on prospective trials of PSMA PET/CT-guided treatment strategies and their impact on survival, as well as evaluation of cost-effectiveness.

REFERENCES

1. American Cancer Society, Cancer Facts & Figures 2022, American Cancer Society, Atlanta, 2022.
2. Eau-eanm-estro-esur-isup-siog,, Guidelines on Prostate Cancer, European Association of Urology, 2022.
3. A.W. Partin, H.B. Carter, D.W. Chan, J.I. Epstein, J.E. Oesterling, R.C. Rock, et al., Prostate specific antigen in the staging of localized prostate cancer: influence of tumor differentiation, tumor volume and benign hyperplasia, J. Urol. 143 (4) (1990) 747–752.
4. J. Morote, M. Lopez, G. Encabo, I.M. de Torres, Effect of inflammation and benign prostatic enlargement on total and percent free serum prostatic specific antigen, Eur. Urol. 37 (5) (2000) 537–540.
5. L.A. Gharzai, R. Jiang, E.M. Jaworski, K. Morales Rivera, R.T. Dess, W.C. Jackson, et al., Meta-analysis of candidate surrogate end points in advanced prostate cancer, NEJM Evid. 2 (4) (2023). EVIDoa2200195.
6. E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2) (2009) 228–247.
7. R. Perez-Lopez, N. Tunariu, A.R. Padhani, W.J.G. Oyen, S. Fanti, H.A. Vargas, et al., Imaging Diagnosis and Follow-up of Advanced Prostate Cancer: Clinical Perspectives and State of the Art, Radiology 292 (2) (2019) 273–286.
8. C. Messiou, G. Cook, A.H. Reid, G. Attard, D. Dearnaley, J.S. de Bono, et al., The CT flare response of metastatic bone disease in prostate cancer, Acta Radiol. 52 (5) (2011) 557–561.
9. L. Fournier, L.F. de Geus-Oei, D. Regge, D.E. Oprea-Lager, M. D'Anastasi, L. Bidaut, et al., Twenty Years On: RECIST as a Biomarker of Response in Solid Tumours an EORTC Imaging Group - ESOI Joint Paper, Front. Oncol. 11 (2021) 800547.
10. S.S. Chang, Overview of prostate-specific membrane antigen, Rev. Urol. 6 (Suppl 10) (2004) S13–S18.
11. M.S. Hofman, N. Lawrentschuk, R.J. Francis, C. Tang, I. Vela, P. Thomas, et al., Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study, Lancet 395 (10231) (2020) 1208–1216.
12. C. Schmidkonz, M. Cordes, T.I. Goetz, O. Prante, T. Kuwert, P. Ritt, et al., 68Ga-PSMA-11 PET/CT derived quantitative volumetric tumor parameters for classification and evaluation of therapeutic response of bone metastases in prostate cancer patients, Ann. Nucl. Med. 33 (10) (2019) 766–775.

13. S. Han, S. Woo, Y.I. Kim, J.L. Lee, A.G. Wibmer, H. Schoder, et al., Concordance between Response Assessment Using Prostate-Specific Membrane Antigen PET and Serum Prostate-Specific Antigen Levels after Systemic Treatment in Patients with Metastatic Castration Resistant Prostate Cancer: A Systematic Review and Meta-Analysis, Diagnostics (basel) 11 (4) (2021).

14. R. Aggarwal, X. Wei, W. Kim, E.J. Small, C.J. Ryan, P. Carroll, et al., Heterogeneous Flare in Prostate-specific Membrane Antigen Positron Emission Tomography Tracer Uptake with Initiation of Androgen Pathway Blockade in Metastatic Prostate Cancer, Eur Urol Oncol. 1 (1) (2018) 78–82.

15. C.S. Denis, F. Cousin, B. Laere, R. Hustinx, B.R. Sautois, N. Withofs, Using (68)Ga-PSMA-11 PET/CT for Therapy Response Assessment in Patients with Metastatic Castration-Resistant Prostate Cancer: Application of EAU/EANM Recommendations in Clinical Practice, J. Nucl. Med. 63 (12) (2022) 1815–1821.

16. Q.A. Shagera, I. Karfis, P. Kristanto, S. Spyridon, R. Diamand, A. Santapau, et al., PSMA PET/CT for Response Assessment and Overall Survival Prediction in Patients with Metastatic Castration-Resistant Prostate Cancer Treated with Androgen Receptor Pathway Inhibitors, J. Nucl. Med. 64 (12) (2023) 1869–1875.

17. S.L. Burgio, V. Conteduca, B. Rudnas, F. Carrozza, E. Campadelli, E. Bianchi, et al., PSA flare with abiraterone in patients with metastatic castration-resistant prostate cancer, Clin. Genitourin. Cancer 13 (1) (2015) 39–43.

18. T. Nelius, S. Filleur, PSA surge/flare-up in patients with castration-refractory prostate cancer during the initial phase of chemotherapy, Prostate 69 (16) (2009) 1802–1807.

19. H.I. Scher, M.J. Morris, W.M. Stadler, C. Higano, E. Basch, K. Fizazi, et al., Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3, J. Clin. Oncol. 34 (12) (2016) 1402–1418.

20. T. Pan, S.A. Einstein, S.C. Kappadath, K.S. Grogg, C. Lois Gomez, A.M. Alessio, et al., Performance evaluation of the 5-Ring GE Discovery MI PET/CT system using the national electrical manufacturers association NU 2–2012 Standard, Med. Phys. 46 (7) (2019) 3025–3033.

21. S. Fanti, K. Goffin, B.A. Hadaschik, K. Herrmann, T. Maurer, S. MacLennan, et al., Consensus statements on PSMA PET/CT response assessment criteria in prostate cancer, Eur. J. Nucl. Med. Mol. Imaging 48 (2) (2021) 469–476.

22. C. Nioche, F. Orlhac, S. Boughdad, S. Reuze, J. Goya-Outi, C. Robert, et al., LIFEx: A Freeware for Radiomic Feature Calculation in Multimodality Imaging to Accelerate Advances in the Characterization of Tumor Heterogeneity, Cancer Res. 78 (16) (2018) 4786–4789.

23. L.M. Mittlmeier, M. Brendel, L. Beyer, N.L. Albert, A. Todica, M.J. Zacherl, et al., Feasibility of Different Tumor Delineation Approaches for (18)F-PSMA-1007 PET/CT Imaging in Prostate Cancer Patients, Front. Oncol. 11 (2021) 663631.

24. O. Jh, M.A. Lodge, R.L. Wahl, Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0, Radiology 280 (2) (2016) 576–584.

25. A. Sella, C.N. Sternberg, I. Skoneczna, S. Kovel, Prostate-specific antigen flare phenomenon with docetaxel-based chemotherapy in patients with androgen independent prostate cancer, BJU Int. 102 (11) (2008) 1607–1609.

26. C. Can, C. Gundogan, O.A. Yildirim, K. Poyraz, Y. Guzel, H. Komek, Role of (68)Ga-PSMA PET/CT parameters in treatment evaluation and survival prediction in prostate cancer patients compared with biochemical response assessment, Hell. J. Nucl. Med. 24 (1) (2021) 25–35.

27. Q.A. Shagera, C. Artigas, I. Karfis, G. Critchi, N.M. Chanza, S. Sideris, et al., (68)Ga-PSMA PET/CT for Response Assessment and Outcome Prediction in Metastatic Prostate Cancer Patients Treated with Taxane-Based Chemotherapy, J. Nucl. Med. 63 (8) (2022) 1191–1198.

28. L. Calderoni, E. Maietti, A. Farolfi, R. Mei, K.S. Louie, M. Groaning, et al., Prostate-Specific Membrane Antigen Expression on PET/CT in Patients with Metastatic Castration-Resistant Prostate Cancer: A Retrospective Observational Study, J. Nucl. Med. 64 (6) (2023) 910–917.

29. B. Grubmuller, S. Rasul, P. Baltzer, H. Fajkovic, D. D'Andrea, F. Berndl, et al., Response assessment using [(68) Ga]Ga-PSMA ligand PET in patients undergoing systemic therapy for metastatic castration-resistant prostate cancer, Prostate 80 (1) (2020) 74–82.

30. T. Ozulker, F. Ozulker, Assessment of the role of Ga-68 PSMA I&T PET/CT in response evaluation to docetaxel therapy in castration resistant prostate cancer patients, Rev Esp Med Nucl Imagen Mol (engl Ed). 39 (5) (2020) 292–298.

31. P.E. Hartrampf, M. Heinrich, A.K. Seitz, J. Brumberg, I. Sokolakis, C. Kalogirou, et al., Metabolic Tumour Volume from PSMA PET/CT Scans of Prostate Cancer Patients during Chemotherapy-Do Different Software Solutions Deliver Comparable Results? J. Clin. Med. 9 (5) (2020).

32. K.A. Zukotynski, U. Emmenegger, S. Hotte, A. Kapoor, W. Fu, A.L. Blackford, et al., Prospective, Single-Arm Trial Evaluating Changes in Uptake Patterns on Prostate-Specific Membrane Antigen-Targeted (18)F-DCFPyL PET/CT in Patients with Castration-Resistant Prostate Cancer Starting Abiraterone or Enzalutamide, J. Nucl. Med. 62 (10) (2021) 1430–1437.

33. A.T. Kuper, D. Kersting, T. Telli, K. Herrmann, A. Rominger, A. Afshar-Oromieh, et al., PSMA-PET follow-up to assess response in patients not receiving PSMA therapy: Is there value beyond localization of disease? Theranostics. 14 (9) (2024) 3623–3633.

34. A. Sanchez-Crespo, Comparison of Gallium-68 and Fluorine-18 imaging characteristics in positron emission tomography, Appl. Radiat. Isot. 76 (2013) 55–62.

35. A. Rizzo, S. Morbelli, D. Albano, G. Fornarini, M. Cioffi, R. Laudicella, et al., The Homunculus of unspecific bone uptakes associated with PSMA-targeted tracers: a systematic review-based definition, Eur. J. Nucl. Med. Mol. Imaging (2024).

36. V. Conteduca, G. Poti, P. Caroli, S. Russi, N. Brighi, C. Lolli, et al., Flare phenomenon in prostate cancer: recent evidence on new drugs and next generation imaging, Ther Adv Med Oncol. 13 (2021), 1758835920987654.

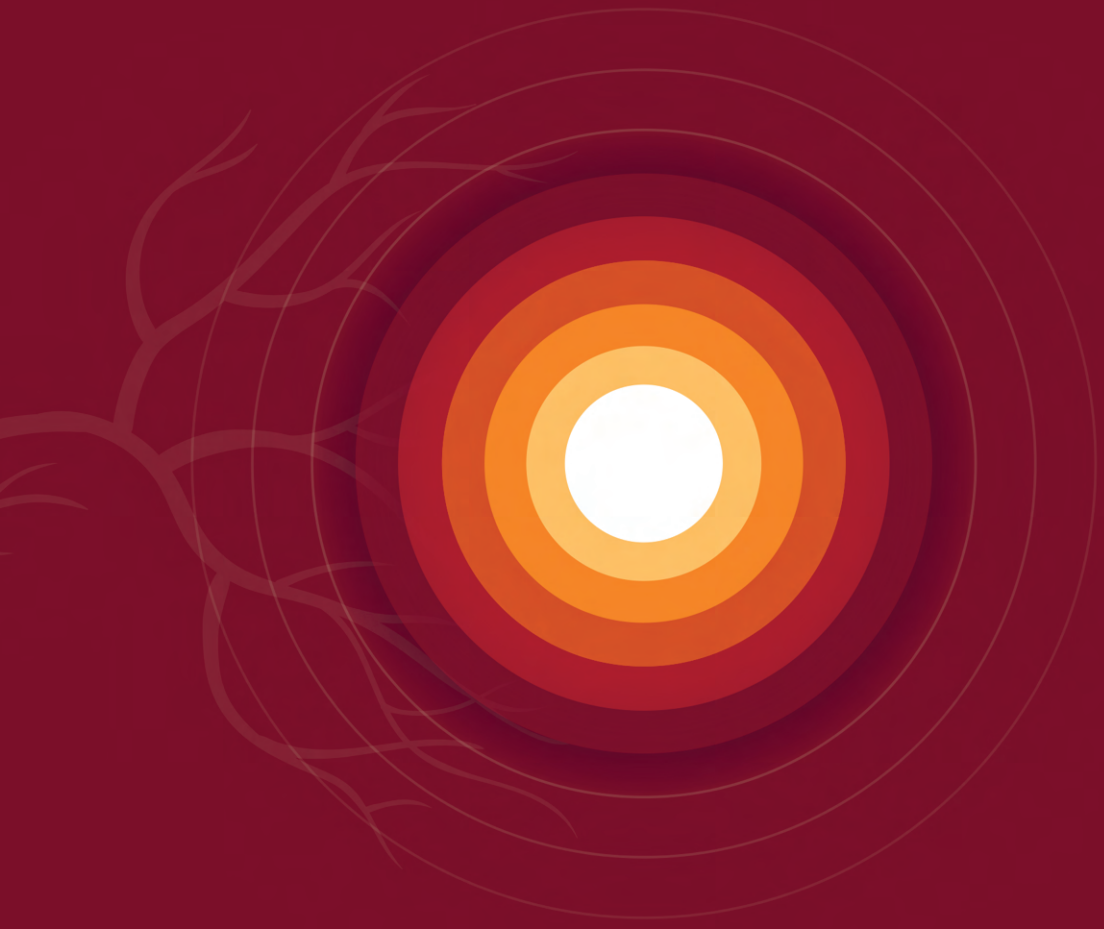
37. P. Bronsert, K. Reichel, J. Ruf, Loss of PSMA Expression in Non-neuroendocrine Dedifferentiated Acinar Prostate Cancer, Clin. Nucl. Med. 43 (7) (2018) 526–528.

38. W. Zhang, T. Wang, Y. Wang, F. Zhu, H. Shi, J. Zhang, et al., Intratumor heterogeneity and clonal evolution revealed in castration-resistant prostate cancer by longitudinal genomic analysis, Transl. Oncol. 16 (2022) 101311.

39. A. Gafita, I. Rauscher, M. Weber, B. Hadaschik, H. Wang, W.R. Armstrong, et al., Novel Framework for Treatment Response Evaluation Using PSMA PET/CT in Patients with Metastatic Castration-Resistant Prostate Cancer (RECIP 1.0): An International Multicenter Study, J. Nucl. Med. 63 (11) (2022) 1651–1658.

PART 2

PSMA PET/CT in non-prostate cancers



CHAPTER 6

Prostate-specific membrane antigen (PSMA) as a potential target for molecular imaging and treatment in bone and soft tissue sarcomas

Review article

Authors
Fleur Kleiburg, Linda Heijmen, Hans Gelderblom, Szymon Kielbasa, Judith Bovée, Lioe-Fee de Geus-Oei.

Published
British Journal of Radiology
2023 Apr;96(1145):20220886
DOI: 10.1259/bjr.20220886

Supplementary materials
https://doi.org/10.1259/bjr.20220886

ABSTRACT

Bone and soft tissue sarcomas are a group of rare malignant tumours with major histological and anatomical varieties. In a metastatic setting, sarcomas have a poor prognosis due to limited response rates to chemotherapy. Radioligand therapy targeting prostate-specific membrane antigen (PSMA) may offer a new perspective. PSMA is a type II transmembrane glycoprotein which is present in all prostatic tissue and overexpressed in prostate cancer. Despite the name, PSMA is not prostate-specific. PSMA expression is also found in a multitude of non-prostatic diseases including a subgroup of sarcomas, mostly in its neovascular endothelial cells. On PET/CT imaging, multiple sarcomas have also shown intense PSMA-tracer accumulation. PSMA expression and PSMA-tracer uptake seem to be highest in patients with aggressive and advanced sarcomas, who are also in highest need of new therapeutic options. Although these results provide a good rationale for the future use of PSMA-targeted radioligand therapy in a selection of sarcoma patients, more research is needed to gain insight into optimal patient selection methods, PSMA-targeting antibodies and tracers, administered doses of radioligand therapy, and their efficacy and tolerability. In this review, mRNA expression of the FOLH1 gene which encodes PSMA, PSMA immunohistochemistry, PSMA-targeted imaging and PSMA-targeted therapy in sarcomas will be discussed.

INTRODUCTION

Sarcomas are a diverse group of malignant tumours that arise from connective tissues, such as bone, cartilage, fat, muscle and blood vessels. These tumours can occur in many different anatomic locations and currently, over 70 histological subtypes are defined [1]. Sarcomas can be divided into two broad categories: soft tissue sarcomas and bone sarcomas, with an estimated incidence of 4.7 per 100,000 /year and 0.8 per 100,000 /year, respectively [2]. Together they represent less than 1% of all new cancer cases [3, 4]. Due to its rarity and its histological and anatomical heterogeneity, diagnosis and optimal tumour management are challenging. Individual treatment plans of sarcoma patients should therefore always be made in multidisciplinary teams of sarcoma reference centres [5, 6]. The golden standard for localized intermediate and high-grade sarcomas is complete surgical resection with wide and microscopically tumour-free (R0) margins. Selected patients, *e.g.* in case of high-risk lesions, can be treated additionally with (neo)adjuvant radiotherapy and/or chemotherapy to improve clinical outcomes. Although complete removal of the tumour is pursued, unfortunately around 30–50% of patients, also depending on tumour characteristics, still develop local recurrences and/or metastases after primary treatment [7–10]. In addition, at least 14–17% of sarcoma patients have distant metastases at presentation [11]. Standard of care in patients with metastatic sarcomas consists of cytotoxic chemotherapy, with doxorubicin being the first choice in most sarcoma types [5, 6]. With the administration of doxorubicin, in combination with or without ifosfamide chemotherapy, treatment response rates of approximately 25% are reached in advanced soft tissue sarcoma [12, 13] and even lower in bone sarcomas. Due to these low response rates to chemotherapy, the five-year survival rates in metastatic soft tissue sarcoma and bone sarcoma are 17 and 31%, respectively [14]. This shows that there is a high need for new effective treatment options that can decrease burden of disease and increase survival, especially in sarcoma patients with advanced disease.

Prostate-specific membrane antigen (PSMA) may offer a new perspective for sarcoma patients. Despite the name, PSMA is not prostate cancer-specific. PSMA, also known as glutamate carboxypeptidase II, is a type II transmembrane glycoprotein which consists of 750 amino acids and is encoded by the FOLH1 (folate hydrolase 1) gene [15]. In prostatic tissue, PSMA is expressed in the secretory epithelial cells, and PSMA is overexpressed in prostate cancer. Further upregulation of PSMA expression is seen in more advanced prostate cancers and

PSMA was shown to be an independent predictor of poor prognosis [16]. Although the exact mechanisms are unknown, PSMA is associated with the activation of PI3K/AKT and cAMP/PKA pathways, which are involved in cell proliferation [17, 18]. The expression pattern of PSMA has made it a well-established target for molecular imaging in prostate cancer. In the last decade, PSMA-targeting PET/CT scans have found their way into clinical practice for primary staging of high-risk prostate cancer patients and in case of biochemical recurrence after primary treatment [19]. Furthermore, PSMA targeting ligands have been labelled with therapeutic nuclides such as lutetium-177 or actinium-225, and these radioligand therapies have achieved beneficial effects in advanced prostate cancer patients with acceptable toxicity [20, 21]. Interestingly, PSMA has been found in the tumour-associated neovascular endothelial cells of a wide variety of other tumours besides prostate cancer, such as renal cell carcinoma, glioblastoma, hepatocellular carcinoma, thyroid cancer, lung cancer, breast cancer, and sarcoma [22]. Since PSMA-targeted radioligand therapy (PSMA-RLT) has demonstrated promising results in patients with prostate cancer, the question arises whether this therapy could also have a beneficial effect in other PSMA-positive tumours, such as sarcomas.

This review aims to give an overview of the available literature about the possible role of PSMA in sarcomas. mRNA expression of the FOLH1 gene, immunohistochemical PSMA expression, PSMA-targeted imaging, PSMA-targeted therapy and the possible future perspectives for sarcomas will be discussed.

METHODS

For the literature search, the following search strategy was used in PubMed:

("Glutamate carboxypeptidase II"[MeSH] OR "Glutamate carboxypeptidase II"[tiab] OR "PSMA"[tiab] OR "Prostate-specific membrane antigen"[tiab] OR "prostate specific membrane antigen"[tiab]) AND ("sarcoma"[MeSH] OR "sarcoma"[tiab] OR "sarcomas"[tiab])

This resulted in 28 articles, of which 15 were relevant for the topic of this review. The excluded articles were either not about PSMA in sarcomas (*n* = 11), or were reviews that cited already included articles (*n* = 2). The same search strategy was used in Scopus and Web of Science, which did not add other relevant articles.

Also, no extra articles were found while reading the included articles and their references. PubMed, Scopus and Web of Science were last checked for new articles on 05-12-2022.

mRNA expression of the FOLH1 gene in sarcomas

PSMA is encoded by the FOLH1 gene, which is localized on chromosome 11p11-p12 and contains 19 exons [23]. As part of the large-scale Pan-Cancer analysis project from The Cancer Genome Atlas Research Network, the FOLH1 mRNA expression levels were determined in over 10.000 tumours from 32 different tumour types, together with a wide variety of other genetic and clinical data. Figure 1 shows the results from this analysis, obtained from cBioPortal [24, 25]. The FOLH1 mRNA expression levels were calculated by the logarithmic transformation of FOLH1 abundance estimates using RSEM [26]. After sortation of FOLH1 mRNA expression levels by median, sarcomas were 12th of the 33 tumour types. There still was a notable difference between the FOLH1 mRNA expression levels in prostate carcinomas and all other carcinomas, which can be seen in Figure 1.

The 251 sarcomas within the Pan-Cancer Atlas were further divided into different subtypes: leiomyosarcoma (n = 99), dedifferentiated liposarcoma (n = 58), undifferentiated pleomorphic sarcoma (n = 50), myxofibrosarcoma (n = 25), synovial sarcoma (n = 10) and malignant peripheral nerve sheath tumour (n = 9). Thus, this means that only a small subset of soft tissue sarcoma subtypes and no bone sarcomas were represented in this sarcoma database. There was a statistically significant difference in FOLH1 mRNA expression levels between the different sarcoma types (Kruskal-Wallis test, $H(5) = 65.587$, $p < 0.001$). Dedifferentiated liposarcomas had higher FOLH1 mRNA expression levels (median = 7.7) compared to malignant peripheral nerve sheath tumours (median = 7.4), undifferentiated pleomorphic sarcomas (median = 6.4), synovial sarcoma (median = 6.1), myxofibrosarcoma (median = 5.5) and leiomyosarcoma (median = 5.5). The maximum FOLH1 mRNA expression level was also highest in dedifferentiated liposarcomas, followed by undifferentiated pleomorphic sarcomas (Figure 2).

Figure 1. Log transformed FOLH1 mRNA expression levels of the 32 tumour types from the TCGA Pan-Cancer Atlas. Sorted by the median in descending order. This figure was obtained from cBioportal.org [24, 25].

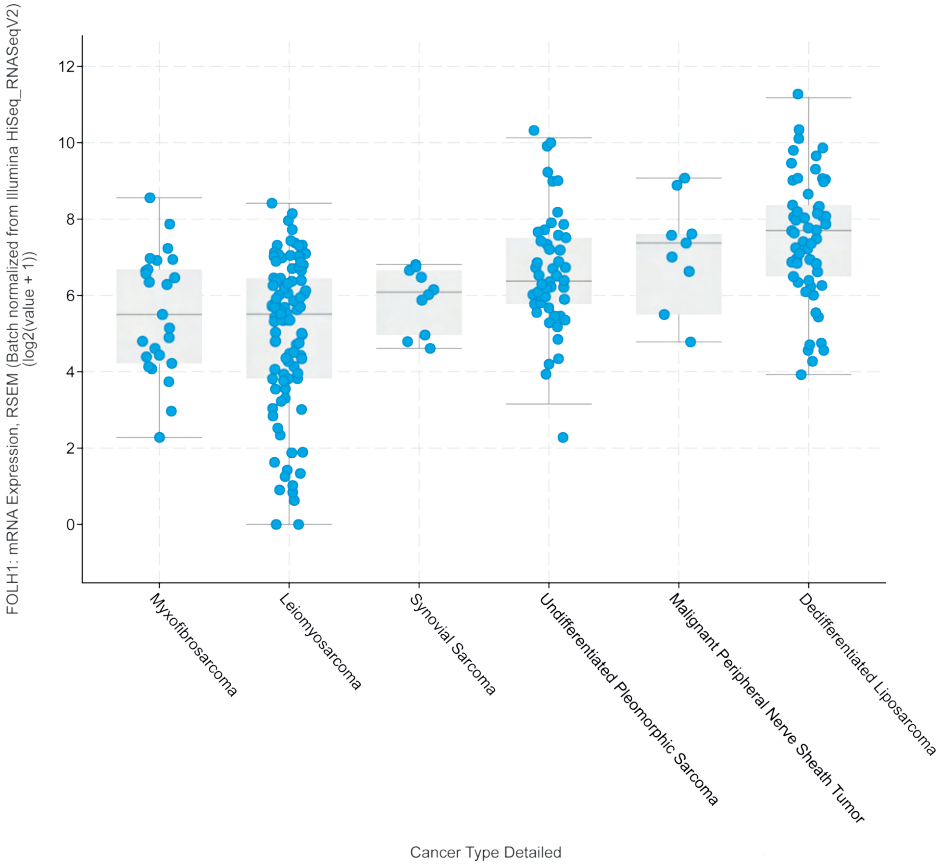


Figure 2. Log transformed FOLH1 mRNA expression levels of myxofibrosarcoma, leiomyosarcoma, synovial sarcoma, undifferentiated pleomorphic sarcoma, malignant peripheral nerve sheath tumour and dedifferentiated liposarcoma. Sorted by the median in ascending order. Of the sarcomas, dedifferentiated liposarcomas had the highest FOLH1 mRNA expression levels. This figure was obtained from cBioportal.org [24, 25].

The Pan-Cancer Atlas also contained overall survival and progression-free survival data. Within the leiomyosarcoma group, higher FOLH1 mRNA expression levels correlated significantly with shorter progression-free survival (Spearman correlation = −0.25, p = 0.015, Figure 3). However, R^2 was 0.06, so only a small percentage of the variation could be attributed to the FOLH1 mRNA expression levels. No such correlation was seen with overall survival. In the other sarcoma subtypes, no correlation was found between FOLH1 mRNA expression levels and progression-free survival or overall survival.

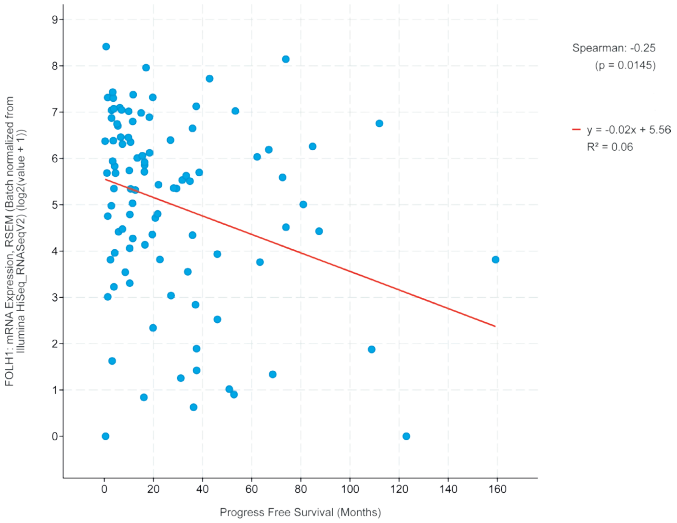


Figure 3. Correlation between log-transformed FOLH1 mRNA expression levels and months of progression-free survival in 100 leiomyosarcomas. Spearman correlation −0.25, $p = 0.015$. $R^2 = 0.06$. This figure was obtained from cBioportal.org [24, 25].

PSMA immunohistochemistry in sarcomas

For determining PSMA expression in formalin-fixed paraffin-embedded (FFPE) tissue samples, immunohistochemistry is the designated technique. Up until now, four articles have investigated immunohistochemical PSMA expression in sarcomas.

Heitkötter et al. analysed 779 samples from 25 different soft tissue and bone tumour types [27], of which 599 were sarcomas. PSMA expression was found to be positive in 151 of 779 soft tissue and bone tumours (19.4%). Similar to other non-prostate tumours, this PSMA expression was found in the tumour-associated neovasculature and was more frequent in sarcomas compared to soft tissue and bone tumours with benign or intermediate biological potential. Strong PSMA expression, defined as moderate staining (readily apparent at 40x magnification) in >5% of the neovasculature or any strong staining of the neovasculature, was found in 43 of 779 soft tissue and bone tumours (5.5%). Table 1 lists the sarcoma subtypes of which over 20% of analysed tumour samples showed any PSMA expression. The sarcoma subtypes with the highest frequency of PSMA-positivity were pleomorphic rhabdomyosarcoma (60%), synovial sarcoma (56%) and pleomorphic liposarcoma (50%). These were also the sarcoma types with the highest number of tumours with strong PSMA expression (40%, 38 and 20%, respectively). As for bone sarcomas, 106 Ewing sarcomas were investigated, of which six were PSMA-positive and none showed strong PSMA expression. No other bone sarcomas were included.

Table 1. Immunohistochemical PSMA expression in different sarcoma subtypes. Different monoclonal antibodies were used.

Authors	Used monoclonal antibody	Sarcoma subtype	Number of tumours with any PSMA expression		Number of tumours with strong PSMA expression [a]	
Heitkötter et al. [27] [b]	3E6	Pleomorphic rhabdomyosarcoma	3/5	(60%)	2/5	(40%)
		Synovial sarcoma	9/16	(56%)	6/16	(38%)
		Pleomorphic liposarcoma	5/10	(50%)	2/10	(20%)
		Undifferentiated pleomorphic sarcoma	15/33	(46%)	6/33	(18%)
		Malignant peripheral nerve sheath tumour	7/21	(33%)	4/21	(19%)
		Leiomyosarcoma	21/66	(32%)	7/66	(11%)
		Endometrial stromal sarcoma	1/4	(25%)	0/4	(0%)
		Dedifferentiated liposarcoma	17/75	(23%)	0/75	(0%)
Zeng et al. [28]	Unknown	Osteosarcoma	21/45	(47%)	-	
Chang et al. [29]	7E11 PM2J004.5 J591 J415 PEQ226.5	Soft tissue sarcoma (specific subtype unknown)	6/7	(86%) [c]	-	
Coskun et al. [30]	EP192	Malignant peripheral nerve sheath tumour	0/25	(0%)	-	

[a] Strong PSMA expression was defined as either moderate staining (readily apparent at 40x magnification) in >5% of the neovasculature or any strong staining of the neovasculature.
[b] From this study, the sarcoma subtypes of which over 20% of analysed tumours were PSMA-positive are included in this table. Additionally, the number of tumours with strong PSMA expression is described in these sarcoma subtypes. All PSMA expression was found in the tumour's neovasculature.
[c] All five monoclonal antibodies gave the same results.

Zeng et al. investigated PSMA expression in osteosarcomas [28]. After immunohistochemical analysis, 21 of 45 osteosarcomas (47%) demonstrated PSMA reactivity in the tumour-associated neovasculature, not in the tumour cells. Interestingly, PSMA expression was significantly associated with tumour size ($p = 0.042$), the presence of pulmonary metastasis ($p < 0.001$) and a worse 5 year survival rate (36.6% vs 63.2%, $p < 0.05$). These findings support the hypothesis that PSMA expression is associated with worse clinical outcome. PSMA expression was not associated with age, gender and location. In two other studies, sarcomas were studied as part of a larger tissue sample cohort. Chang et al. studied 20 benign and 12 malignant tissue types, including seven soft tissue sarcomas [29]. Of these, six turned out PSMA-positive. No further histological information on these soft tissue sarcomas is described. Coskun et al. investigated PSMA expression, along with STAT3 and VEGF expression, in 25 malignant peripheral nerve sheath tumours (MPNST) [30]. None of the 25 MPNSTs were PSMA-positive.

It is important to take into account that up until now, a wide variety of monoclonal antibodies (mAbs) have been developed to use for PSMA immunohistochemistry and that in the previously described articles, several different mAbs are used (Table 1). The 7E11 mAb was the firstly used anti-PSMA antibody, which binds to the intracellular epitope of PSMA. More recently developed mAbs, such as mAb 3E6, used by Heitkötter et al. [27], often bind to the extracellular epitope of PSMA. Chang et al. [29] compared two mAbs that bind to the intracellular PSMA domain (7E11 and PM2J004.5) to three other mAbs that bind to the extracellular PSMA domain (J591, J415 and PEQ226.5). All five anti-PSMA mAbs reacted strongly with the neovasculature of the analysed malignant tumours, including soft tissue sarcomas, so there was no difference found by these authors. Coskun et al. [30] describe that 0/25 (0%) MPNSTs showed any PSMA expression, while Heitkötter et al. [27] report that 7/21 (33%) MPNSTs were PSMA-positive, by the use of mAb EP192 and mAb 3E6, respectively. It is difficult to conclude if the mAb may account for this difference, as no comparative studies between these mAbs have been conducted.

When looking specifically at the seven different soft tissue sarcoma subtypes of which FOLH1 mRNA expression levels have been determined in the Pan-Cancer Atlas, it is noticeable that the mean FOLH1 mRNA expression levels do not necessarily correspond to the immunohistochemical PSMA positivity rates from Heitkötter et al. Dedifferentiated liposarcomas had the highest mean FOLH1 mRNA expression, but only 22.67% of the investigated dedifferentiated liposarcomas

showed any PSMA expression and none showed strong PSMA expression. On the other hand, synovial sarcomas were only fifth in median FOLH1 mRNA expression but had the highest number of PSMA-positive tumours (56.3%) and tumours with strong PSMA expression (37.5%) of these seven subtypes. Thus, based on these limited data that are available, there does not seem to be a correlation between mRNA expressions of FOLH1 and protein expression of PSMA.

PSMA-targeted PET/CT imaging in sarcomas

Because there is evidence available about the presence of PSMA in sarcomas, the question arises whether PSMA-tracer uptake can be seen on the PSMA-targeted PET/CT scans that have been developed for prostate cancer. Available literature about PSMA-targeted imaging in sarcomas consists of case reports, as no case series or prospective studies have been published yet. Table 2 describes each of these case reports.

First of all, three liposarcomas have shown PSMA uptake; a well-differentiated, dedifferentiated and pleomorphic liposarcoma [31–33]. It is remarkable that in the dedifferentiated liposarcoma, PSMA seemed to differentiate between the lipomatous and non-lipomatous regions, where the latter was PSMA-positive. The highest tumour-to-background ratio was seen on the PSMA PET/CT scan of pleomorphic liposarcoma (SUV_{max} = 13). Plouznikoff et al. and Mathew et al. reported two patients with PSMA-positive undifferentiated pleomorphic sarcoma in the left obturator muscle and the right posterior chest wall, respectively, one of which was likely radiation-induced [34, 35]. As for bone sarcomas, one Ewing sarcoma and two osteosarcomas with PSMA uptake have been described [36–38]. Two of them were females, for which the PSMA PET/CT scans were specifically made to assess the amount of PSMA-tracer binding in the tumour, while in all other case reports the PSMA-avid sarcomas were incidental findings in patients with prostate cancer. In one patient with osteosarcoma, PSMA could differentiate between areas of fibrous dysplasia and areas of malignant transformation to osteosarcoma in the skull bones. When looking at sarcomas in a metastatic setting, one of the osteosarcoma patients and additionally one patient with angiosarcoma and two patients with leiomyosarcoma had multiple PSMA-avid metastases [39–41]. Interestingly, especially in these patient cases high PSMA uptake with high SUV_{max} values were reported. The highest SUV_{max} values were described in high-grade osteosarcoma, localized in the sternum and multiple lung, bone and liver metastases (SUV_{max} values of 10.6–35.1). An example of one of our own patients with PSMA-avid metastatic sarcoma is shown in Figure 4.

Table 2. An overview of all published case reports describing PSMA-tracer accumulation in sarcomas on PET/CT imaging. The SUV_{max} values are included in this table if described in the case report

Authors	Gender	Age	Scan	Diagnosis	Description of PSMA-tracer uptake	Other
Malik et al. [31]	Male	76	^{68}Ga-PSMA PET/CT	Well-differentiated liposarcoma in the left sartorius muscle	Mild uptake	
Inanir et al. [32]	Male	77	^{68}Ga-PSMA PET/CT	Dedifferentiated liposarcoma, 42cm (craniocaudally) retroperitoneal mass	Visible uptake in the non-lipomatous component, but no uptake in the lipomatous region	[^{18}F]FDG PET/CT matched findings, but with a lower tumour-to-background ratio
Militano et al. [33]	Male	74	^{68}Ga-PSMA PET/CT	Pleomorphic liposarcoma of the left quadriceps	SUV_{max} = 13	8.5x6x11.2cm mass
Plouznikoff et al. [34]	Male	67	^{68}Ga-PSMA PET/CT	Undifferentiated soft tissue sarcoma in the left internal obturator muscle, most likely radiation-induced	Visible uptake above the background	FNCLCC grade 2
Mathew et al. [35]	Male	72	^{68}Ga-PSMA PET/CT	High-grade pleomorphic sarcoma in the right posterior chest wall	Intense PSMA uptake	
Parihar et al. [36]	Female	19	[^{68}Ga]Ga-PSMA-11 PET/CT	Ewing sarcoma in the left iliac bone	SUV_{max} = 7.4	[^{18}F]FDG PET/CT: SUV_{max} = 5.1
Sasikumar et al. [37]	Female	60	^{68}Ga-PSMA PET/CT	Fibrous dysplasia with areas of osteosarcoma in left side skull bones	Clear uptake in osteosarcoma, not in fibrous dysplasia	99mTC-MDP: could not differentiate between osteosarcoma and fibrous dysplasia

Table 2. *Continued.*

Authors	Gender	Age	Scan	Diagnosis	Description of PSMA-tracer uptake	Other
Can et al. [38]	Male	75	^{68}Ga-PSMA PET/CT	High-grade osteosarcoma in the sternum with multiple lung, bone and liver metastases	SUV_{max} = 16.9 (sternum), 10.6 (lung), 23.1 (bone), 35.1 (liver)	[^{18}F]FDG PET/CT: SUV_{max} = 14.6 (sternum), 3.1 (lung), 9.5 (bone), 7.9 (liver)
Marafi et al. [39]	Male	59	^{18}F-PSMA PET/CT	Angiosarcoma in the scalp with metastases in ribs, iliac bones, femur and right tibia	Increased uptake	
Juptner et al. [40]	Female	50	^{68}Ga-PSMA PET/CT	Leiomyosarcoma of the vena cava inferior with metastases in bone, lung, liver and muscle	SUV_{max} = 14.5 (bone), 1.7 (lung), 12.9 (liver), 16.5 (gluteus maximus muscle), 3.7 (vastus lateralis muscle)	Received one application of 6.0 GBq [^{177}Lu]Lu-PSMA-617
Digklia et al. [41]	Female	Mid-50s	[^{68}Ga]Ga-PSMA-11 PET/CT	Uterine leiomyosarcoma with pulmonary and peritoneal metastases	SUV_{max} = 8.9 (lung)	Received two applications of [^{177}Lu]Lu-PSMA-I&T in combination with nivolumab

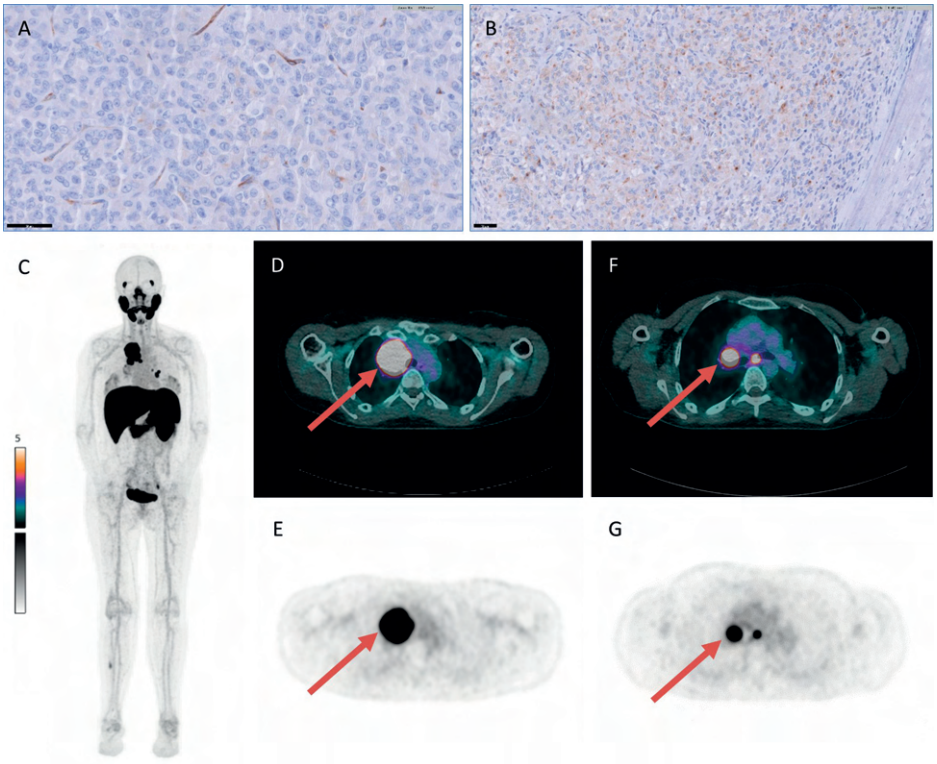


Figure 4. A 33-year-old female with an epithelioid malignant peripheral nerve sheath tumour in the right ankle. PSMA immunohistochemistry on the biopsy material of this tumour revealed both focal neovascular PSMA expression (A) and cytoplasmic, dot-like PSMA staining in 30% of tumour cells (B).Nineteen months post-operatively, conventional imaging detected multiple mediastinal and pulmonary metastases. A [^{18}F]JK-PSMA-7 PET/CT scan was experimentally made to assess tumoural PSMA-tracer binding. The [^{18}F]JK-PSMA-7 PET/CT scan showed good tracer accumulation in mediastinal metastases (SUV_{max} = 21.5, see D and E), pulmonary metastases (SUV_{max} = 14.8, see F and G) and one bone metastasis in the right fibula (SUV_{max} = 3.0). C: whole-body maximal intensity projection, D and F: fused PET/CT images, E and G: PET images. These results from the Leiden University Medical Centre (Leiden, The Netherlands) have not been published before. Informed consent for publishing the data and images was obtained from the patient.

The potential pitfall of PSMA-targeted PET/CT imaging is that not all PSMA-tracer uptake besides physiological uptake can be attributed to malignant lesions. Occasionally, PSMA expression and PSMA-tracer uptake are seen in benign neoplasms, such as haemangiomas and schwannomas [22, 27]. Without careful interpretation, such lesions might be easily mistaken for metastases [42–46]. It is important to take this possibility into account while interpreting PSMA PET/CT scans of sarcoma patients, as this would otherwise lead to false-positive findings.

PSMA-targeted therapy in sarcomas

While for prostate cancer PSMA-RLT is still often given in a research setting, already two case reports have been published describing the administration of ^{177}Lu-PSMA-RLT to a patient with metastasized leiomyosarcoma, both also described in Table 2 [40, 41].

The first patient was a 50-year-old female who was diagnosed with a leiomyosarcoma of the vena cava inferior in 2015 (TNM pT2pN0(0/9)pR0 FNCLCC Grade 1) [40]. In 2016, the patient underwent selective internal radiotherapy for several liver metastases. In 2017, routine diagnostics revealed new metastatic lesions in bone, lung and muscle. As routine systemic therapies were denied by the patient, a diagnostic PSMA PET/CT scan was performed to assess the feasibility of PSMA-RLT. Because of low SUV_{max} values, re-evaluation with PSMA PET/CT was done one year later, which showed clear progression of disease with multiple new lesions and increased SUV_{max} values. The lesions in both lungs, liver, left gluteus maximus muscle, right vastus lateralis muscle and multiple bone lesions were treated with one application of 6.0 GBq [^{177}Lu]Lu-PSMA-617. The radionuclide therapy was well tolerated by the patient, but intratherapeutic whole body scans revealed moderate PSMA uptake in the left gluteus maximus muscle and left os ilium and weak uptake in all other lesions. Therefore, no other [^{177}Lu]Lu-PSMA-617 cycles were given. Three months later, further progression of the disease was seen. The images of this patient are shown in Figure 5.

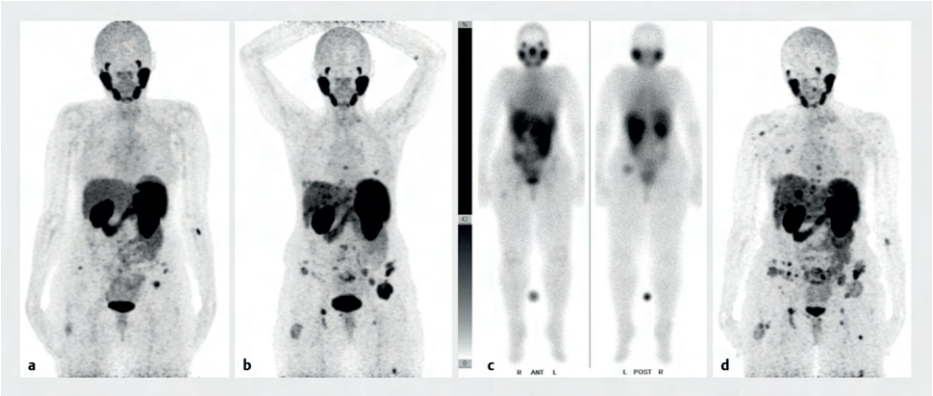


Figure 5. A 50-year-old female patient with metastasized leiomyosarcoma has received one application of 6.0 GBq [^{177}Lu]Lu-PSMA-617. This figure was obtained from Jüptner et al. [40]. A: First ^{68}Ga-PSMA PET/CT scan in July 2017, with several lung and bone metastases and singular lesions in the liver, in the left gluteus maximus muscle and in the right vastus lateralis muscle. B: Second ^{68}Ga-PSMA PET/CT scan in April 2018, with progression of the disease with increasing PSMA accumulation in the existing metastases and multiple newly emerged lesions. C: Intratherapeutic whole body scan 24 hours after injection of 6 GBq [^{177}Lu]Lu-PSMA-617, with poor fixation of the radiotracer in the multiple metastases. A dosimetrical phantom is placed between the legs. R = right, L = left, ANT = anterior, POST = posterior. D: Post therapeutic ^{68}Ga-PSMA PET/CT scan in August 2018, with progression of the PSMA-positive lesions in lung, liver, bone and muscle. The leading lesion in the left gluteus maximus muscle decreased because of previous external beam radiation. Copyrights for this figure were acquired through the Copyright Clearance Centre.

Another female patient was diagnosed with uterine leiomyosarcoma with peritoneal metastases in 2018, in her mid-50s [41]. After several palliative chemotherapy treatments, tumour progression was observed. Due to the presence of tumour-infiltrating lymphocytes and a lack of standard treatment options, off-label nivolumab treatment was initiated in February 2021. Four months later, the tumour growth rate was +36.5% per month (compared to +23.8% per month before nivolumab was started). To test the feasibility of PSMA-RLT, a PSMA PET/CT scan was performed, which showed PSMA uptake in lung, adrenal and peritoneal lesions. Two cycles of [^{177}Lu]Lu-PSMA-I&T with a two-month interval were administered in combination with nivolumab. Four months after the last dose, the tumour growth rate reduced to +11.3% per month. The lung lesion with the highest PSMA uptake (SUV_{max} = 8.9) showed a considerable size reduction. At this time, nivolumab treatment was stopped. The administered dose of [^{177}Lu]Lu-PSMA-I&T and its tumour retention time, however, were not described in this case report.

Future perspectives

To assess the feasibility of PSMA-targeted imaging and therapy in patients with sarcomas, it is important to know whether or not PSMA expression is seen in the tumour, and which factors determine effective PSMA-RLT. Previously published literature reveals that not all sarcomas, but definitely a subgroup of sarcomas show (strong) PSMA expression in their tumour-associated neovasculature, and (high) PSMA-tracer uptake on PET/CT imaging. These results provide a rationale that PSMA-RLT can be successful in a selection of sarcoma patients.

In prostatic tissue, PSMA expression is upregulated in case of malignant transformation and is further upregulated in more advanced stages of disease. Several studies show that PSMA expression is an independent prognostic factor in prostate cancer patients, associated with worse survival.16 Similar patterns can be observed in sarcomas. PSMA immunohistochemistry studies revealed that sarcoma types had significantly higher PSMA expression compared to tumour types with benign or intermediate biological potential [27]. Furthermore, in osteosarcomas, PSMA expression was significantly associated with tumour size, the presence of pulmonary metastasis and a worse 5 year survival rate [28]. In previously published case reports of PSMA-tracer uptake in sarcomas on PET/CT imaging, PSMA was able to differentiate between dedifferentiated liposarcoma and lipomatous regions [32] and between osteosarcoma and fibrous dysplasia [37], showing visibly higher PSMA-tracer uptake in the malignant lesions. Although not every case report has described SUV_{max} values, metastasized sarcomas seem to have more intense PSMA-tracer uptake compared to non-metastasized sarcomas [38, 40]. Lastly, one patient with metastasized leiomyosarcoma who received multiple PSMA PET/CT scans showed a noticeable increase in SUV_{max} values after one year of disease progression [40]. These observations suggest that if present, PSMA expression seems to be highest in patients with aggressive and/or advanced sarcomas, similar to prostate cancer. These are also the patient groups that are in highest need of new therapeutic options and could benefit the most from PSMA-RLT.

Currently, there is no evidence for an optimal selection method yet to determine whether a sarcoma patient is likely to respond to PSMA-RLT. The most evident option seems to select eligible patients by using PSMA immunohistochemistry on biopsy or resection material to identify tumours with high PSMA expression. In prostate cancer, the percentage of PSMA-expressing tumour cells correlates significantly with SUV_{max} on PSMA PET/CT imaging [47]. However, no studies

have investigated the correlation between PSMA immunohistochemistry and SUV_{max} on PSMA PET/CT imaging in sarcomas yet. Therefore, no definition of 'high PSMA expression', enough for significant PSMA-tracer uptake in sarcomas on PSMA PET/CT imaging, is available. As sarcomas have mainly shown PSMA expression on the neovascular endothelial cells instead of on the tumour cells itself (with some rare exceptions), the radiotracer uptake is expected to be less than in prostate carcinoma. However, the case reports of *e.g.* Militano et al., Can et al. and Jüptner et al. show that high SUV_{max} values over 12 can be reached in sarcomas, which generally is considered enough to explore the possibility of PSMA-RLT [48, 49]. Additionally, several case reports that describe ^{177}Lu-PSMA-RLT treatment in other non-prostatic malignancies with neovascular PSMA expression, such as glioblastoma multiforma, show that good tumour retention (in a lesion with a SUV_{max} of 10.3) and a significant reduction in tumour size and symptoms can be achieved, with acceptable toxicity [50, 51]. It is also important to consider that a direct correlation between PSMA-tracer uptake on PSMA PET/CT imaging and the intratumoural dose after PSMA-RLT is not self-evident, as the 6.0 GBq of [^{177}Lu]Lu-PSMA-617 that was administered to a patient with metastasized leiomyosarcoma, showed limited retention in the tumour 24 h after injection compared to the diagnostic ^{68}Ga-PSMA PET/CT scan that was obtained beforehand.

Although the currently available literature suggests that there might be a selection of sarcoma patients that could benefit from PSMA-RLT, it should be realized that this suggestion is based on a limited amount of patient studies and a few number of clinical cases. For a successful treatment with PSMA-RLT, two factors are of great importance: 1) the achieved intratumoural radioactive dose, which is influenced by multiple factors such as the total administered radiation dose, the tumoural reachability, the radiotracer's affinity towards PSMA, its retention time and the level of PSMA expression, and 2) the radiosensitivity of the tumour. Further investigation is necessary to gain knowledge about each of these aspects regarding sarcoma patients, *e.g.* by comparing different PSMA-targeting antibodies and tracers, evaluating their tumour dosimetry and assessing PSMA-RLT efficacy and toxicity profiles with different doses. However, first of all, more insight is needed into what tumour characteristics predict significant intratumoural binding of PSMA-ligands. For this, currently, one trial is recruiting patients to compare PSMA immunohistochemistry in biopsy material with, in case of high PSMA expression, PSMA-tracer accumulation on PET/CT imaging (clinicaltrials.gov, NCT05522257). Furthermore, in end-stage metastatic prostate cancer, ^{177}Lu- and ^{225}Ac-labelled PSMA have proven to prolong overall and progression-free

survival with acceptable toxicity [20, 21]. Recently, the first results on ^{89}Zr-labelled PSMA ligands in prostate cancer have been published, having the advantage of a longer half-life and allowing acquisition at later time points after injection [52, 53]. This might result in higher tumour-to-background ratios, better assessment of tumour retention and improved patient selection for PSMA-RLT compared to ^{18}F or ^{68}Ga. Developments in this field and the increasing knowledge about the role of PSMA in different neoplasms should be closely monitored and used for the rationale regarding PSMA-RLT in sarcoma patients as well.

CONCLUSION

Strong PSMA expression and good PSMA-tracer accumulation are observed in a selection of sarcoma patients, which seems to be more prevalent in aggressive and advanced sarcomas. These patient groups are also in highest need of new therapeutic options, as their 5-year survival rates are low. The results from previous literature have laid the foundation for further research that is needed to investigate the possible efficacy of PSMA-RLT in sarcomas and how eligible sarcoma patients could be selected in a reliable way. Hopefully, additional insights will be given by the currently recruiting prospective study (clinicaltrials.gov, NCT05522257).

REFERENCES

1. WHO Classification of Tumours Editorial Board. *WHO Classification of tumours of soft tissue and bone*. 5th edn. Lyon, France: IARC Press; 2020.
2. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in europe: report from the RARECARE project. *Eur J Cancer* 2013; 49: 684-95. https://doi.org/10.1016/j.ejca.2012.09.011
3. National Cancer Institute. SEER Cancer Stat Facts: Soft Tissue Cancer. Bethesda, MD. Available from: https://seer.cancer.gov/statfacts/html/soft.html
4. SEER Cancer Stat Facts: Bone and Joint Cancer. National Cancer Institute. Bethesda, MD [Available from: https://seer.cancer.gov/statfacts/html/bones.html.
5. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2021; 32: 1348-65. https://doi.org/10.1016/j.annonc.2021.07.006
6. Strauss SJ, Frezza AM, Abecassis N, Bajpai J, Bauer S, Biagini R, et al. Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN paedcan clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2021; 32: 36-1520. https://doi.org/10.1016/j.annonc.2021.08.1995
7. Sawamura C, Matsumoto S, Shimoji T, Okawa A, Ae K. How long should we follow patients with soft tissue sarcomas? *Clin Orthop Relat Res* 2014; 472: 842-48. https://doi.org/10.1007/s11999-013-3076-6
8. Smolle MA, Sande M van de, Callegaro D, Wunder J, Hayes A, Leitner L, et al. Individualizing follow-up strategies in high-grade soft tissue sarcoma with flexible parametric competing risk regression models. *Cancers (Basel)* 2019; 12(1): 47. https://doi.org/10.3390/cancers12010047
9. Rothermundt C, Whelan JS, Dileo P, Strauss SJ, Coleman J, Briggs TW, et al. What is the role of routine follow-up for localised limb soft tissue sarcomas? A retrospective analysis of 174 patients. *Br J Cancer* 2014; 110: 2420-26. https://doi.org/10.1038/bjc.2014.200
10. Smeland S, Bielack SS, Whelan J, Bernstein M, Hogendoorn P, Krailo MD, et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (european and American osteosarcoma study) cohort. *Eur J Cancer* 2019; 109: 36-50. https://doi.org/10.1016/j.ejca.2018.11.027
11. American Cancer Society. Cancer Facts & Figures 2017. Atlanta: American Cancer Society; 2017.

12. Van Glabbeke M, van Oosterom AT, Oosterhuis JW, Mouridsen H, Crowther D, Somers R, et al. Prognostic factors for the outcome of chemotherapy in advanced soft tissue sarcoma: an analysis of 2,185 patients treated with anthracycline-containing first-line regimens -- a European organization for research and treatment of cancer soft tissue and bone sarcoma group study. *J Clin Oncol* 1999; 17: 150-57. https://doi.org/10.1200/JCO.1999.17.1.150

13. Sleijfer S, Ouali M, van Glabbeke M, Krarup-Hansen A, Rodenhuis S, Le Cesne A, et al. Prognostic and predictive factors for outcome to first-line ifosfamide-containing chemotherapy for adult patients with advanced soft tissue sarcomas: an exploratory, retrospective analysis on large series from the European organization for research and treatment of Cancer-Soft tissue and bone sarcoma group (EORTC-STBSG). *Eur J Cancer* 2010; 46: 72-83. https://doi.org/10.1016/j.ejca.2009.09.022

14. Cancer Statistics Explorer Network SEER*Explorer. National Cancer Institute. Available from. Available from: https://seer.cancer.gov/statistics-network/explorer/application.html

15. Chang SS. Overview of prostate-specific membrane antigen. *Rev Urol* 2004; 6:S13-8.

16. Vlachostergios PJ, Niaz MJ, Sun M, Mosallaie SA, Thomas C, Christos PJ, et al. Prostate-specific membrane antigen uptake and survival in metastatic castration-resistant prostate cancer. *Front Oncol* 2021; 11:630589. https://doi.org/10.3389/fonc.2021.630589

17. Kaittanis C, Andreou C, Hieronymus H, Mao N, Foss CA, Eiber M, et al. Prostate-Specific membrane antigen cleavage of vitamin B9 stimulates oncogenic signaling through metabotropic glutamate receptors. *J Exp Med* 2018; 215: 159-75. https://doi.org/10.1084/jem.20171052

18. Bedolla RG, Wang Y, Asuncion A, Chamie K, Siddiqui S, Mudryj MM, et al. Nuclear versus cytoplasmic localization of filamin A in prostate cancer: immunohistochemical correlation with metastases. *Clin Cancer Res* 2009; 15: 788-96. https://doi.org/10.1158/1078-0432.CCR-08-1402

19. Fanti S, Goffin K, Hadaschik BA, Herrmann K, Maurer T, MacLennan S, et al. Consensus statements on PSMA PET/CT response assessment criteria in prostate cancer. *Eur J Nucl Med Mol Imaging* 2021; 48: 469-76. https://doi.org/10.1007/s00259-020-04934-4

20. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. *N Engl J Med* 2021; 385: 1091-1103. https://doi.org/10.1056/NEJMoa2107322

21. Sathekge M, Bruchertseifer F, Vorster M, Lawal IO, Knoesen O, Mahapane J, et al. Predictors of overall and disease-free survival in metastatic castration-resistant prostate cancer patients receiving 225ac-PSMA-617 radioligand therapy. *J Nucl Med* 2020; 61: 62–69. https://doi.org/10.2967/jnumed.119.229229

22. de Galiza Barbosa F, Queiroz MA, Nunes RF, Costa LB, Zaniboni EC, Marin JFG, et al. Nonprostatic diseases on PSMA PET imaging: a spectrum of benign and malignant findings. *Cancer Imaging* 2020; 20(1): 23. https://doi.org/10.1186/s40644-020-00300-7

23. O'Keefe DS, Su SL, Bacich DJ, Horiguchi Y, Luo Y, Powell CT, et al. Mapping, genomic organization and promoter analysis of the human prostate-specific membrane antigen gene. *Biochim Biophys Acta* 1998; 1443:113-27. https://doi.org/10.1016/s0167-4781(98)00200-0

24. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cbio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; 2: 401-4. https://doi.org/10.1158/2159-8290.CD-12-0095

25. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cbioportal. *Sci Signal* 2013; 6: 269. https://doi.org/10.1126/scisignal.2004088

26. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011; 12: 323. https://doi.org/10.1186/1471-2105-12-323

27. Heitkotter B, Trautmann M, Grunewald I, Bogemann M, Rahbar K, Gevensleben H, et al. Expression of PSMA in tumor neovasculature of high grade sarcomas including synovial sarcoma, rhabdomyosarcoma, undifferentiated sarcoma and MPNST. *Oncotarget* 2017;8: 4268-76. https://doi.org/10.18632/oncotarget.13994

28. Zeng C, Ke Z-F, Yang Z, Wang Z, Yang S-C, Luo C-Q, et al. Prostate-Specific membrane antigen: a new potential prognostic marker of osteosarcoma. *Med Oncol* 2012; 29: 2234-39. https://doi.org/10.1007/s12032-011-0089-4

29. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, Gaudin PB. Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res* 1999; 59: 3192–98.

30. Coskun SK, Gamsizkan M, Yalcınkaya U, Sungur MA. Stat3, VEGF, and PSMA expression patterns in malignant peripheral nerve sheath tumors, malignant melanomas, and glioblastomas: does staining percentage and intensity have an effect on survival? *J Environ Pathol Toxicol Oncol* 2022; 41: 45-53. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2021039719

31. Malik D, Mittal BR, Kumar R, Singh H, Bhattacharya A, Singh SK. Incidental detection of tracer avidity in liposarcoma on 68Ga-labeled prostate-specific membrane antigen PET/CT. *Clin Nucl Med* 2018; 43: e334-35. https://doi.org/10.1097/RLU.0000000000002189

32. Inanir S, Kesim S, Ergelen R, Tınay İ, Turkoz HK. 68Ga-PSMA PET/CT in giant retroperitoneal liposarcoma. *Clin Nucl Med* 2019; 44: e612-13. https://doi.org/10.1097/RLU.0000000000002762

33. Militano V, Afaq A, Bomanji J. 68Ga-prostate-specific membrane antigen PET/CT: incidental finding of a liposarcoma. *Clin Nucl Med* 2019; 44: e90-92. https://doi.org/10.1097/RLU.0000000000002389

34. Plouznikoff N, Woff E, Artigas C, Alexiou J, Flamen P. Incidental detection of a radiation-induced soft-tissue sarcoma on 68Ga-PSMA PET/CT in a patient previously treated for prostate cancer. *Clin Nucl Med* 2019; 44: e501-2. https://doi.org/10.1097/RLU.0000000000002592

35. Mathew B, Agrawal A, Purandare NC, Ramadwar M, Menon S, Bakshi G, et al. Incidental detection of pleomorphic sarcoma on 68Ga-PSMA PET/CT in a patient with prostate cancer. *Clin Nucl Med* 2020; 45:v e120-21. https://doi.org/10.1097/RLU.0000000000002760

36. Parihar AS, Sood A, Mittal BR, Kumar R, Singh H, Dhatt SS. 68Ga-PSMA-HBED-CC PET/CT and 18F-FDG PET/CT in Ewing sarcoma. *Clin Nucl Med* 2020; 45: e57-58. https://doi.org/10.1097/RLU.0000000000002764

37. Sasikumar A, Joy A, Pillai MRA, Alex TM, Narayanan G. 68Ga-PSMA PET/CT in osteosarcoma in fibrous dysplasia. *Clin Nucl Med* 2017; 42: 446-47. https://doi.org/10.1097/RLU.0000000000001646

38. Can C, Gundoğan C, Komek H. Is 68Ga-prostate-specific membrane antigen PET/CT superior than 18F-FDG PET/CT for evaluation of metastatic osteosarcoma? *Clin Nucl Med* 2021; 46: e233-35. https://doi.org/10.1097/RLU.0000000000003320

39. Marafi F, Esmail A, Usmani S. Prostate-Specific membrane antigen expression in metastatic angiosarcoma detected on 18F-PMSA PET/CT: a new potential prognostic marker. *Clin Nucl Med* 2022; 47: e147-48. https://doi.org/10.1097/RLU.0000000000003780

40. Juptner M, Marx M, Zuhayra M, Lutzen U. Experimental 177Lu-PSMA-617 radioligand therapy in a patient with extended metastasized leiomyosarcoma. *Nuklearmedizin* 2019; 58: 328-30. https://doi.org/10.1055/a-0914-2486

41. Digklia A, Boughdad S, Homicsko K, Dromain C, Trimech M, Dolcan A, et al. First on the efficacy of combined 177Lutetium-PSMA with immunotherapy outside prostatecancer. *J Immunother Cancer* 2022; 10: 10.

42. Chausse G, Laufer J, Abikhzer G, Probst S. Splenic hemangioma as a potential pitfall on PSMA-targeted 18F-DCFPyL PET/CT. *Clin Nucl Med* 2019; 44: 255-56. https://doi.org/10.1097/RLU.0000000000002428

43. Bhardwaj H, Stephens M, Bhatt M, Thomas PA. Prostate-Specific membrane antigen PET/CT findings for hepatic hemangioma. *Clin Nucl Med* 2016; 41: 968–69. https://doi.org/10.1097/RLU.0000000000001384

44. Jochumsen MR, Vendelbo MH, Hoyer S, Bouchelouche K. Subcutaneous lobular capillary hemangioma on 68Ga-PSMA PET/CT. *Clin Nucl Med* 2017; 42: e214-15. https://doi.org/10.1097/RLU.0000000000001542

45. Kanthan GL, Izard MA, Emmett L, Hsiao E, Schembri GP. Schwannoma showing avid uptake on 68Ga-PSMA-HBED-CC PET/CT. *Clin Nucl Med* 2016; 41: 703-4. https://doi.org/10.1097/RLU.0000000000001281

46. Gualberto RJC, Nister M, Castro P de, Arantes PBO. Schwannoma: a rare cause of false-positive 68Ga-PSMA PET/CT uptake in the evaluation of metastatic prostate cancer. *Urol Case Rep* 2022; 41: 101974. https://doi.org/10.1016/j.eucr.2021.101974

47. Ruschoff JH, Ferraro DA, Muehlematter UJ, Laudicella R, Hermanns T, Rodewald A-K, et al. What's behind 68Ga-PSMA-11 uptake in primary prostate cancer PET? investigation of histopathological parameters and immunohistochemical PSMA expression patterns. *Eur J Nucl Med Mol Imaging* 2021; 48: 4042-53. https://doi.org/10.1007/s00259-021-05501-1

48. Kratochwil C, Fendler WP, Eiber M, Baum R, Bozkurt MF, Czernin J, et al. EANM procedure guidelines for radionuclide therapy with 177Lu-labelled PSMA-ligands (177Lu-PSMA-RLT). *Eur J Nucl Med Mol Imaging* 2019; 46: 2536-44. https://doi.org/10.1007/s00259-019-04485-3

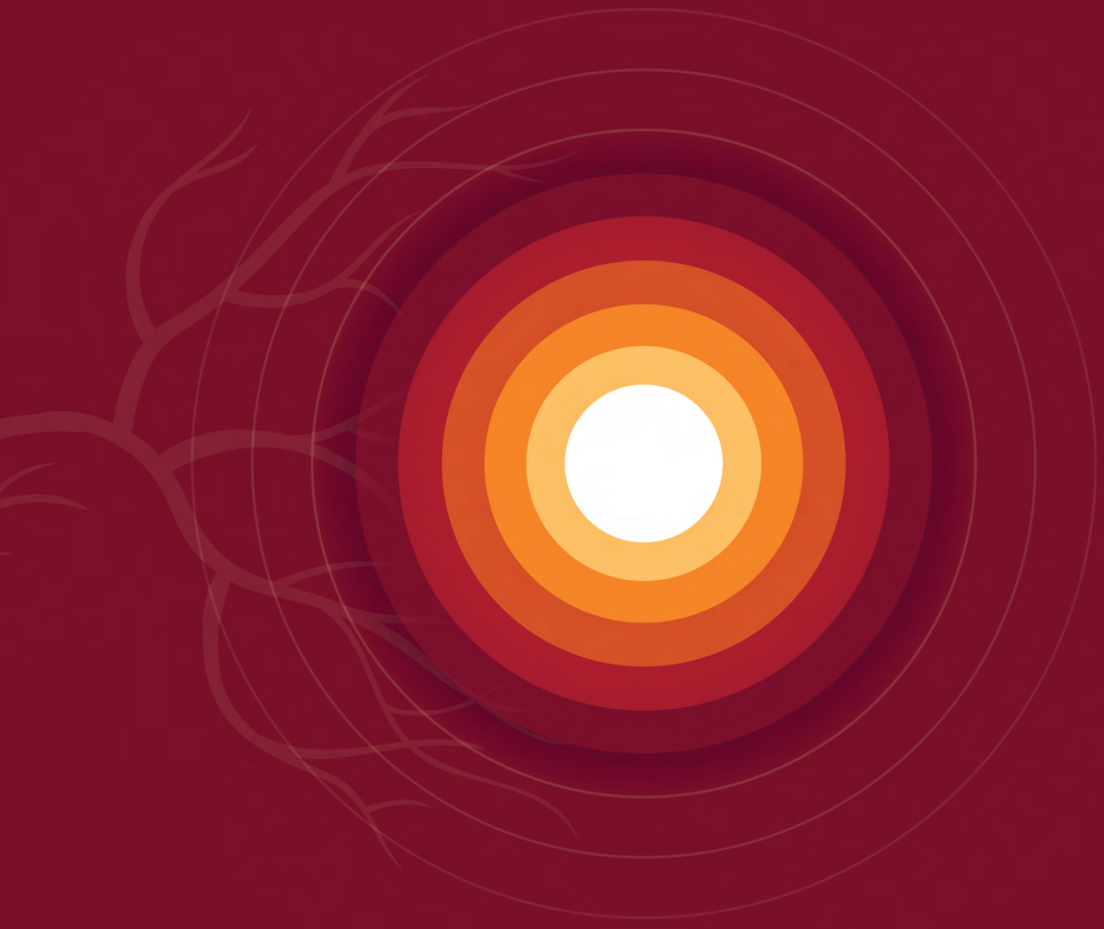
49. Jansen BHE, Kramer GM, Cysouw MCF, Yaqub MM, deB, Lavalaye J, et al. Healthy tissue uptake of (68)Ga-prostate-specific membrane antigen, (18)F-DCFPyL, (18)F-fluoromethylcholine, and (18)F-dihydrotestosterone. *J Nucl Med Mol Imaging* 2019; 60: 7-1111.

50. Kunikowska J, Charzyńska I, Kuliński R, Pawlak D, Maurin M, Krolicki L. Tumor uptake in glioblastoma multiforme after IV injection of [177Lu]Lu-PSMA-617. *Eur J Nucl Med Mol Imaging* 2020; 47: 1605-6. https://doi.org/10.1007/s00259-020-04715-z

51. Kumar A, Ballal S, Yadav MP, ArunRaj ST, Haresh KP, Gupta S, et al. 177Lu-/68Ga-PSMA theranostics in recurrent glioblastoma multiforme: proof of concept. *Clin Nucl Med* 2020; 45: e512-13. https://doi.org/10.1097/RLU.0000000000003142

52. Prive BM, Derks YHW, Rosar F, Franssen GM, Peters SMB, Khreish F, et al. 89Zr-labeled PSMA ligands for pharmacokinetic PET imaging and dosimetry of PSMA-617 and PSMA-I&T: a preclinical evaluation and first in man. *Eur J Nucl Med Mol Imaging* 2022; 49: 2064-76. https://doi.org/10.1007/s00259-021-05661-0

53. Rosar F, Bartholoma M, Maus S, Prive BM, Khreish F, Franssen GM, et al. 89Zr-PSMA-617 PET/CT may reveal local recurrence of prostate cancer unidentified by 68Ga-PSMA-11 PET/CT. *Clin Nucl Med* 2022; 47: 435-36. https://doi.org/10.1097/RLU.0000000000004108



CHAPTER 7

PSMA expression and PSMA PET/CT imaging in metastatic soft tissue sarcoma patients, results of a prospective study

Authors
Fleur Kleiburg, Tom van der Hulle, Hans Gelderblom, Marije Slingerland, Frank Speetjens, Luuk Hawinkels, Petra Dibbets-Schneider, Floris van Velden, Martin Pool, Suk Wai Lam, Judith Bovée, Linda Heijmen, Lioe-Fee de Geus-Oei

Published
European Journal of Nuclear Medicine and Molecular Imaging
2025 Aug;52(10):3690-3699
DOI: 10.1007/s00259-025-07224-z

Supplementary materials
https://doi.org/10.1007/s00259-025-07224-z

ABSTRACT

Background

Prostate-specific membrane antigen (PSMA) expression has been observed in a subset of soft tissue sarcomas, mainly in the neovascular endothelial cells. This feasibility study aimed to evaluate PSMA expression and PSMA PET/CT imaging in metastatic soft tissue sarcoma, providing important insights for potential future exploration of PSMA-targeted radioligand therapy.

Methods

This prospective single-center study included adult patients with metastatic soft tissue sarcoma, with measurable disease (lesion diameter > 1 cm), available biopsy/resection material, ECOG/WHO performance status of 0–2 and either no prior systemic treatment, progressive disease during/after treatment, or stable disease/partial response with the last dose > 8 weeks prior. Immunohistochemical PSMA staining was performed on previously obtained biopsy or resection material. In case of high PSMA expression, a [^{18}F]-JK-PSMA-7 PET/CT scan evaluated tracer uptake, with adequate uptake defined as SUV_{max} > 8.

Results

Of 25 included patients, 11 (44%) had high PSMA expression: 4/11 leiomyosarcomas, 3/4 dedifferentiated liposarcomas, 2/5 undifferentiated pleomorphic sarcomas, 1/2 myxofibrosarcomas and 1/1 malignant peripheral nerve sheath tumour. Five of 11 patients agreed to a [^{18}F]-JK-PSMA-7 PET/CT, of which 3 had lesions that showed adequate tracer uptake (SUV_{max} 16.7–10.7). However, uptake across all metastatic lesions was highly heterogeneous (median SUV_{max} = 3.8; range –0.5 16.7), indicating that these patients are unlikely to benefit sufficiently from PSMA-targeted therapy. The study was therefore terminated prematurely.

Conclusions

PSMA expression and PSMA tracer uptake in metastatic soft tissue sarcoma were highly heterogeneous. A deeper understanding of PSMA biology and improved patient selection criteria are essential for future application of PSMA-targeted radioligand therapy in this disease.

Trial registration: clinicaltrials.gov, NCT05522257. Registered 31-08-2022.

INTRODUCTION

Soft tissue sarcomas represent a heterogeneous group of rare malignancies arising from mesenchymal tissues, with an incidence of approximately 5 cases per 100,000 individuals annually [1]. It can develop in many different anatomical locations and over 70 histological subtypes have been identified [2]. Due to its diverse and heterogeneous origin, it can manifest with a wide range of clinical presentations and variable responses to treatment, making the diagnosis and management of soft tissue sarcomas challenging. For metastatic soft tissue sarcomas, standard of care consists of cytotoxic chemotherapy, with doxorubicin being the first choice for the majority of soft tissue sarcoma subtypes [3]. However, approximately 14% of patients respond to doxorubicin treatment [3, 4], and the five-year survival rate for metastatic soft tissue sarcoma is only 17% [5]. This highlights the need to explore new effective treatment options to improve patient outcomes.

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein best known for its upregulated expression in the epithelium of prostate cancer cells, where it serves as an important target for molecular imaging (PSMA-targeted PET/CT scans [6]) and radioligand therapy (e.g. [^{177}Lu]Lu-PSMA-617 and [^{225}Ac]Ac-PSMA-617 [7, 8]). Interestingly, studies have demonstrated PSMA expression in the tumour-associated neovascular endothelial cells of various other malignancies, including soft tissue sarcomas [9]. This was first described in 2017 by Heitkötter et al., who analysed 779 sarcoma samples and reported strong neovascular PSMA expression in various sarcoma entities, such as pleomorphic rhabdomyosarcoma (40% of samples) and synovial sarcoma (38% of samples) [10]. Malignant soft tissue tumours showed evidently higher PSMA expression compared to tumours with benign and intermediate biological potential. Multiple case reports have confirmed PSMA tracer uptake in patients with soft tissue sarcoma, with uptake ranging from mild to intense in e.g. liposarcoma, undifferentiated pleomorphic sarcoma, angiosarcoma and leiomyosarcoma [11]. Notably, high PSMA tracer uptake has been observed particularly in aggressive histological subtypes and metastatic disease, with maximum standardised uptake values (SUV_{max}) of up to 17 [12–14]. In one case, PSMA tracer uptake differentiated dedifferentiated liposarcoma from lipomatous regions [15], while in another case, tracer uptake increased with progression of metastatic leiomyosarcoma [13]. These findings suggest that PSMA expression, if present, becomes more pronounced in more advanced soft tissue sarcomas. Therefore, we hypothesised that effective PSMA-ligand binding may be achievable in selected patients with PSMA-positive

metastatic soft tissue sarcoma, potentially enabling PSMA-targeted radioligand therapy (PSMA-RLT) in this patient population most in need of new treatment options. However, no prospective studies have been performed yet to investigate this potential.

The aim of this prospective feasibility study was to investigate immunohistochemical PSMA expression in metastatic soft tissue sarcomas and to evaluate tracer uptake on PSMA PET/CT imaging in patients with confirmed PSMA-expressing soft tissue sarcomas. This will provide important insights for potential future exploration of PSMA theranostics in soft tissue sarcomas.

METHODS

Study design

The study was a single-center, open-label, feasibility study in patients with metastatic soft tissue sarcomas, conducted at the Leiden University Medical Center (Leiden, The Netherlands). It was a non-randomized, non-blinded study to assess the level of PSMA expression in biopsy or resection material from soft tissue sarcomas and, in case of confirmed high PSMA expression, to assess the amount of tumoural PSMA-tracer binding on a [^{18}F]-JK-PSMA-7 PET/CT scan (in short: PSMA PET/CT scan). This study was approved by the Medical Ethics Committee Leiden The Hague Delft and was registered on clinicaltrials.gov (NCT05522257, registry date 31-08-2022).

Eligibility criteria

The inclusion criteria were: (1) diagnosis of metastatic (nodal or distant) soft tissue sarcoma; (2) age ≥ 18 years at the time of written informed consent; (3) recent (< 8 weeks) standard imaging (with CT or [^{18}F]FDG PET/CT) with measurable disease (lesion diameter > 1 cm); (4) biopsy or resection available of the primary tumour and/or metastasis; (5) ECOG/WHO performance status of 0–2; (6) either no previous systemic therapy for advanced soft tissue sarcoma, or, previous systemic therapy for advanced soft tissue sarcoma with progression of disease during or after discontinuation of systemic therapy, or, previous systemic therapy for advanced soft tissue sarcoma with partial response or stable disease where the last dose of systemic therapy was given > 8 weeks before.

The exclusion criteria were: (1) women who were pregnant and/or lactating; (2) medical or psychiatric conditions that compromised the patient's ability to give informed consent; (3) known hypersensitivity to drugs comparative to [^{18}F]-JK-PSMA-7, any of their excipients or to any component of [^{18}F]-JK-PSMA-7; (4) inability to undergo PET/CT scanning, e.g. claustrophobia, body weight higher than the weight limit of the scanner or inability to tolerate lying down for the duration of a PET/CT scan.

Study procedures

In all eligible patients, immunohistochemical PSMA staining was performed on formalin-fixed paraffin-embedded slides of biopsy or resection material that was obtained as part of standard clinical practice. Immunohistochemistry was performed with the anti-PSMA antibody (clone: D7I8E) (Cell Signalling, Danvers, MA, USA) at a dilution of 1:40. The EnVision detection system was used and all steps were performed on the DAKO Omnis (Agilent Technologies, Santa Clara, CA, USA). Antigen retrieval was carried out with a low pH, followed by incubation with the primary antibody for 27.5 min with the addition of a rabbit linker (10 min incubation time). PSMA expression levels were assessed by one pathologist and categorised into no expression, low expression or high expression as defined by Heitkötter et al. [10]. High PSMA expression was defined as moderate staining intensity in > 5% of the neovasculature or the presence of any strong staining.

An intravenous injection with a fixed dose of 359 ± 36 MBq [^{18}F]-JK-PSMA-7 was administered 90 min before the PET/CT scan was acquired [16]. Images were obtained using the Philips Vereos (Philips Healthcare, Best, The Netherlands) or the Omni Legend 32 cm PET/CT scanner (GE Healthcare, Chicago, Illinois, United States of America), located at the Leiden University Medical Center. The scan range was from crown to mid-thigh, or from crown to toe in case the primary tumour was located in the lower extremities, and acquisition was carried out in supine position. All PET images underwent iterative reconstruction, compliant with the EARL1 harmonization criteria to ensure comparable SUVs [17]. All reported lesions on standard imaging were assessed on PSMA PET/CT and a 3-dimensional volume of interest was inserted around each lesion with a diameter > 1 cm. Thereafter, SUV_{max} values were extracted to quantify PSMA tracer uptake. As PSMA uptake is generally underestimated in smaller lesions due to the partial-volume effect [18], no quantification was performed in lesions < 1 cm.

Study objectives

The primary study objective was to determine the number of patients in which a SUV_{max} higher than 8 was reached. The cut-off of 8 was chosen to identify patients that might benefit from PSMA-RLT in the future. The *EANM procedure guidelines for radionuclide therapy with 177Lu-labelled PSMA-ligands* state that there is no consensus yet on the definition of "adequate" uptake for therapy in prostate cancer patients, but suggest to use the definition from the LuPSMA trial: $SUV_{max, tumour} > 1.5 * SUV_{mean, liver}$ [19, 20]. However, reference-organ variability between different PSMA tracers becomes an issue here. The LuPSMA trial used [^{68}Ga]Ga-PSMA-11 PET/CT scans, on which healthy liver tissue has an average SUV_{mean} of 4.8, while the uptake in healthy liver tissue on [^{18}F]-JK-PSMA-7 is more than twice as high [16, 21]. In order to still have a comparable threshold, we chose an absolute threshold of $SUV_{max} > 8$ (at least 1.5 * 4.8) to investigate which lesions might have adequate uptake for potential treatment. In this feasibility study we aimed to perform a PSMA PET/CT scan in 15 patients. The protocol stated that the study would be terminated early if a total of 5 PSMA PET/CT scans showed no adequate tracer uptake in all or the majority of metastases.

Statistical analysis

As this was a feasibility study, study analyses were performed to derive preliminary results that may provide insights for future research. Descriptive statistics were used to describe the study outcomes. IBM SPSS Statistics (version 25 or higher) was used to derive these descriptive statistics.

RESULTS

Patient characteristics

A total of 25 patients were included for immunohistochemical PSMA staining. Their characteristics are described in Table 1. Seven different histological soft tissue sarcoma entities were seen, of which leiomyosarcoma was the most common (44%). At diagnosis, 5 patients (20%) had metastatic disease and the majority had FNCLCC (Fédération Nationale des Centres de Lutte Contre le Cancer) grade 2 (48%). The median time from diagnosis to study inclusion was 18 months (range 0–143 months). Two patients had received systemic therapy before study inclusion; both had progressive disease during or after previous systemic therapy. The other patients had not received any systemic therapy.

Table 1. Patient characteristics (n = 25). *FNCLCC (Fédération Nationale des Centres de Lutte Contre Le Cancer) grade unknown in 8 patients

Characteristic	Value	
Age in years, median (range)	68	(33–84)
Sex, n (%)		
Male	15	
Female	10	(40%)
FNCLCC grade at diagnosis, n (%)*		
1	1	(4%)
2	12	(48%)
3	4	(16%)
Histological type, n (%)		
Leiomyosarcoma	11	(44%)
Dedifferentiated liposarcoma	5	(20%)
Undifferentiated pleomorphic sarcoma	4	(16%)
Myxofibrosarcoma	2	(8%)
Malignant peripheral nerve sheath tumour	1	(4%)
Sclerosing epithelioid fibrosarcoma	1	(4%)
Dermatofibrosarcoma protuberans	1	(4%)

PSMA expression levels

High PSMA expression was seen in 11 patients (44%); 4/11 leiomyosarcomas, 2/5 dedifferentiated liposarcomas, 3/4 undifferentiated pleomorphic sarcomas, 1/2 myxofibrosarcoma and 1/1 malignant peripheral nerve sheath tumour (MPNST), see Table 2. Low PSMA expression was seen in 7 patients (28%); 4/11 leiomyosarcomas, 2/5 dedifferentiated liposarcomas and 1/2 myxofibrosarcomas. No PSMA expression was seen in 7 patients (28%); 3/11 leiomyosarcomas, 1/5 dedifferentiated liposarcomas, 1/4 undifferentiated pleomorphic sarcomas, 1/1 sclerosing epithelioid fibrosarcoma and 1/1 dermatofibrosarcoma protuberans. In four patients, both neovascular and cellular PSMA expression were observed; two leiomyosarcomas (one with high and one with low PSMA expression), one dedifferentiated liposarcoma (with low PSMA expression) and one MPNST (with high PSMA expression). The other patients showed only neovascular PSMA expression.

Table 2. Number of patients with high PSMA expression per histological subtype of soft tissue sarcoma

Histological subtype	High PSMA expression	
Leiomyosarcoma	4/11	(36%)
Dedifferentiated liposarcoma	2/5	(40%)
Undifferentiated pleomorphic sarcoma	3/4	(75%)
Myxofibrosarcoma	1/2	(50%)
Malignant peripheral nerve sheath tumour	1/1	(100%)
Sclerosing epithelioid fibrosarcoma	0/1	(0%)
Dermatofibrosarcoma protuberans	0/1	(0%)

PSMA PET/CT scans

Of the eleven patients with high PSMA expression, five agreed to undergo a PSMA PET/CT scan. The other six either did not want to participate (*n* = 3), or were not asked due to rapid clinical deterioration (*n* = 3). The immunohistochemical PSMA expression and PSMA PET/CT images of scanned patients can be seen in Figures 1 and 2. Two patients were scanned with the Philips Vereos PET/CT scanner and three with the Omni Legend 32 cm PET/CT scanner. The patient characteristics, including details on PSMA expression and PSMA tracer uptake, are displayed in Table 3. In four patients immunohistochemical PSMA staining was performed on material from the primary tumour, and in one patient it was performed on resection material from a lung metastasis. In this patient, the primary tumour, which had been resected nine years earlier, was also assessed and showed no PSMA expression. An SUV_{max} > 8 was reached in three out of five patients; patient 1 had SUV_{max} = 16.7, patient 2 had SUV_{max} = 11.2 and patient 3 had SUV_{max} 10.7. Additionally, patient 4 showed moderate tracer uptake with an SUV_{max} = 6.0, and patient 5 showed no visual tracer uptake above the background. When analysing all lesions individually, high heterogeneity in tracer uptake was observed, see Figure 3. Of 41 analysed lesions, 5 lesions (12%) had SUV_{max} > 8 and the median SUV_{max} of all lesions was 3.8 (range 0.5–16.7). Due to the heterogeneity, the included patients were not considered suitable for potential PSMA-targeted radioligand monotherapy and therefore, even though sufficient tracer uptake was seen in some lesions, the study was stopped early.

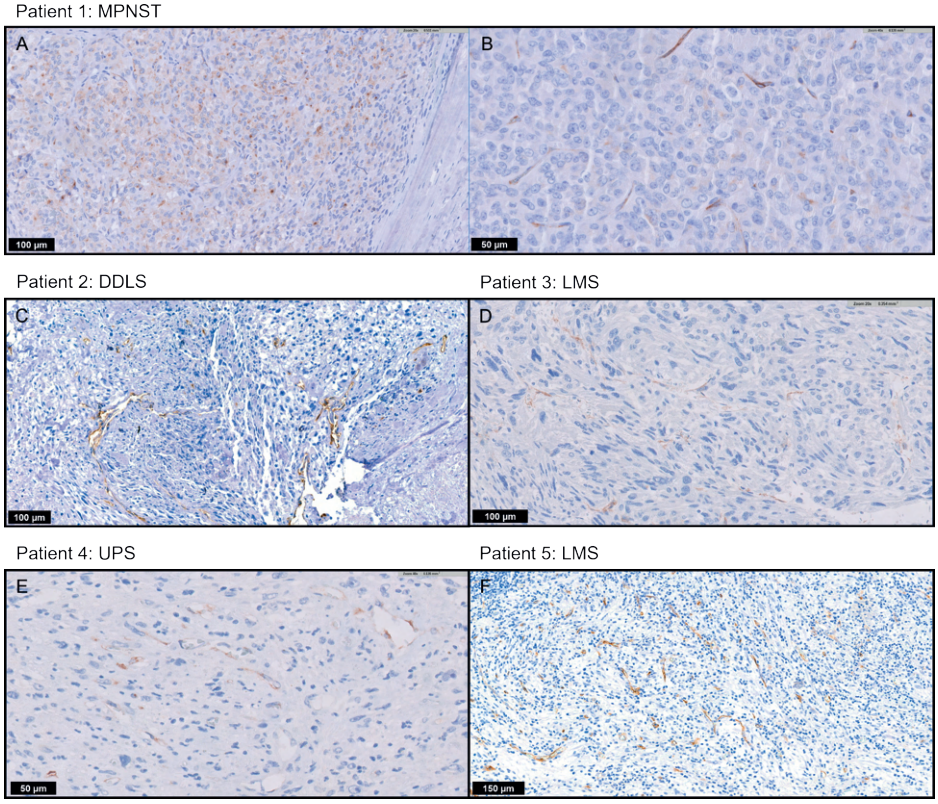


Figure 1. Immunohistochemical PSMA expression of the five patients that underwent PSMA PET/CT imaging. MPNST = malignant peripheral nerve sheath tumour, DDLS = dedifferentiated liposarcoma, LMS = leiomyosarcoma, UPS = undifferentiated pleomorphic sarcoma. Patient 1 had both cellular PSMA expression in approximately 30% of tumour cells (A) and focal neovascular PSMA expression (B). Patients 2–5 had neovascular PSMA expression in > 5% of blood vessels (C-F)

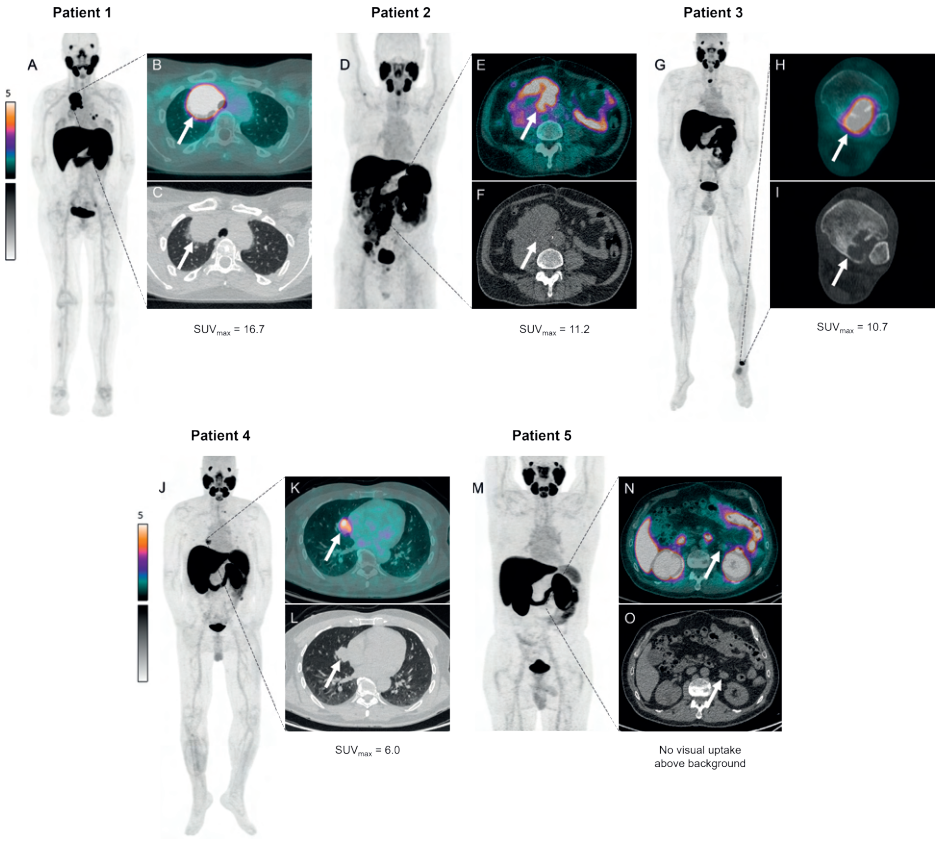


Figure 2. PSMA PET/CT images of the five scanned patients. White arrows indicate either the lesion with the highest SUV_{max} (patients 1 to 4, SUV_{max} = 6.0–16.7), or the largest lesions (patient 5, lesion caudal to left renal vein, no visual uptake above the background). A, D, G, J, M: maximum intensity projection (MIP) images. B, E, H, K, N: fused PET EARL1 and low-dose CT images. C, F, I, L, O: low-dose CT images

Table 3. Patient and disease characteristics, PSMA expression results and PSMA PET/CT results of the five scanned patients. None of these patients had received prior systemic treatment for soft tissue sarcoma

Patient number	Age	Sex	Initial diagnosis	Assessed biopsy/ resection material	Interval biopsy to inclusion	Localisation of PSMA expression	Lesions with SUV_{max} > 8 on PSMA PET/CT scan	Total number of lesions > 1 cm
1	33	F	Malignant peripheral nerve sheath tumour in right distal nervus tibialis, T1N0M0	Resection primary tumour	18 months	Cellular expression (~ 30%) and focal neovascular expression	Mediastinum: SUV_{max} = 16.7 Right hilum: SUV_{max} = 12.7 Subcarinal: SUV_{max} = 10.0	7
2	71	M	Dedifferentiated liposarcoma in right funiculus, T2N1M0	Biopsy primary tumour	2 months	Neovascular strong expression (~ 20%)	Retroperitoneal: SUV_{max} = 11.2	16
3	57	M	Leiomyosarcoma in left medial malleolus, T1N0M0	Resection lung metastasis left superior lobe*	6 months	Neovascular moderate expression (~ 20%)	Left tibia: SUV_{max} = 10.7	9
4	57	M	Undifferentiated pleomorphic sarcoma in right tibia, T1N0M0	Resection primary tumour	6 months	Neovascular moderate to strong expression (~ 40%)	None	6
5	61	M	Leiomyosarcoma in left adrenal gland, T2M0M0	Resection primary tumour	24 months	Neovascular moderate expression (~ 5%), a few vessels with strong expression	None	3

* 9 years after initial diagnosis. In this patient, immunohistochemical PSMA staining was also done on the resection specimen of the primary tumour (from 9 years earlier), which showed no PSMA expression.

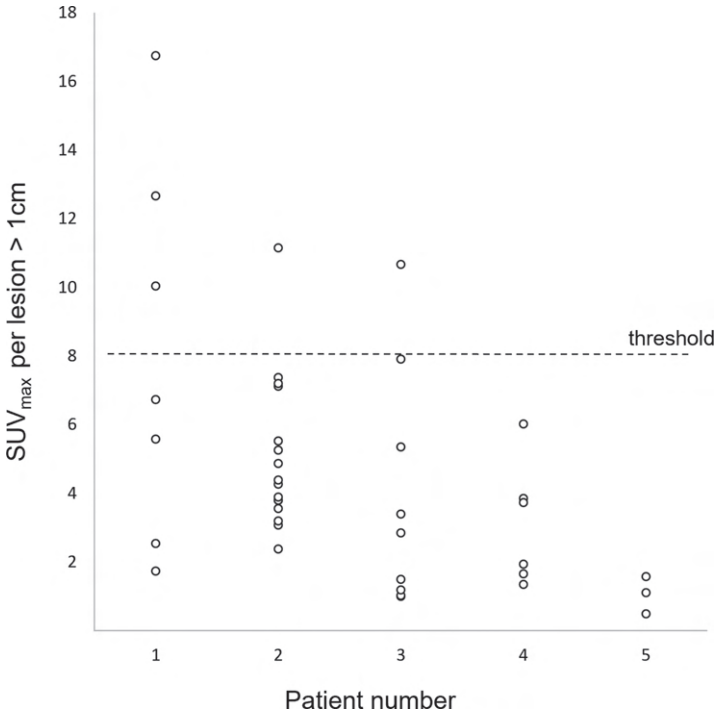


Figure 3. SUV_{max} values per lesion with a diameter > 1 cm. Five lesions had a SUV_{max} above the study threshold of 8. Immunohistochemical PSMA expression was done on resected lesions in all five patients, making these lesions unavailable for evaluation on PSMA PET/CT

DISCUSSION

This study presents the first prospective data on PSMA expression and PSMA PET/CT imaging in patients with metastatic soft tissue sarcoma. PSMA expression was observed in a substantial proportion of patients, with variations across histological subtypes. PSMA PET/CT imaging showed adequate tracer binding in select lesions, however, within the patients in this study the tracer uptake was considered too heterogeneous for potential PSMA-RLT.

Of 25 included patients, representing seven different soft tissue sarcoma entities, 18 (72%) demonstrated PSMA-positive biopsy results, of which 11 (44%) showed high PSMA expression. These cases included leiomyosarcoma, dedifferentiated liposarcoma, undifferentiated pleomorphic sarcoma, myxofibrosarcoma and malignant peripheral nerve sheath tumour. Although our cohorts were small, the

percentages of high PSMA expression in this study were higher than those reported by Heitkötter et al. [10], who applied the same definition. In their analysis of 779 tissue samples from a broad range of soft tissue and bone tumours, including 599 malignant tumours, they found PSMA expression in 20% of malignant tumours, with high PSMA expression in 7%. The difference in high PSMA expression rate was also evident within specific histological subtypes, for instance, 36% (4/11) of leiomyosarcomas in our study had high PSMA expression, compared to 11% (7/66) of leiomyosarcomas in the cohort of Heitkötter et al. Based on the association between PSMA expression and tumour aggressiveness, also within sarcomas [11], we hypothesise that the increased rates of high PSMA expression in this study stem from the inclusion criteria, which selected only patients who had developed metastatic soft tissue sarcoma. This finding is particularly relevant as these are the patients most in need of new treatment options. Interestingly, in one scanned patient a lung metastasis showed high PSMA expression, whereas the primary tumour resected nine years earlier had showed no PSMA expression, supporting the idea that PSMA expression can increase as the disease progresses. Furthermore, we observed a higher rate of cellular PSMA expression (4/25) compared to Heitkötter et al. (1/599), which may have implications for the feasibility of PSMA-RLT, as tumour cell expression could enhance treatment efficacy.

Five patients with high PSMA expression underwent PSMA PET/CT imaging, of which three met the criterium of SUV_{max} > 8. This indicates that adequate tracer binding may be achieved in over half of patients with high PSMA expression. However, PSMA uptake across all lesions was heterogeneous, with SUV_{max} values ranging from 0.5 to 16.7 (median 3.8) in lesions with a diameter of > 1 cm. Consequently, PSMA-RLT was not considered a feasible option for these patients and the study was stopped early. The decision to stop the study early was based on the pre-specified criteria and ethical considerations, as continuing with the methods and selection criteria of this study was unlikely to yield positive results given the observed heterogeneity. No research has yet investigated the underlying causes of heterogeneity in PSMA uptake within soft tissue sarcoma patients. We hypothesise that this variability may be due the evolution of different cancer subclones, each with varying levels of aggressiveness and PSMA expression. Other potential factors include differences in tumour microenvironment and in neovascularisation, which may promote PSMA expression in different ways, particularly as soft tissue sarcomas can occur in a wide variety of anatomical locations. In addition, two sarcoma patients treated with PSMA-RLT have been described in previous literature, both with metastatic leiomyosarcoma and

heterogeneous PSMA uptake. In one case, the patient received a single dose of [^{177}Lu]Lu-PSMA-617 (6.0 GBq), but treatment was discontinued due to poor radiotracer fixation in metastases observed on a whole body scan [13]. In the second case, two cycles of [^{177}Lu]Lu-PSMA-I&T (dose unspecified) were combined with nivolumab, and post-treatment SPECT/CT showed marked uptake in lung metastases. However, treatment was stopped due to progressive disease [14]. These cases support the fact that there are still hurdles to overcome for potential PSMA-targeted treatment in sarcoma patients.

When considering PSMA-RLT in sarcoma patients, but also non-prostate tumours in general, several challenges must be addressed. Firstly, unlike prostate cancer where PSMA is expressed directly on tumour cells, PSMA is mostly restricted to neovascular endothelial cells in sarcoma and other non-prostate tumours [22]. Although the beta particle range of ^{177}Lu-PSMA (approximately 1–2 mm) enables irradiation of surrounding tumour cells, the lower density of neovascular endothelial cells compared to tumour cells reduces available targets in the tumour. Secondly, PSMA expression and tracer uptake are often highly heterogeneous across lesions within the same patient, resulting in uneven radiation dose distribution. Thirdly, studies suggest that PSMA tracers in tumours with neovascular PSMA expression may exhibit faster washout, further diminishing the radiation dose. Fourthly, prior systemic treatments may have influenced PSMA expression, as some therapies have been reported to modulate PSMA levels. Lastly, the radiosensitivity of the targeted tumour has to be taken into account. Within soft tissue sarcoma cell lines there is a broad spectrum of radiosensitivities [23], meaning that some histological subtypes need higher absorbed radiation doses compared to others to achieve effective treatment. These factors collectively highlight the need for good understanding of PSMA biology and well-considered patient selection to reach potential effective PSMA-RLT.

Despite these challenges, some non-prostate cancers have shown successful response to ^{177}Lu-PSMA treatment. For example, three glioblastoma patients achieved good tumour radiation doses, tumour shrinkage and improvement in performance status [24]. Similarly, multiple patients with adenoid cystic carcinoma achieved good clinical responses, such as pain reduction and symptom relief [24]. These results may be explained by the fact that glioblastoma is one of the most vascularised tumours, and that adenoid cystic carcinoma is known to be one of the few non-prostate tumours that expresses PSMA on tumour cells rather than its neovascular endothelial cells. These examples highlight the importance of patient

selection strategies that consider tumour vascularisation and cellular PSMA expression patterns. However, the current literature is limited, and publication bias must be considered.

This study has several limitations. As this was an exploratory feasibility study with a small sample size, it was not possible to test for significant differences or associations, and no robust conclusions could be drawn. The diversity of the included soft tissue sarcoma subtypes added further complexity to comparisons, a common challenge in sarcoma research due to the wide variety of histological subtypes in combination with the rarity of tumours. Also, patients were selected based on immunohistochemical PSMA staining of biopsy or resection samples that were already previously obtained in a clinical setting. Sampling errors or long intervals between biopsy or resection and the development of metastases may have resulted in missed cases of high PSMA expression. In all scanned patients the immunohistochemical PSMA staining was performed on resected lesions, so the direct correlation between PSMA expression and PSMA tracer uptake could not be evaluated. Additionally, PSMA expression levels were assessed by one pathologist without a second reader, introducing potential variability, and patients were scanned at a single time point, so tracer retention time and absorbed radiation doses could not be assessed. Even though two different PET/CT scanners were used in this study, comparability of PET quantification was maximised by using EARL harmonisation. This standardisation helps to reduce potential variability in image quantification, thus ensuring reliable comparisons. No relevant differences in tumoural tracer accumulation are to be expected between [^{18}F]-JK-PSMA-7, the tracer used in this study, and other commonly used PSMA tracers such as [^{68}Ga]Ga-PSMA-11 [25].

For future studies investigating PSMA-targeted imaging and treatment in (soft tissue) sarcomas, it is crucial to focus on refining patient selection criteria to optimise identification of potentially eligible patients for PSMA-RLT without missing patients, while minimising unnecessary scans with no or insufficient tracer uptake and thus unnecessary patient burden and costs. For that, a broader cohort of patients has to be analysed. Also, limiting the time interval between tissue sampling and study inclusion may better capture relevant PSMA expression. To ensure consistency in immunohistochemical PSMA expression results, in future studies the assessment of inter-observer variability between pathologists, or automated quantification should be considered. To ensure consistent quantification of PSMA uptake across different scanners and studies, continued attention should be paid

to PET harmonisation guidelines. A deeper understanding of PSMA biology is important for advancing the potential application of PSMA-RLT. Areas of interest include mechanisms that promote PSMA expression, both neovascular and cellular, factors underlying the observed heterogeneity in tracer uptake, and the influence of prior systemic treatments on PSMA expression. Additionally, the effect of neovascular PSMA expression on PSMA ligand binding and retention has to be analysed. Hopefully, the results of study NCT05420727, which aims to investigate the effect of [^{177}Lu]Lu-PSMA-I&T in soft tissue sarcoma patients, will provide additional insights. In the future, perhaps exploring different theranostic targets simultaneously, such as PSMA and fibroblast activation protein inhibitors (FAPI), may lead to a more personalised theranostic approach for sarcoma patients.

CONCLUSIONS

Although 44% of included patients exhibited high PSMA expression, and three out of five scanned patient had metastatic lesions with sufficient PSMA tracer uptake (SUV_{max} 10.7–16.7), the uptake across all lesions was deemed too heterogeneous to achieve adequate radiation doses. Consequently, this limits the potential for future effective radioligand treatment. To make PSMA-targeted radioligand treatment viable for patients with metastatic soft tissue sarcoma, a deeper understanding of PSMA biology and improved patient selection criteria are crucial. Larger trials are needed to further build on these preliminary results and explore potential strategies to improve patient selection and PSMA ligand binding. If current challenges can be overcome, PSMA theranostics may still hold promise for selected sarcoma patients.

REFERENCES

1. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. Eur J Cancer. 2013;49:684– 95. https://doi.org/10.1016/j.ejca.2012.09.011.
2. WHO Classification of Tumours Editorial Board. WHO Classification of tumours of soft tissue and bone. 5th ed. Lyon, France: IARC; 2020.
3. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up(). Ann Oncol. 2021;32:1348–65. https://doi.org/10.1016/j.annonc.2021.07.006.
4. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol. 2014;15:415–23. https://doi.org/10.1016/S1470-2045(14)70063-4.
5. SEER Cancer Stat Facts. Soft Tissue Cancer. National Cancer Institute. Bethesda MD.
6. Fendler WP, Eiber M, Beheshti M, Bomanji J, Calais J, Ceci F, et al. PSMA PET/CT: joint EANM procedure guideline/SNMMI procedure standard for prostate cancer imaging 2.0. Eur J Nucl Med Mol Imaging. 2023;50:1466–86. https://doi.org/10.1007/s00259-022-06089-w.
7. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177-PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2021;385:1091–103. https://doi.org/10.1056/NEJMoa2107322.
8. Sathekge M, Bruchertseifer F, Vorster M, Lawal IO, Knoesen O, Mahapane J, et al. Predictors of Overall and Disease-Free Survival in Metastatic Castration-Resistant Prostate Cancer Patients Receiving (225)Ac-PSMA-617 Radioligand Therapy. J Nucl Med. 2020;61:62–9. https://doi.org/10.2967/jnumed.119.229229.
9. de Galiza Barbosa F, Queiroz MA, Nunes RF, Costa LB, Zaniboni EC, Marin JFG, et al. Nonprostatic diseases on PSMA PET imaging: a spectrum of benign and malignant findings. Cancer Imaging. 2020;20:23. https://doi.org/10.1186/s40644-020-00300-7.
10. Heitkotter B, Trautmann M, Grunewald I, Bogemann M, Rahbar K, Gevensleben H, et al. Expression of PSMA in tumor neovasculature of high grade sarcomas including synovial sarcoma, rhabdomyosarcoma, undifferentiated sarcoma and MPNST. Oncotarget. 2017;8:4268–76. https://doi.org/10.18632/oncotarget.13994.
11. Kleiburg F, Heijmen L, Gelderblom H, Kielbasa SM, Bovee JV, De Geus-Oei LF. Prostate-specific membrane antigen (PSMA) as a potential target for molecular imaging and treatment in bone and soft tissue sarcomas. Br J Radiol. 2023;96:20220886. https://doi.org/10.1259/bjr.20220886.

12. Militano V, Afaq A, Bomanji J. 68Ga-Prostate-Specific Membrane Antigen PET/CT: Incidental Finding of a Liposarcoma. Clin Nucl Med. 2019;44:e90–2. https://doi.org/10.1097/RLU.0000000000002389.

13. Juptner M, Marx M, Zuhayra M, Lutzen U. Experimental 177Lu- PSMA-617 radioligand therapy in a patient with extended metastasized leiomyosarcoma. Nuklearmedizin. 2019;58:328–30. https://doi.org/10.1055/a-0914-2486.

14. Digklia A, Boughdad S, Homicsko K, Dromain C, Trimech M, Dolcan A, et al. First communication on the efficacy of combined < sup > 177 Lutetium-PSMA with immunotherapy outside prostate cancer. J Immunother Cancer. 2022;10. https://doi.org/10.1136/jitc-2022-005383.

15. Inanir S, Kesim S, Ergelen R, Tinay I, Turkoz HK. 68Ga-PSMA PET/CT in Giant Retroperitoneal Liposarcoma. Clin Nucl Med. 2019;44:e612–3. https://doi.org/10.1097/RLU.0000000000002762.

16. Hohberg M, Kobe C, Krapf P, Tager P, Hammes J, Dietlein F, et al. Biodistribution and radiation dosimetry of [(18)F]-JK-PSMA-7 as a novel prostate-specific membrane antigen-specific ligand for PET/CT imaging of prostate cancer. EJNMMI Res. 2019;9:66. https://doi.org/10.1186/s13550-019-0540 -7.

17. Boellaard R, O'Doherty MJ, Weber WA, Mottaghy FM, Lonsdale MN, Stroobants SG, et al. FDG PET and PET/CT: EANM procedure guidelines for tumour PET imaging: version 1.0. Eur J Nucl Med Mol Imaging. 2010;37:181–200. https://doi.org/10.1007/s00259-009-1297-4.

18. Soret M, Bacharach SL, Buvat I. Partial-volume effect in PET tumor imaging. J Nucl Med. 2007;48:932–45. https://doi.org/10.2967/jnumed.106.035774.

19. Kratochwil C, Fendler WP, Eiber M, Baum R, Bozkurt MF, Czernin J, et al. EANM procedure guidelines for radionuclide therapy with (177)Lu-labelled PSMA-ligands ((177)Lu-PSMA-RLT). Eur J Nucl Med Mol Imaging. 2019;46:2536–44. https://doi.org/10.1007/s00259-019-04485-3.

20. Hofman MS, Violet J, Hicks RJ, Ferdinandus J, Thang SP, Akhurst T, et al. [(177)Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): a single-centre, single-arm, phase 2 study. Lancet Oncol. 2018;19:825–33. https://doi.org/10.1016/S1470-2045(18)30198-0.

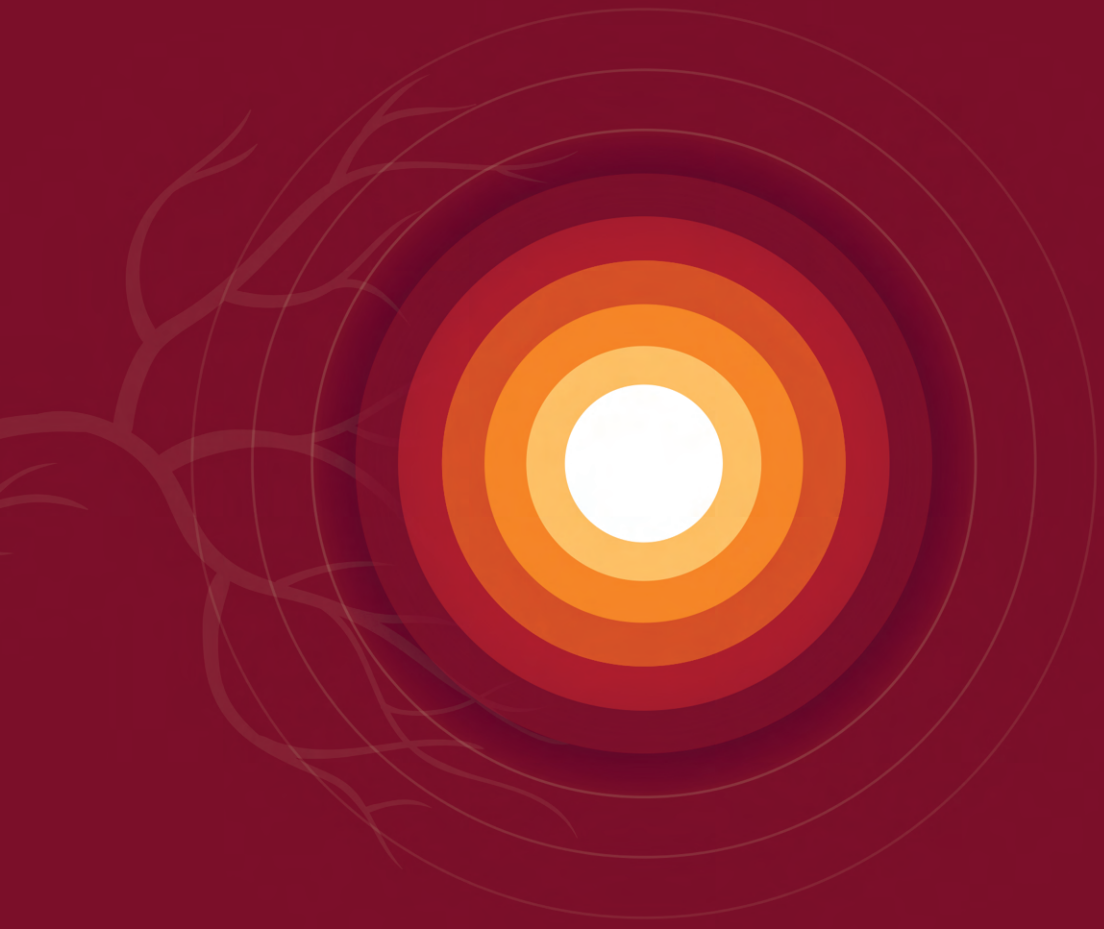
21. Jansen BHE, Kramer GM, Cysouw MCF, Yaqub MM, de Keizer B, Lavalaye J, et al. Healthy Tissue Uptake of (68)Ga-Prostate- Specific Membrane Antigen, (18)F-DCFPyL, (18)F-Fluoromethylcholine, and (18)F-Dihydrotestosterone. J Nucl Med. 2019;60:1111–7. https://doi.org/10.2967/jnumed.118.222505.

22. Uijen MJM, Derks YHW, Merkx RIJ, Schilham MGM, Roosen J, Prive BM, et al. PSMA radioligand therapy for solid tumors other than prostate cancer: background, opportunities, challenges, and first clinical reports. Eur J Nucl Med Mol Imaging. 2021;48:4350–68. https://doi.org/10.1007/s00259-021-05433-w.

23. Haas RL, Floot BGJ, Scholten AN, van der Graaf WTA, van Houdt W, Schrage Y, et al. Cellular Radiosensitivity of Soft Tissue Sarcoma. Radiat Res. 2021;196:23–30. https://doi.org/10.1667/RADE-20-00226.1.

24. Wang JH, Kiess AP. PSMA-targeted therapy for non-prostate cancers. Front Oncol. 2023;13:1220586. https://doi.org/10.3389/fonc.2023.1220586.

25. Dietlein F, Hohberg M, Kobe C, Zlatopolskiy BD, Krapf P, Endepols H, et al. An (18) F-Labeled PSMA Ligand for PET/CT of Prostate Cancer: First-in-Humans Observational Study and Clinical Experience with (18)F-JK-PSMA-7 During the First Year of Application. J Nucl Med. 2020;61:202–9. https://doi.org/10.2967/jnumed.119.229542



CHAPTER 8

Prostate-specific membrane antigen targeted PET/CT imaging in patients with colon, gastric and pancreatic cancer

Authors
Floris Vuijk, Fleur Kleiburg, Wyanne Noortman, Linda Heijmen, Shirin Feshtali Shahbazi, Floris van Velden, Victor Baart, Shadhvi Bhairosingh, Bert Windhorst, Luuk Hawinkels, Petra Dibbets-Schneider, Neanke Bouwman, Stijn Crobach, Arantza Fariña-Sarasqueta, Andreas Marinelli, Daniela Oprea-Lager, Rutger-Jan Swijnenburg, Frist Smit, Alexander Vahrmeijer, Lioe-Fee de Geus-Oei, Denise Hilling, Marije Slingerland

Published
Cancers
2022 Dec;14(24):6209
DOI: 10.3390/cancers14246209

Supplementary materials
https://doi.org/10.3390/cancers14246209

ABSTRACT

Background

Current imaging modalities frequently misjudge disease stage in colorectal, gastric and pancreatic cancer. As treatment decisions are dependent on disease stage, incorrect staging has serious consequences. Previous preclinical research and case reports indicate that prostate-specific membrane antigen (PSMA)-targeted PET/CT imaging might provide a solution to some of these challenges. This prospective clinical study aims to assess the feasibility of [^{18}F]DCFPyL PET/CT imaging to target and visualize primary colon, gastric and pancreatic cancer.

Methods

In this prospective clinical trial, patients with colon, gastric and pancreatic cancer were included and underwent both [^{18}F]DCFPyL and [^{18}F]FDG PET/CT scans prior to surgical resection or (for gastric cancer) neoadjuvant therapy. Semiquantitative analysis of immunohistochemical PSMA staining was performed on the surgical resection specimens, and the results were correlated to imaging parameters.

Results

The results of this study demonstrate detection of the primary tumour by [^{18}F] DCFPyL PET/CT in 7 out of 10 patients with colon, gastric and pancreatic cancer, with a mean tumour-to-blood pool ratio (TBR) of 3.3 and mean SUV_{max} of 3.6. However, due to the high surrounding uptake, visual distinction of these tumours was difficult, and the SUV_{max} and TBR on [^{18}F]FDG PET/CT were significantly higher than on [^{18}F]DCFPyL PET/CT. In addition, no correlation between PSMA expression in the resection specimen and SUV_{max} on [^{18}F]DCFPyL PET/CT was found.

Conclusions

The detection of several gastrointestinal cancers using [^{18}F]DCFPyL PET/CT is feasible. However, low tumour expression and high uptake physiologically in organs/background hamper the clear distinction of the tumour. As a result, [^{18}F] FDG PET/CT was superior in detecting colon, gastric and pancreatic cancers.

INTRODUCTION

Gastrointestinal cancers are among the most prevalent cancers worldwide, with colorectal cancer being the third, gastric cancer the fifth and pancreatic cancer the twelfth most common type of cancer, respectively [1]. Currently, the diagnostic workup of suspected gastrointestinal tumours includes a combination of endoscopy, computed tomography (CT), magnetic resonance imaging (MRI), [^{18}F]FDG positron emission tomography–computed tomography (PET/CT), ultrasound and even diagnostic laparoscopy, depending on the tumour type. Curative treatment for all three cancers still consists of surgical resection of the primary tumour and, if indicated, chemo(radio)therapy [2].

Although these imaging modalities are frequently used in the clinic, they lack sensitivity or specificity in specific diagnostic entities, leading to over- or undertreatment. In colon cancer, for example, imaging modalities (e.g., CT) are currently insufficient in determining nodal stage. As a result, early colorectal cancers with low risk for lymph node metastases (10–15%) might currently undergo unnecessary oncologic bowel resection, while in the majority of these patients (85–90%), local treatment would suffice. In gastric cancer, the sensitivity of CT to detect distant and peritoneal metastasis is 14–65% and 22–33%, respectively [3–5]. Recent results from the PLASTIC trial indicated a high detection rate for the primary tumour of 79%; however, it also found the limited additional value of [^{18}F]FDG PET/CT in gastric cancer staging [6]. Especially for signet cell, mucinous and poorly differentiated gastric carcinomas, [^{18}F]FDG PET/CT is difficult, as they tend to be less metabolically active [7]. Even more complicating is the physiological uptake of [^{18}F]FDG in the stomach wall, frequently masking the primary tumour. This results in an underestimation of the tumour stage, from which incorrect treatment choices are made. Finally, in pancreatic cancer, as much as 13% of Whipple procedures are currently being performed for benign disease [8]. Additionally, a high rate of early recurrence after resection is seen (28%) [9], indicating the presence of micro-metastases at the time of resection. Possibly, molecular imaging such as PET/CT could provide information on tumour biology.

Prostate-specific membrane antigen (PSMA)-targeted PET/CT imaging might provide a solution to some of these challenges. PSMA is a metallopeptidase that is expressed by prostate cells. Increased expression is found in prostate carcinoma, making it a well-established target for molecular imaging. PSMA-targeted PET/

CT imaging has quickly evolved in the past few years and is now being adopted into the standard-of-care in the primary staging and follow-up of prostate cancer.

Recently, PSMA expression was also reported in other cancer types, including colorectal, gastric and pancreatic cancer [10,11]. PSMA expression is found on the endothelium of newly formed vasculature, which is essential for nutrient supply in all cancers. By immunohistochemical analysis, approximately 85% of colorectal cancer, 66% of gastric cancer and 84% of pancreatic cancer patients demonstrated expression of PSMA in capillaries within the tumour bed, which can be selectively targeted by [^{18}F]DCFPyL [10,11]. In addition, our group demonstrated sustained PSMA expression after neoadjuvant treatment in pancreatic cancer using immunohistochemistry analysis [12]. Three case reports in patients with synchronous prostate cancer and colorectal, gastric, or pancreatic cancer suggested the feasibility of PSMA-targeted PET/CT for detection of the primary tumour and/or its metastases [10,13–15]. Recently, a larger study including 19 pancreatic cancer patients demonstrated positive uptake in 18 of these, and allowed for the distinction of malignant from benign pancreatic lesions, with a sensitivity and specificity of 84.2% and 90.5%, respectively [16]. Aside from being a target for molecular imaging, PSMA could also serve as a target for theranostics [17] ([^{177}Lu]Lu-PSMA, [^{225}Ac]Ac-PSMA).

As a first step towards the clinical use of PSMA-targeted imaging in non-prostate cancer, this feasibility study aimed to assess the feasibility of using [^{18}F]DCFPyL PET/CT imaging to target and visualize primary colon, gastric and pancreatic cancer.

METHODS

Patient population

This is a bi-centre, non-randomized prospective clinical trial. Patients admitted to the Leiden University Medical Center (Leiden, The Netherlands) and Haaglanden Medical Centrum (HMC, The Hague, The Netherlands), and diagnosed with (histologically proven) T3-4N0-2M0-1 colon, T3-4N0-2M0-1 gastric, or pancreatic cancer, were included. No sample size calculation was possible due to the exploratory nature of this study. Gastric cancer patients received neoadjuvant therapy before surgery, consisting of 4 courses of fluorouracil, leucovorin, oxaliplatin and docetaxel. The other patients (colon and pancreatic cancer)

underwent surgery without prior therapy. Clinical and pathological data were obtained from medical records. No follow-up was performed. The study was conducted in concordance with the Declaration of Helsinki, and the laws and regulations of the Netherlands. The study was approved by a certified medical ethics review board (Leiden Den Haag Delft) and the local review board of the HMC. All subjects provided written informed consent prior to any study-related activities. The study was registered in the Netherlands Trial Register (NL-8919). The goal was to include 30 patients. An early stopping rule was implemented in case interim analyses after 10 patients showed lower tumour accumulation on [^{18}F]DCFPyL PET/CT than on [^{18}F]FDG PET/CT (significant difference in average SUV_{max} [^{18}F]FDG and [^{18}F]DCFPyL).

Data acquisition and image reconstruction

As part of this trial, patients underwent both [^{18}F]DCFPyL and [^{18}F]FDG PET/CT prior to surgery (colon and pancreatic cancer patients) or start of neoadjuvant therapy (gastric cancer patients). There were ≥24 h between scans. [^{18}F]DCFPyL was chosen due to its favourable renal clearance. All PET/CT scans were acquired on a Vereos digital PET/CT scanner (Philips Healthcare, Best, The Netherlands), except one single [^{18}F]DCFPyL PET/CT scan that was acquired on a GE Discovery MI 5-Ring digital PET/CT scanner (GE, Boston, MA, USA) (the other scan from this patient was acquired on the Vereos scanner). Both PET systems are EARL-accredited. Patients underwent a low-dose CT scan (120 kV, 35 mA_{eff}) for attenuation correction purposes prior to the PET scan. Patients received an average dose of 198.9 ± 38.4 MBq [^{18}F]DCFPyL and were scanned after an average of 120.8 ± 5.7 min post-injection [18,19]. [^{18}F]FDG was dosed using the quadratic formula with a factor of 379 MBq·min·bed^{-1}·kg^{-2}, resulting in an average dose of 155.8 ± 93.5 MBq [^{18}F]FDG, and patients were scanned 63.4 ± 10.6 min post-injection. Before [^{18}F]FDG PET/CT, patients fasted for 6 h and were prehydrated with 1 L of water. A blood glucose threshold of <11.0 mmol/L was set for patients undergoing [^{18}F]FDG PET/CT. For both scans, a PET scan of the abdomen was performed in the case of colon or pancreatic cancer, and a PET scan of the abdomen to skull base was performed in the case of gastric cancer. As the detection of distant metastases or staging was not the primary aim of this study, only partial body scans were performed to minimize radiation exposure. All scans were acquired for a duration of 5 min per bed position. [^{18}F]DCFPyL and [^{18}F]FDG PET/CT images were reconstructed in accordance with EANM guidelines for tumour [^{18}F]FDG PET imaging version 2.0 with a 4 mm^3 voxel size [20].

Quantitative image analysis

PET/CT analysis was performed by two experienced, board-certified nuclear medicine physicians (L.G., L.H.) using Sectra IDS7 software (version 21.2; Sectra AB, Linköping, Sweden). The volumes of interest (VOI) were delineated using LIFEx (version 6.30; Inserm, Orsay, France) [21]. Various lesional body-weighted standardized uptake values (SUV), i.e., maximum (SUV_{max}), minimum (SUV_{min}), mean (SUV_{mean}) and peak (SUV_{peak}), as well as volumetric parameters tumour volume (TV_{DCFPyL} for [^{18}F]DCFPyL or MTV for [^{18}F]FDG) and total lesion uptake (TL_{DCFPyL} for [^{18}F]DCFPyL or TLG for [^{18}F]FDG), defined as SUV_{mean} × tumour volume), were extracted for all patients from both scans [22]. TV_{DCFPyL}, $TL_{DCFPyL,}$ MTV and TLG were determined with an isocontour set at 45% of the maximum uptake for [^{18}F]DCFPyL PET/CT scans [22] and 50% of the maximum uptake for [^{18}F]FDG PET/CT scans [20]. Uptake on both PET/CTs was considered positive when the $SUV_{max} \geq 2.5$. Tumours were considered detectable on PET/CT imaging when a tumour-to-blood pool ratio (TBR) ≥ 2 was observed. The blood pool was delineated using a 3 × 3 pixel region of interest (ROI) in the descending aorta (the ascending aorta was not in the field of view in colon or pancreatic cancer patients) on 5 consecutive slices of the CT scan, yielding the blood pool activity used for the calculation of TBR [23]. TBR was determined by dividing the SUV_{peak} of the tumour by the SUV_{peak} of the aortic blood pool.

Immunohistochemistry

PSMA expression in the resection specimens (after neoadjuvant therapy in gastric cancer) was visualized using immunohistochemistry on formalin-fixed paraffin-embedded tumour tissue sections (4 µm). Endoglin was used as the gold standard for identifying activated endothelial cells [24]. After deparaffinization in xylene and rehydration, endogenous peroxidase activity was blocked with 0.3% H_2O_2 (20 min). Antigen retrieval was performed by boiling slides in Tris-EDTA buffer (pH 9.0) for PSMA and citrate buffer (pH 6.0) for endoglin at 95 °C (10 min), followed by overnight incubation with the primary antibodies (mouse anti-PSMA (Dako, Clone 3E6, no. N1611, 1.64 µg/mL), or goat anti-endoglin (R&D systems, BAF1097, 1.0 µg/mL)). Next, slides were incubated for 30 min at room temperature with the secondary antibodies (anti-mouse, anti-goat (Envision, Dako, Glostrup, Denmark)). Lastly, immunoreactions were visualized using 3,3'diaminobenzidine substrate buffer (Dako, Glostrup, Denmark) and counterstained using hematoxylin. Placental tissue was used as a positive control for endoglin staining, and prostate cancer tissue was used as positive control for PSMA staining. Negative controls were included in the experiments.

The evaluation of PSMA expression was performed by an experienced, board-certified gastrointestinal pathologist (S.C.) using the semi-quantitative H-score [25,26]. This resulted in a score ranging of 0–300 and considered both staining intensity (0–3) as well as the percentage (0–100%) of target cells stained. The endoglin staining was used as the gold standard (100% staining) for neo-angiogenesis (pre-existing vasculature was excluded from the analyses by visual identification). Higher scores indicate more PSMA expression.

Statistical analysis

Statistical analysis and figure editing were performed using SPSS (version 25; IBM SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (version 8; GraphPad Software, Inc., San Diego, CA, USA). Due to the small sample size, all data are displayed as mean ± standard deviation. Imaging parameters of patients between [^{18}F]DCFPyL and [^{18}F]FDG PET/CT were compared using the independent samples *t*-test. The correlation between [^{18}F]DCFPyL SUV_{max} and H-score was evaluated using a logistic regression analysis, and displayed as the r^2 and concurrent *p*-value. A *p*-value < 0.05 was considered significant.

RESULTS

Ten patients were included in this clinical trial in the period from August 2020 until May 2021. After the interim analysis of 10 patients, low [^{18}F]DCFPyL SUV_{max} values in primary tumours compared to surrounding organs were seen in all but one patient (in contrast to high [^{18}F]FDG SUV_{max} values), and the study was prematurely terminated. Six women and four men were included, who were on average 65.3 ± 11.9 years old. All patients underwent both [^{18}F]DCFPyL and [^{18}F]FDG PET/CT, except one (patient 5) who did not undergo the [^{18}F]FDG PET/CT, as this was not part of standard-of-care diagnostics (cT2-3 gastric carcinoma). Of the 10 included patients, 4 patients were diagnosed with colon cancer, 3 with gastric cancer, and 3 with pancreatic cancer. Two patients had a well-differentiated adenocarcinoma, three were scored as well/moderate, two as moderate and three as poor. Patient characteristics are further depicted in Table 1.

Quantitative analysis of PET/CT scans

Of the nine [^{18}F]FDG PET/CT scans, 100% demonstrated positive uptake (SUV_{max} ≥ 2.5) with a mean SUV_{max} of 14.9 ± 14.5; 25.4 ± 17.0 for colon cancer, 6.1 ± 2.4 for gastric cancer and 6.8 ± 3.3 for pancreatic cancer. Of the 10 [^{18}F]DCFPyL PET/

CT scans, 6 (60%) demonstrated positive expression with a mean SUV_{max} of 3.6 ± 2.5; 4.2 ± 3.9 for colon cancer, 2.7 ± 0.7 for gastric cancer and 3.6 ± 1.4 for pancreatic cancer. Examples of colon, gastric and pancreatic cancer scans are displayed in Figure 1, Figure 2 and Figure 3, respectively. The primary tumour was detectable (TBR ≥2) on 6 out of 9 (67%) [^{18}F]FDG PET/CT scans (3/4 colon, 1/2 gastric, 2/3 pancreatic tumours) and on 7 out of 10 (70%) [^{18}F]DCFPyL PET/CT scans (3/4 colon, 1/3 gastric, 3/3 pancreatic tumours). The mean TBR on [^{18}F]FDG PET/CT was 13.0 ± 8.0 for colon cancer, 2.3 ± 0.9 for gastric cancer and 3.2 ± 1.6 for pancreatic cancer.

The mean TBR on [^{18}F]DCFPyL was 3.3 ± 2.7 for colon cancer, 1.9 ± 0.5 for gastric cancer and 2.3 ± 0.5 for pancreatic cancer. For all patients except one (patient 1), volumetric PET/CT-derived parameters could not be extracted due to the relatively low tumour uptake of [^{18}F]DCFPyL and the high uptake in surrounding tissue. The SUV_{max} and TBR on [^{18}F]FDG were significantly higher compared to [^{18}F]DCFPyL (p = 0.028 and p = 0.049, respectively). Although the primary metastatic sites were included in the field of view of the scans, no previously unknown lesions were found on [^{18}F]DCFPyL or [^{18}F]FDG PET/CT. Figure 4 shows maximal intensity projections of both [^{18}F]FDG and [^{18}F]DCFPyL PET/CT scans, indicating the much more intense uptake of [^{18}F]FDG compared to [^{18}F]DCFPyL. In one patient (patient 1), additional parameters could be extracted from both [^{18}F]DCFPyL and [^{18}F]FDG PET/CT. When comparing the [^{18}F]DCFPyL to [^{18}F]FDG PET/CT for this patient, the SUV_{max} was 9.9 versus 45.5, SUV_{mean} was 6.4 versus 28.4, SUV_{min} was 4.5 versus 22.8, SUV_{peak} was 8.4 versus 41.0, TBR was 7.3 versus 20.4, TV_{DCFPyL} was 13.6 cm^3 versus MTV 59.4 cm^3, and TL_{DCFPyL} was 87.6 versus TLG 1686.1, as displayed in Table 2.

Table 1. Overview of patient characteristics.

No Figure	Age	Tumour Location	Tumour Differentiation	cTNM Stage *	pTNM Stage	Max Diameter (mm) **	SUV_{max} [^{18}F] DCFPyL	SUV_{max} [^{18}F]FDG	TBR [^{18}F] DCFPyL	TBR [^{18}F]FDG	H-Score
1	72	Colon adenocarcinoma	Well/moderate	cT3/4N1M0	pT3N0M0	180	9.9	45.5	7.3	20.4	120
2	68	Colon adenocarcinoma	Well/moderate	cT4N2M0	pT4N0M0	80	1.9	29.1	2.3	15.6	225
3	73	Colon adenocarcinoma	Poor	cT4N0M0	pT4N0M0	50	3.3	22.5	2.4	14.1	60
4	58	Colon adenocarcinoma	Well/moderate	cTxN0M0	pT4N0M0	15	1.5	4.5	1.2	1.7	80
5	38	Signet ring cell gastric carcinoma	Poor	cT2-3N0M0	ypT3N0M0	42	3.5	n.a.	1.9	n.a.	0
6	71	Tubular gastric adenocarcinoma	Moderate	cT4N1M0	ypT3N1M0	25	2.5	7.8	2.3	2.9	150
7	50	Tubular gastric adenocarcinoma	Poor	cT3N0M0	ypT4N1M0	45	2.1	4.4	1.4	1.6	0
8	70	PDAC	Well	cTxN0M0	pT2N1M0	22	3.3	3.6	2.0	1.3	150
9	76	PDAC	Moderate	cTxN0M0	pT2N1M0	28	2.4	6.8	2.0	4.3	30
10	63	PDAC	Well	cTxN2M0	pT2N2M0	35	5.1	10.1	2.8	3.9	0

Abbreviations: TNM stage, tumour, nodal and metastatic status; SUV, standardized uptake value; n.a., not available; H-score, immunohistochemical staining score; PDAC, pancreatic ductal adenocarcinoma. * Pathological TNM stage for colon and pancreatic cancer patients, initial clinical TNM stage for gastric cancer patients (as neoadjuvant therapy was given after [^{18}F]DCFPyL PET/CT). ** Diameter measured at pathological examination.

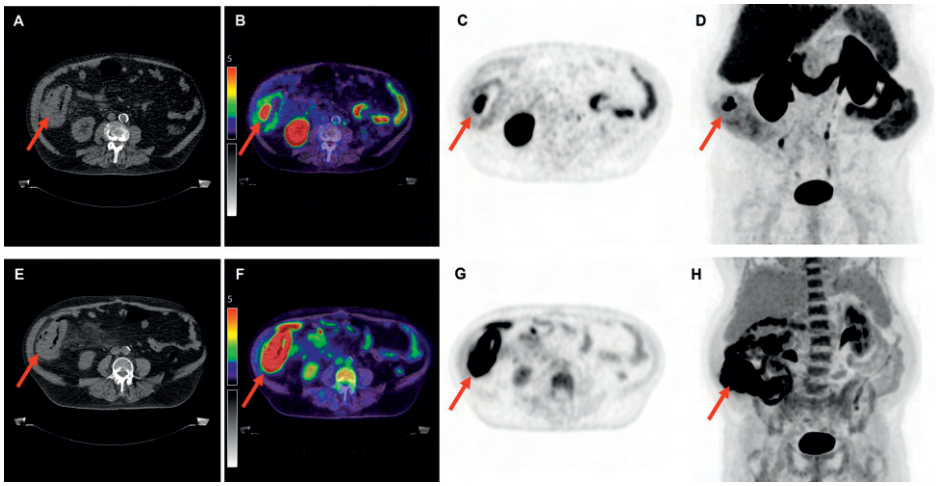


Figure 1. Overview of imaging modalities of a patient with pT3N0M0 colon carcinoma (patient 1). The arrows indicate (upper row) a lesion with intense [^{18}F]DCFPyL expression with an SUV_{max} of 9.9 and (bottom row) a lesion with [^{18}F]FDG uptake with an SUV_{max} of 45.5. From left to right: low-dose CT (A,E), fused PET/CT (B,F), PET (C,G), and the maximal intensity projection (MIP, (D,H)). Image scale SUV 0-5.

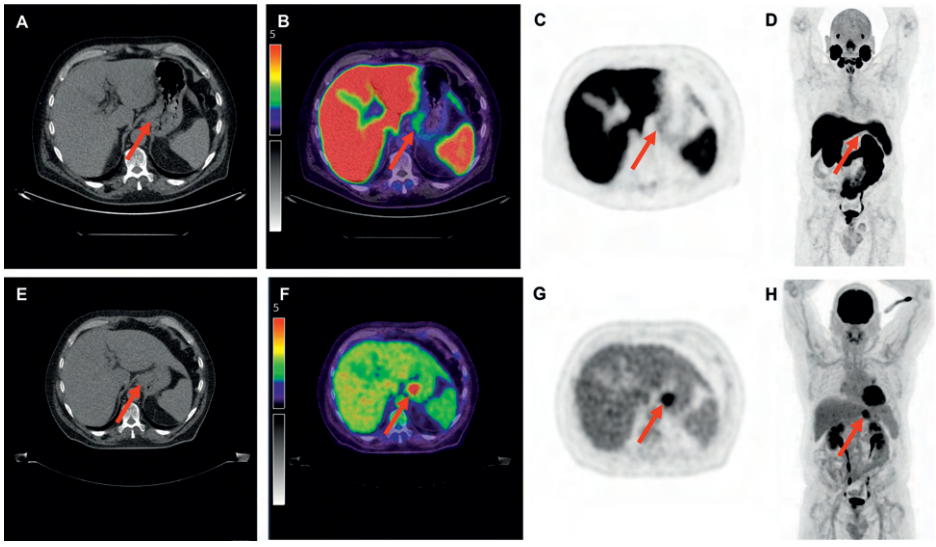


Figure 2. Overview of imaging modalities of a patient with cT4N1M0 tubular gastric carcinoma (patient 6). The arrows indicate (upper row) a lesion with light [^{18}F]DCFPyL expression with an SUV_{max} of 2.5 and (bottom row) a lesion with [^{18}F]FDG uptake with an SUV_{max} of 7.8. From left to right: low-dose CT (A,E), fused PET/CT (B,F), PET (C,G), and the maximal intensity projection (MIP, (D,H)). Image scale SUV 0-5.

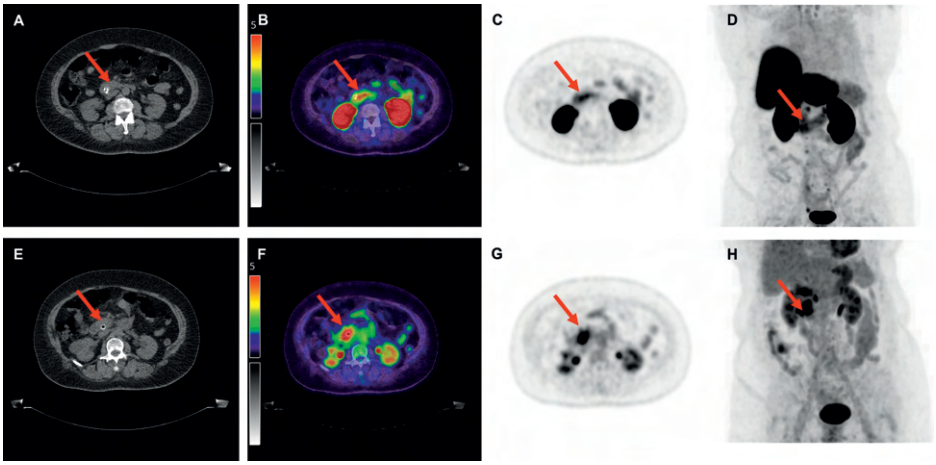


Figure 3. Overview of imaging modalities of a patient with pT2N2M0 pancreatic ductal adenocarcinoma (patient 10). The arrows indicate (upper row) a lesion with moderate to intense [^{18}F]DCFPyL expression with an SUV_{max} of 5.1 and (bottom row) a lesion with [^{18}F]FDG uptake with an SUV_{max} of 10.1. From left to right: low-dose CT (A,E), fused PET/CT (B,F), PET (C,G), and the maximal intensity projection (MIP, (D,H)). Image scale SUV 0-5.

Table 2. Overview of extended imaging parameters of patient 1.

	[^{18}F]DCFPyL	**[^{18}F]FDG**
SUV_{max}	9.9	45.5
SUV_{mean}	6.4	28.4
SUV_{min}	4.5	22.8
SUV_{peak}	8.4	41.0
TBR	7.3	20.4
TV_{DCFPyL}/MTV (cm^3)	13.6	59.4
TL_{DCFPyL}/TLG	87.6	1686.1

Abbreviations: SUV, standardized uptake value; TBR, tumour to blood pool ratio; TV_{DCFPyL}, tumour volume on [^{18}F]DCFPyL PET/CT; MTV, metabolic tumour volume; TL_{DCFPyL}, total lesion uptake on [^{18}F]DCFPyL PET/CT; TLG, total lesion glycolysis.

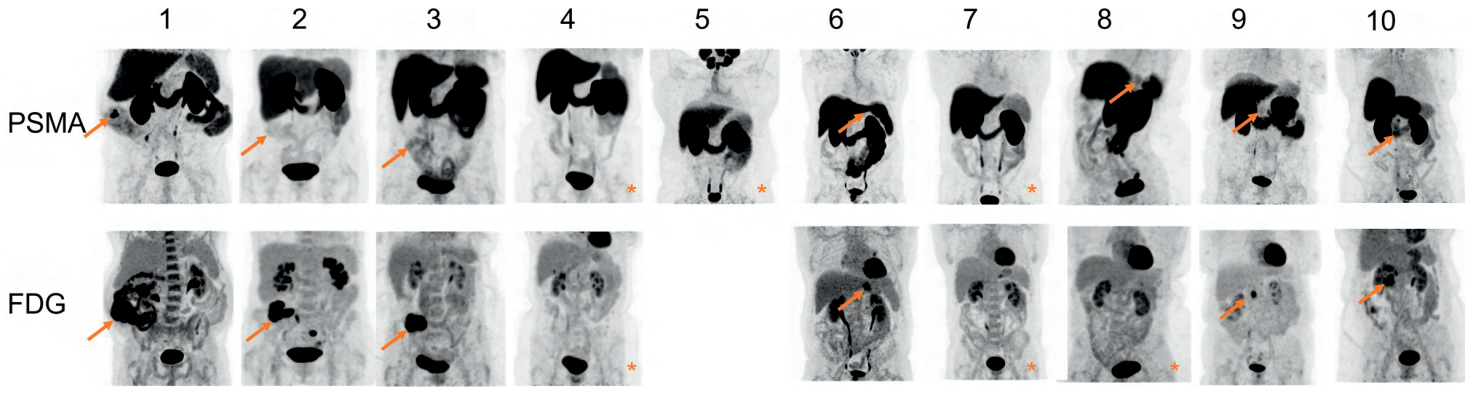


Figure 4. Maximum Intensity Projection (MIP) PET images of all included patients. The arrows indicate the location of the primary tumour. In the MIP PET images with an asterisk the primary tumour was not visible. [^{18}F]FDG PET/CT of patient 5 was not performed as this was not the standard of care due to his cT2-3 gastric tumour. Patient numbers are identical to Table 1.

Immunohistochemical analysis

Immunohistochemistry resulted in a general mean H-score of 81.5 ± 77.8—121.3 ± 73.5 for colon cancer, 50.0 ± 86.6 for gastric cancer, and 60.0 ± 79.4 for pancreatic cancer. [^{18}F]DCFPyL SUV_{max} was not correlated to the PSMA H-score (R^2 0.0001, p = 0.997; Figure 5). Figure 6 shows examples of immunohistochemical staining for the PSMA of the patients displayed in Figure 1, Figure 2 and Figure 3.

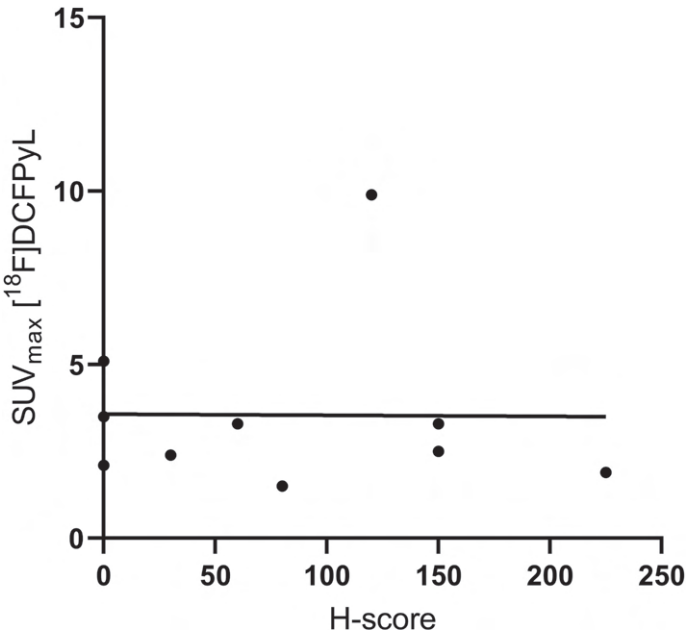


Figure 5. Scatterplot of [^{18}F]DCFPyL SUV_{max} values with associated H scores. Abbreviations: SUV_{max}, maximal standardized uptake value.

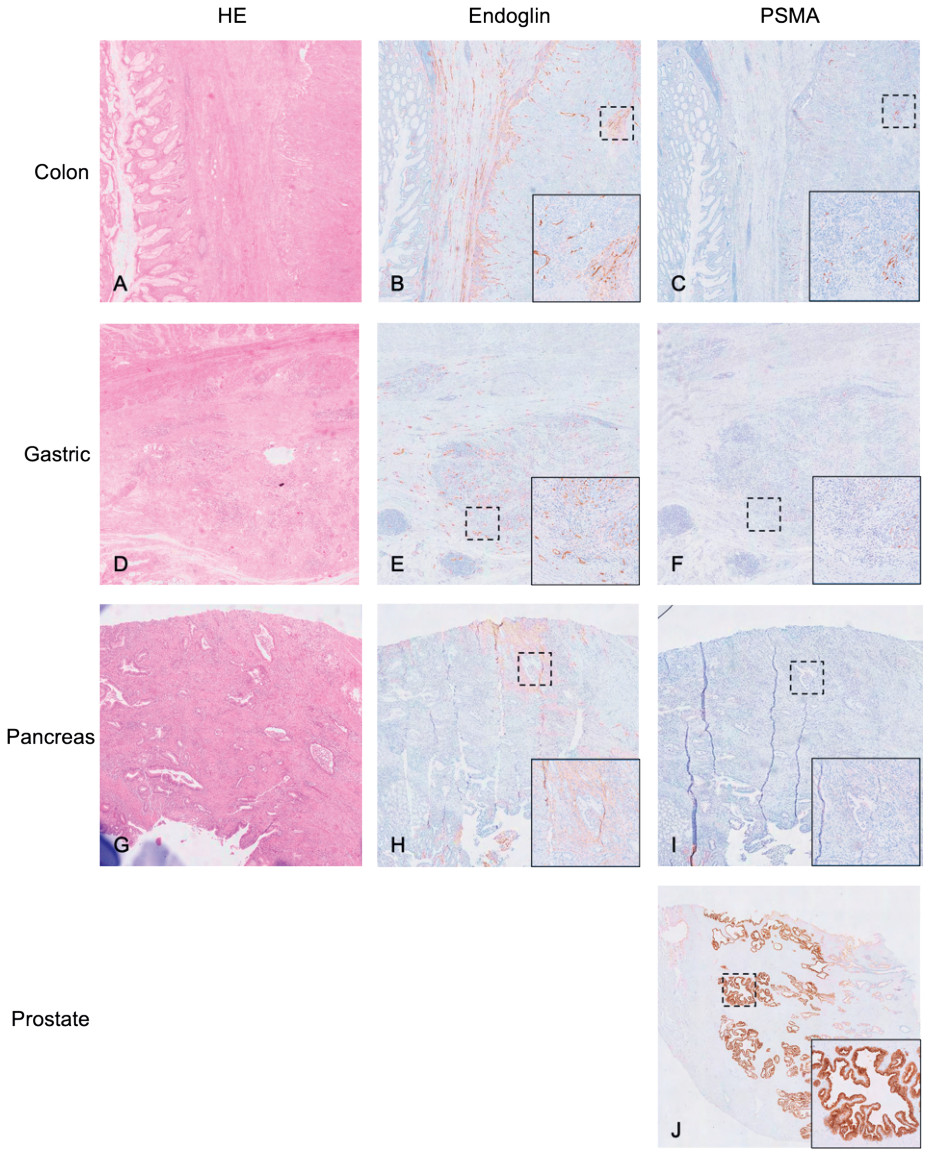


Figure 6. Overview of immunohistochemical staining results. This figure displays Hematoxylin and Eosin (HE), endoglin and PSMA staining of, respectively, colon ((A–C), H-score 120), gastric ((D–F), H-score 150) and pancreatic cancer ((G–I), H-score 0). As a positive control, the PSMA staining was performed on prostate cancer tissue ((J), H-score 300). Overview images were made at 1–2× magnification, zoom images at 10× magnification.

DISCUSSION

Results from this study demonstrate the detection of the primary tumour by [^{18}F]DCFPyL PET/CT in 7 out of 10 patients (3/4 colon, 1/3 gastric, 3/3 pancreatic cancers), with a mean TBR of 3.3 and mean SUV_{max} of 3.6. However, due to the low contrast and high level of uptake in the surrounding tissue, the visual distinction of these tumours was difficult, and the SUV_{max} and TBR on [^{18}F]DCFPyL PET/CT were significantly lower compared to [^{18}F]FDG PET/CT. In addition, no correlation between PSMA expression in the tumour bed in the resected specimen and SUV_{max} on [^{18}F]DCFPyL PET/CT was found.

Previous literature has reported on PSMA-targeted PET tracers to detect gastrointestinal tumours. This includes incidental findings and studies with a large number of patients. In four (suspected) prostate cancer patients, colorectal cancer was unexpectedly found, with an SUV_{max} varying from 7.4 to 19.6 [13–15, 27]. A second study, including metastatic colorectal cancer patients, found a mean SUV_{max} in three patients for the primary tumour of 7.9 ± 2.5 (using [^{68}Ga]Ga-PSMA-11) [28]. This was higher when compared to our found mean SUV_{max} of 4.2 ± 3.9 in three colon cancer patients. As in our study, the SUV_{max} on [^{18}F]FDG PET/CT was significantly higher than on PSMA PET/CT (23.7–43.7, n = 2). Unfortunately, as these patients did not undergo surgery, no correlation to PSMA expression in the resection specimen was available. Most recently, a larger study by Krishnaraju et al. including 40 patients with pancreatic lesions was conducted (21 benign (wide variety of lesions) and 19 malignant) [16]. The ^{68}Ga-PSMA PET/CT was positive in 18 out of 19 pancreatic cancers, and the median SUV_{max} of malignant lesions was significantly higher compared to benign lesions (SUV_{max} 7.4 (IQR 4.5) versus 3.5 (IQR 1.6), $p < 0.001$). The sensitivity and specificity of the visual assessment of ^{68}Ga-PSMA in detecting malignant pancreatic lesions were 94.7% and 90.5%, respectively. Using a quantitative SUV_{max} cut-off value of 4.8, ^{68}Ga-PSMA detected malignant disease with a sensitivity of 84.2% and specificity of 90.5%. The study by Krishnaraju et al. found a considerably higher PSMA uptake in pancreatic cancers compared to our study (median SUV_{max} 7.4 versus median SUV_{max} of 3.3 in our study). Interestingly, the study by Krishnaraju et al. also performed [^{18}F]FDG PET/CT in each patient; however, the median SUV_{max} values of both PET tracers were similar ([^{18}F]FDG 7.6, ^{68}Ga-PSMA 7.4), and the SUV_{max} values of [^{18}F]FDG PET/CT were comparable to our study (mean SUV_{max} [^{18}F]FDG 6.8). The difference in PSMA uptake between these studies currently remains unexplained, but could be influenced by the differences in pharmacokinetic properties and targeting

characteristics (e.g., affinity, binding site) between [^{18}F]DCFPyL and ^{68}Ga-PSMA [29,30]. In addition, no proper pharmacokinetics studies with ^{68}Ga-PSMA were performed, as have been performed for [^{18}F]DCFPyL (including arterial and venous sampling).

The relatively low uptake of [^{18}F]DCFPyL in this study is probably due to the low PSMA expression on the tumours. As is visualized in Figure 6, PSMA expression in the tumour bed of these cancers is significantly lower compared to prostate cancer. Although the endothelial expression of PSMA was visually intense, it was only seen in a low number of angiogenic endothelial cells. However, the IHC results for colon cancer, for example, were in line with previous literature, as all four patients expressed PSMA at varying levels. The physiological uptake of [^{18}F] DCFPyL in the target organs has previously been described by Giesel et al., who found a median SUV_{max} of 2.95 in the pancreas, but did not find any notable uptake in the stomach or colon (n = 12) [31]. [^{18}F]DCFPyL is, however, the most suitable tracer for the detection of gastrointestinal cancers due to its favourable renal clearance, as its alternative, [^{18}F]PSMA-1007, shows predominant hepatobiliary excretion leading to an even higher background signal in both liver and intestines, which interferes with potentially pathological tracer accumulation, especially in these cancers [31]. The low uptake of [^{18}F]DCFPyL in patients with a high H-score could indicate the tracer was not able to penetrate into the tumour core enough. In general, it might be possible that higher-grade tumours (such as included in the study by Cuda et al. [28]) express higher degrees of PSMA. In addition, it is unclear what effect neoadjuvant therapy in gastric cancer patients could have had on the immunohistochemical staining of PSMA.

Possible limitations of this study include the limited sample size, which is due to the premature termination of the trial. However, results from the included 10 patients demonstrate a clear pattern of high background and low tumour uptake, hampering clear tumour identification. As these results appear to be valid for most patients, we believe these results are representative of a larger population of the selected cancer types and thereby provide relevant information. To the best of our knowledge, this is one of the first prospective studies to include patients with gastrointestinal cancers and perform both [^{18}F]DCFPyL as well as [^{18}F]FDG PET/CT, and provide correlation to immunohistochemical expression of PSMA.

CONCLUSIONS

In conclusion, the detection of colon, gastric and pancreatic cancer using [^{18}F] DCFPyL PET/CT imaging is feasible. However, low tumour uptake and high uptake in other organs hamper the clear distinction of tumour mass. In this study, [^{18}F] FDG PET/CT was found to be superior in detecting colon, gastric and pancreatic cancers. These results do not encourage further investigation into the application of [^{18}F]DCFPyL PET/CT imaging in these cancers. However, this may be different for other PSMA-targeted tracers.

REFERENCES

1. Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today; International Agency for Research on Cancer: Lyon, France, 2020. Available online: https://gco.iarc.fr/today (accessed on 9 February 2022).
2. Cunningham, D.; Allum, W.H.; Stenning, S.P.; Thomposon, J.N.; Van de Velde, C.J.H.; Nicolson, M.; Scarffe, J.H.; Lofts, F.J.; Falk, S.J.; Iveson, T.J.; et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N. Engl. J. Med. 2006, 355, 11–20.
3. Choi, J.Y.; Shim, K.-N.; Kim, S.-E.; Jung, H.-K.; Jung, S.-A.; Yoo, K. The Clinical Value of 18F-Fluorodeoxyglucose Uptake on Positron Emission Tomography/Computed Tomography for Predicting Regional Lymph Node Metastasis and Non-curative Surgery in Primary Gastric Carcinoma. Korean J. Gastroenterol. 2014, 64, 340–347.
4. Seevaratnam, R.; Cardoso, R.; McGregor, C.; Lourenco, L.; Mahar, A.; Sutradhar, R.; Law, C.; Paszat, L.; Coburn, N. How useful is preoperative imaging for tumor, node, metastasis (TNM) staging of gastric cancer? A meta-analysis. Gastric Cancer 2012, 15, 3–18.
5. Wang, Z.; Chen, J.-Q. Imaging in assessing hepatic and peritoneal metastases of gastric cancer: A systematic review. BMC Gastroenterol. 2011, 11, 1–14.
6. Gertsen, E.C.; Brenkman, H.J.F.; van Hillegersberg, R.; van Sandick, J.; van Berge Henegouwen, M.I.; Gisbertz, S.S.; Luyer, M.D.P.; Nieuwenhuijzen, G.A.P.; van Lanschot, J.J.B.; Lagarde, S.M.; et al. 18F-Fludeoxyglucose-Positron Emission Tomography/Computed Tomography and Laparoscopy for Staging of Locally Advanced Gastric Cancer: A Multicenter Prospective Dutch Cohort Study (PLASTIC). JAMA Surg. 2021, 156, e215340.
7. Smyth, E.; Schöder, H.; Strong, V.E.; Capanu, M.; Kelsen, D.P.; Coit, D.G.; Shah, M.A. A prospective evaluation of the utility of 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography and computed tomography in staging locally advanced gastric cancer. Cancer 2012, 118, 5481–5488.
8. Gerritsen, A.; Dutch Pancreatic Cancer Group; Molenaar, I.Q.; Bollen, T.L.; Nio, C.Y.; Dijkgraaf, M.G.; Van Santvoort, H.C.; Offerhaus, G.J.; Brosens, L.A.; Biermann, K.; et al. Preoperative Characteristics of Patients with Presumed Pancreatic Cancer but Ultimately Benign Disease: A Multicenter Series of 344 Pancreatoduodenectomies. Ann. Surg. Oncol. 2014, 21, 3999–4006.
9. Tummers,W.S.; Groen, J.V.; Mulder, B.G.S.; Farina-Sarasqueta, A.; Morreau, J.; Putter, H.; Van De Velde, C.J.; Vahrmeijer, A.L.; Bonsing, B.A.; Mieog, J.S.; et al. Impact of resection margin status on recurrence and survival in pancreatic cancer surgery. Br. J. Surg. 2019, 106, 1055–1065.

10. Haffner, M.C.; Kronberger, I.E.; Ross, J.S.; Sheehan, C.E.; Zitt, M.; Mühlmann, G.; Öfner, D.; Zelger, B.; Ensinger, C.; Yang, X.J.; et al. Prostate-specific membrane antigen expression in the neovasculature of gastric and colorectal cancers. Hum. Pathol. 2009, 40, 1754–1761.

11. Ren, H.; Zhang, H.; Wang, X.; Liu, J.; Yuan, Z.; Hao, J. Prostate-specific membrane antigen as a marker of pancreatic cancer cells. Med Oncol. 2014, 31, 1–6.

12. Vuijk, F.A.; de Muynck, L.D.A.N.; Franken, L.C.; Busch, O.R.; Wilmink, J.W.; Besselink, M.G.; Bonsing, B.A.; Bhairosingh, S.S.; Kuppen, P.J.K.; Mieog, J.S.D.; et al. Molecular targets for diagnostic and intraoperative imaging of pancreatic ductal adenocarcinoma after neoadjuvant FOLFIRINOX treatment. Sci. Rep. 2020, 10, 1–9.

13. Huang, Y.-T.; Fong,W.; Thomas, P. Rectal Carcinoma on 68Ga-PSMA PET/CT. Clin. Nucl. Med. 2016, 41, e167–e168.

14. Hangaard, L.; Jochumsen, M.R.; Vendelbo, M.H.; Bouchelouche, K. Metastases from Colorectal Cancer Avid on 68Ga-PSMA PET/CT. Clin. Nucl. Med. 2017, 42, 532–533.

15. Stoykow, C.; Huber-Schumacher, S.; Almanasreh, N.; Jilg, C.; Ruf, J. Strong PSMA Radioligand Uptake by Rectal Carcinoma: Who Put the 'S' in PSMA? Clin. Nucl. Med. 2017, 42, 225–226.

16. Krishnaraju, V.S.; Kumar, R.; Mittal, B.R.; Sharma, V.; Singh, H.; Nada, R.; Bal, A.; Rohilla, M.; Singh, H.; Rana, S.S. Differentiating benign and malignant pancreatic masses: Ga-68 PSMA PET/CT as a new diagnostic avenue. Eur. Radiol. 2021, 31, 2199–2208.

17. Sartor, O.; de Bono, J.; Chi, K.N.; Fizazi, K.; Herrmann, K.; Rahbar, K.; Tagawa, S.T.; Nordquist, L.T.; Vaishampayan, N.; El-Haddad, G.; et al. Lutetium-177–PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. N. Engl. J. Med. 2021, 385, 1091–1103.

18. Jansen, B.H.E.; Yaqub, M.; Voortman, J.; Cysouw, M.C.F.; Windshorst, A.D.; Schuit, R.C.; Kramer, G.M.; van den Eertwegh, A.J.M.; Schwarte, L.A.; Hendrikse, N.H.; et al. Simplified Methods for Quantification of 18F-DCFPyL Uptake in Patients with Prostate Cancer. J. Nucl. Med. 2019, 60, 1730–1735.

19. Wondergem, M.; van der Zant, F.M.; Knol, R.J.J.; Lazarenko, S.V.; Pruim, J.; de Jong, I. 18F-DCFPyL PET/CT in the Detection of Prostate Cancer at 60 and 120 Minutes: Detection Rate, Image Quality, Activity Kinetics, and Biodistribution. J. Nucl. Med. 2017, 58, 1797–1804.

20. Boellaard, R.; Delgado-Bolton, R.; Oyen,W.J.G.; Giammarile, F.; Tatsch, K.; Eschner,W.; Verzijlbergen, F.J.; Barrington, S.F.; Pike, L.C.; Weber, W.A.; et al. FDG PET/CT: EANM procedure guidelines for tumour imaging: Version 2.0. Eur. J. Nucl. Med. Mol. Imaging 2015, 42, 328–354.

21. Nioche, C.; Orlhac, F.; Boughdad, S.; Reuzé, S.; Goya-Outi, J.; Robert, C.; Pellot-Barakat, C.; Soussan, M.; Frouin, F.; Buvat, I. LIFEx: A Freeware for Radiomic Feature Calculation in Multimodality Imaging to Accelerate Advances in the Characterization of Tumor Heterogeneity. Cancer Res. 2018, 78, 4786–4789.

22. Schmuck, S.; von Klot, C.A.; Henkenberens, C.; Sohns, J.M.; Christiansen, H.; Wester, H.-J.; Ross, T.L.; Bengel, F.M.; Derlin, T. Initial Experience with Volumetric 68Ga-PSMA I&T PET/CT for Assessment of Whole-Body Tumor Burden as a Quantitative Imaging Biomarker in Patients with Prostate Cancer. J. Nucl. Med. 2017, 58, 1962–1968.

23. Jansen, B.H.E.; Cysouw, M.C.F.; Vis, A.N.; van Moorselaar, R.J.A.; Voortman, J.; Bodar, Y.J.L.; Schober, P.R.; Hendrikse, N.H.; Hoekstra, O.S.; Boellaard, R.; et al. Repeatability of Quantitative 18F-DCFPyL PET/CT Measurements in Metastatic Prostate Cancer. J. Nucl. Med. 2020, 61, 1320–1325.

24. Jung, I.; Gurzu, S.; Raica, M.; Cîmpean, A.M.; Szentirmay, Z. The differences between the endothelial area marked with CD31 and CD105 in colorectal carcinomas by computer-assisted morphometrical analysis. Rom. J. Morphol. Embryol. 2009, 50, 239–243.

25. Hirsch, F.R.; Varella-Garcia, M.; Bunn, P.A., Jr.; Di Maria, M.V.; Veve, R.; Bremnes, R.M.; Barón, A.E.; Zeng, C.; Franklin, W.A. Epidermal Growth Factor Receptor in Non–Small-Cell Lung Carcinomas: Correlation Between Gene Copy Number and Protein Expression and Impact on Prognosis. J. Clin. Oncol. 2003, 21, 3798–3807.

26. John, T.; Liu, G.; Tsao, M.-S. Overview of molecular testing in non-small-cell lung cancer: Mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. Oncogene 2009, 28, S14–S23.

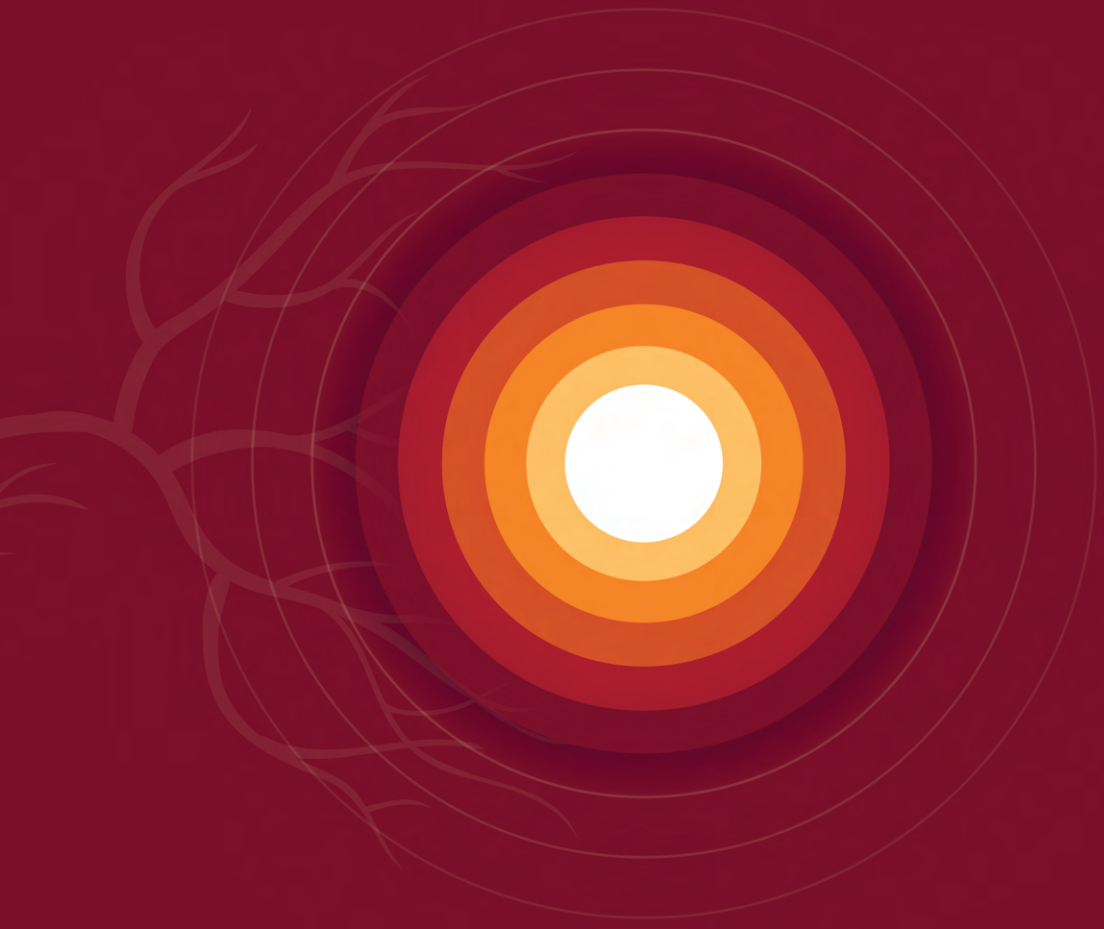
27. Arçay, A.; Eiber, M.; Langbein, T. Incidental Finding of Colon Carcinoma Related to High Uptake in 18F-PSMA-1007 PET. Clin. Nucl. Med. 2020, 45, 561–562.

28. Cuda, T.J.; Riddell, A.D.; Liu, C.; Whitehall, V.L.; Borowsky, J.;Wyld, D.K.; Burge, M.E.; Ahern, E.; Griffin, A.; Lyons, N.J.; et al. PET Imaging Quantifying 68Ga-PSMA-11 Uptake in Metastatic Colorectal Cancer. J. Nucl. Med. 2020, 61, 1576–1579.

29. Ferreira, G.; Iravani, A.; Hofman, M.S.; Hicks, R.J. Intra-individual comparison of 68Ga-PSMA-11 and 18F-DCFPyL normal-organ biodistribution. Cancer Imaging 2019, 19, 23.

30. Man, K.D.; Laeken, N.V.; Schelfhout, V.; Fendler, W.P.; Lambert, B.; Kersemans, K.; Piron, S.; Lumen, N.; Decaestecker, K.; Fonteyne, V.; et al. 18F-PSMA-11 Versus 68Ga-PSMA-11 Positron Emission Tomography/Computed Tomography for Staging and Biochemical Recurrence of Prostate Cancer: A Prospective Double-blind Randomised Cross-over Trial. Eur. Urol. 2022, 82, 501–509.

31. Giesel, F.L.; Will, L.; Lawal, I.; Lengana, T.; Kratochwil, C.; Vorster, M.; Neels, O.; Reyneke, F.; Harberkon, U.; Kopka, K.; et al. Intraindividual Comparison of 18F-PSMA-1007 and 18F-DCFPyL PET/CT in the Prospective Evaluation of Patients with Newly Diagnosed Prostate Carcinoma: A Pilot Study. J. Nucl. Med. 2018, 59, 1076–1080.



CHAPTER 9

General discussion and future perspectives

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Stage migration in prostate cancer

Since the introduction of PSMA PET/CT, which has demonstrated superior accuracy over conventional imaging with CT and bone scintigraphy, changes in the staging of prostate cancer patients have been described [1]. These shifts primarily involve upstaging with the identification of (small) lesions previously unidentified by conventional imaging, but may also involve downstaging as PSMA PET/CT is associated with a reduced rate of false positives [2]. These changes in staging can have important clinical implications, since treatment decisions are often based on disease stage. For example, if PSMA PET/CT detects distant metastases in a patient, the treatment approach may change from curative to palliative.

Compared to conventional imaging, PSMA PET/CT can shift disease stage between localised disease (N0M0), nodal disease (N1M0) or distant metastatic disease (M1). The proPSMA trial reported nodal or distant upstaging in 14% of high-risk prostate cancer patients, with 5% being upstaged to M1 [1]. However, in the THUNDER study cohort, which included patients with very high-risk prostate cancer who were all referred for curative-intent radiation therapy, nodal or distant upstaging was observed in 30% of patients, with 13% being upstaged to M1 (*Chapter 2*). This was more than double the rate observed in the proPSMA trial, which also included patients considered for radical prostatectomy. The increased upstaging rate in the THUNDER study cohort could likely be attributed to the very high-risk profile of patients, with each high-risk factor being more prevalent than in the proPSMA trial (*Chapter 2; Table 1*). Additionally, the number of high-risk factors was significantly associated with the risk of upstaging by PSMA PET/CT (upstaging to miM1 disease; OR = 1.86 per additional high-risk factor, 95% C.I. [1.13-3.07]). To the best of our knowledge, no previous prospective study has described such correlation. These findings provide a rationale for prioritising the use of PSMA PET/CT for staging patients with multiple high-risk factors. Ravi et al. [3] showed that increasing numbers of high-risk factors were associated with poorer survival outcomes. This suggests that such patients may have more aggressive disease, leading to both higher rates of upstaging on PSMA PET/CT and worse prognosis. However, the optimal treatment strategy for patients upstaged by PSMA PET/CT remains unclear. To assess whether PSMA PET/CT-guided treatment adaptation improves patient outcome, one of the aims of the currently recruiting phase 3 THUNDER trial (clinicaltrials.gov NCT06282588) is to evaluate whether the addition of androgen receptor inhibitor darolutamide to standard-of-care radiotherapy and

androgen deprivation therapy (ADT) improves metastasis-free survival in upstaged high-risk prostate cancer patients (Figure 1). Results of the THUNDER trial are pending.

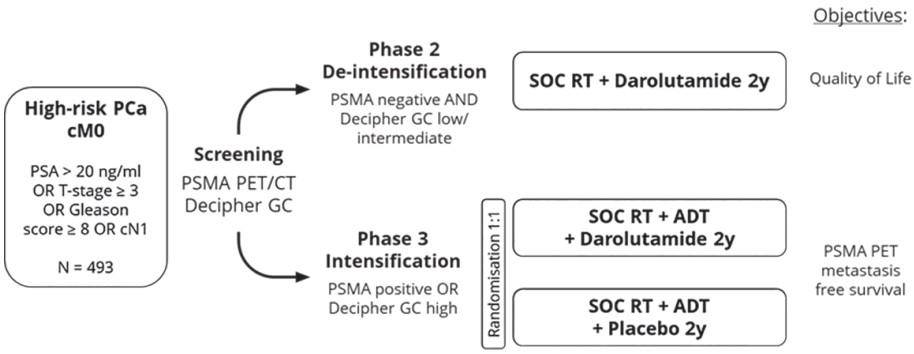


Figure 1. THUNDER study flowchart. PSMA PET/CT and the Decipher Genomic Classifier (GC) score, which is a gene expression assay, are being used for risk stratification and guide treatment intensification or de-intensification strategies. In the phase 3 intensification trial, patients are treated with standard of care (SOC) radiation therapy (RT) and androgen deprivation therapy (ADT), with randomisation between addition of darolutamide or placebo for 2 years. In the phase 2 non-randomised de-intensification trial, patients are treated with SOC RT + darolutamide for 2 years. PSMA positive = extra-prostatic disease on PSMA PET/CT. Decipher GC high = score >0.6.

Redefining low- and high-volume disease in metastatic hormone-sensitive prostate cancer (mHSPC)

In patients with mHSPC, PSMA PET/CT has also led to disease upstaging; due to its superior sensitivity, some patients would be reclassified from low- to high-volume disease. As the criteria for low- and high-volume disease are based on conventional imaging, the introduction of PSMA PET/CT has led to uncertainty around how to stratify patients and which treatment to select, particularly in borderline cases. For the translation of conventional imaging-based criteria to PSMA PET/CT-based criteria, we propose using PSMA-derived total tumour volume (PSMA-TV) as a biomarker to base treatment decisions on. Of all imaging parameters, PSMA-TV was considered to be the most robust prognostic factor for OS with a 39% increased risk per doubling (*Chapter 3*), independent of WHO performance status and administered treatment. Furthermore, PSMA-TV represents an accurate reflection of tumour burden, unlike earlier definitions which mainly relied on the number and location of bone metastases, and ignored lymph node burden and lesion volumes. In our retrospective study of 204 patients (*Chapter 3*), the optimal PSMA PET/CT-based classification for high-volume disease in terms of survival was: PSMA-TV ≥ 150 mL or any visceral metastasis (HR = 3.01, 95% C.I. [1.88-

4.80]). However, we do not recommend using this threshold in a clinical setting. Using a binary cut-off will oversimplify clinical reality; patients below and above this threshold sill represent a heterogeneous group, and survival continues to decrease with higher tumour volumes. Furthermore, this retrospective study carries a substantial risk of overfitting. Therefore, we propose the development and use of survival prediction models that incorporate PSMA-TV and other clinically relevant parameters as continuous inputs. This approach would allow for more individualised survival prediction, and would better reflect the heterogeneity in mHSPC patients.

PSMA-TV has also been recognised in prior studies as a robust prognostic factor. Karpinski et al. developed a nomogram for overall survival that also included PSMA-TV, along with TNM-stage and SUV_{mean} [4]. However, this nomogram was developed using a heterogeneous patient population across all prostate cancer disease stages, with synchronous mHSPC patients comprising less than 7% of the cohort. Moreover, the nomogram did not incorporate any clinical prognostic factors. In addition to WHO performance status, previous studies have identified age, PSA level and Gleason score as prognostically relevant in metastatic prostate cancer [5, 6]. We hypothesise that in our cohort of 204 synchronous mHSPC patients, the sample size may have restricted the statistical power to detect additional significant predictors beyond PSMA-TV and WHO performance status. For the future development of a robust and generalisable prediction tool, we recommend to evaluate these additional parameters in a larger, multicentre cohort to more accurately assess their potential additional value.

Survival prediction in metastatic castration-resistant prostate cancer (mCRPC)

Patients with mCRPC are typically treated sequentially, starting with an androgen receptor targeted agents (ARTA), followed by chemotherapy, with only 20% of patients receiving further systemic therapies [7]. This framework largely follows a “one size fits all” model, with limited nuance for individual patient differences. To further help with personalised treatment decision-making in this heterogeneous disease, we have demonstrated that PSMA PET/CT has strong prognostic value in mCRPC patients as well. Similar to findings in mHSPC patients (*Chapter 3*), PSMA-TV was the strongest predictor of overall survival in mCRPC patients treated with an ARTA or chemotherapy (*Chapter 4*). A doubling of PSMA-TV was associated with a 41% increased mortality risk, independent of baseline PSA level, haemoglobin level and line of mCRPC treatment. These findings are consistent with previous

studies that have identified PSMA-TV as a prognostic marker in the mCRPC setting [4, 8-10]. As most studies included relatively small patient cohorts, the next step is to establish a larger, multicentre database to enhance statistical power. This would enable more robust and precise estimates of the prognostic value of PSMA-TV. Such data will be valuable for clinical decision-making in mCRPC, as prognosis is limited and therapeutic choices must be carefully weighed against toxicity and impact on quality of life. While our study did not include WHO performance status or comorbidities, it would be interesting to include these parameters in future analyses as they are known to affect prognosis as well [5].

In addition to confirming the prognostic value of PSMA-TV, *Chapter 4* presents two novel findings. First, although baseline PSA levels alone were not predictive in univariate analysis, we observed that a lower PSA level in combination with higher PSMA-TV was associated with poorer survival outcomes. This finding motivated a post-hoc analysis introducing PSA density (defined as PSA / PSMA-TV), which was also significantly associated with prognosis (HR = 0.76 per doubling, $p < 0.01$). We hypothesise that lower PSA density may reflect tumour dedifferentiation, serving as a potential surrogate for more aggressive disease biology. Second, we found that DmaxVox, a measure for the maximal spatial spread of PSMA-positive lesions in centimetres, also demonstrated prognostic value (HR = 1.31 per 10 cm increase, $p = 0.011$), and may serve as a practical alternative to PSMA-TV. Although DmaxVox has previously been studied in the context of PSMA-RLT, our results suggest that it can have broader applicability as a general imaging biomarker in both mCRPC and mHSPC (*Chapter 3*: HR = 1.23 per 10 cm increase, $p < 0.001$), particularly when total tumour segmentation is not feasible.

In both *Chapters 3* and *Chapter 4*, PSMA-TV outperformed TL-PSMA, SUV_{mean} and SUV_{max} as an imaging biomarker. A possible explanation is that parameters incorporating SUV values can be confounded by tumour heterogeneity. For instance, lower SUV could indicate biologically less aggressive disease, or it could indicate dedifferentiated, more aggressive disease, complicating their interpretability. For this reason, we propose PSMA-TV as the most robust PET-derived parameter for quantifying tumour burden and recommend its use in future clinical trials in metastatic prostate cancer.

Automatic tumour segmentation

To enable the routine use of whole-body PET parameters such as PSMA-TV in clinical practice, it is essential that a fully automated and user-friendly segmentation

method is in place. As dr. Buvat explained at the PSMA Conference in 2025, we need a "one-push-button solution" to make this feasible. Manual or semi-automated segmentation remains too time-consuming and resource-intensive for clinical workflows. Significant progress has already been made in this area, particularly through the application of artificial intelligence, and several automated tools have been developed (e.g. Lesion ID Pro from MIM software and PYLARIFY AI from EXINI Diagnostics). However, it is important to note that automated segmentation still requires interpretation and validation by clinical experts, as certain PSMA PET/CT pitfalls exist. For example, physiological uptake in ganglia or the bowel may be mistaken for pathological lymph nodes, or unspecific bone uptake may be mistaken for metastasis.

Despite these advancements, challenges remain. There is currently no consensus on the optimal tumour segmentation method or threshold. In this thesis, we used a fixed threshold of SUV = 4, based on previous literature [11]. A fixed threshold was preferred over a relative threshold, as in the case of treatment response evaluation, disease progression or response would too greatly influence the segmentation threshold. Additionally, differences in scan protocols (type of tracer, type of scanner, image reconstruction algorithms, injection-to-scan interval) can influence quantitative results. For this, the standardisation efforts led by EARL (EANM Research Ltd.) guidelines remain essential to ensure reproducibility and comparability across different scanners and institutions.

Radiomics

Radiomics, i.e. the extraction of a large number of features from images, have been a topic of interest for years in radiology. A segmented lesion consists of pixels, or voxels, each with a different intensity value (SUV). With radiomics, such a lesion can be analysed in many different ways by extracting shape features (e.g. volume, sphericity), intensity-based features (e.g. SUV_{max}, SUV-kurtosis, SUV-IQR, TL-PSMA) and texture features (e.g. Gray-Level Co-occurrence Matrix (GLCM); how often pairs of intensity values occur at a defined distance and direction, therefore a measure of heterogeneity).

To better understand lesion-level characteristics associated response to treatment in CRPC patients, we conducted a preliminary radiomic analysis with twelve shape features, intensity-based features and GLCM features, hypothesising that these features could predict lesion progression and thereby add to more personalised treatment decision-making (*Chapter 4*). Those features were primarily chosen

as they have been associated with patient outcome in previous literature. In our analysis, including 241 separate lesions, there were several intensity-based and GLCM features associated with progression on follow-up PSMA PET/CT after 3-4 months in univariable logistic regression. However, in multivariate analysis most of these associations did not hold. Only SUV_{mean} correlated with lesion progression, independent of lesion location and line of treatment. For lesion location, bone lesions showed the highest progression rates, potentially due to their dense, poorly vascularised environment and the protective role of the bone marrow.

Because this preliminary analysis evaluated only twelve literature-based features, it is possible that relevant radiomic features were overlooked. Perhaps using more advanced techniques such as machine learning are needed to achieve predictive ability. Furthermore, as imaging techniques continue to become more accurate with smaller pixels and better signal to noise ratios, radiomic features will more accurately reflect tumour characteristics. We recommend future research to focus on assembling large homogeneous datasets with robust harmonisation strategies on imaging data. Furthermore, multicollinearity among radiomic features should be addressed and feature selection methods should be used to minimise overfitting and prevent p-hacking.

Treatment response evaluation

For treatment response evaluation in metastatic prostate cancer, clinicians have long relied on monitoring PSA levels, often in combination with other biochemical markers such as ALP, LDH and Hb levels, and screening for potential new metastases with conventional imaging. We demonstrated that in mCRPC patients, PSMA PET/CT outperforms PSA levels in assessing treatment response, and could better differentiate short- and long-term survivors (*Chapter 5*). Importantly, PSMA PET/CT enabled the early identification of non-responders by detecting progressive disease on imaging despite stable or declining PSA values. To our knowledge, this is one of the first studies to assess the use of PSMA PET/CT at predefined time points for treatment response evaluation, thereby demonstrating its potential to detect treatment resistance earlier than PSA monitoring. Applying a minimum interval of three months for ARTA treatment and three cycles for chemotherapy before evaluation minimised the risk of false-positive findings due to flare. Ultimately, the early detection of non-responders can allow for timely discontinuation of ineffective therapies, reducing unnecessary toxicity and costs, and initiation of potentially effective therapies at an earlier stage. This could potentially improve patient outcomes, given that tumour burden (PSMA-TV) is

associated with survival. On the other hand, PSMA PET/CT might provide early identification of favourable responders who might benefit from treatment de-escalation strategies such as lowering the dose or intermittent treatment regimens.

As mCRPC is known as a heterogeneous disease, both between patients and within patients, tumour progression and patient prognosis are often driven by the most treatment-resistant subclone. Consequently, an imaging-based assessment capable of showing mixed responses, such as partially responding lesions alongside progressing or new lesions, can provide a more accurate reflection of disease biology than PSA values. Furthermore, qualitative (visual) response assessment on PSMA PET/CT outperformed quantitative assessment (e.g. percentage change in PSMA-TV), likely due to this heterogeneity (*Chapter 5*). Qualitative response assessment can capture progressive or new lesions, whereas quantitative assessment may not reflect this.

Several challenges remain in the evaluation of treatment response with PSMA PET/CT. One key issue is that multiple response criteria exist, including those proposed by EANM, PPP and RECIP, each with different definitions [12-14]. To ensure comparability across clinical trials and clinical practices, standardisation of response assessment and reporting is required. Furthermore, the optimal imaging time points for response assessment have yet to be defined.

Finally, before PSMA PET/CT for treatment response evaluation can be integrated into clinical practice, cost-effectiveness analyses are important. Earlier detection of progression may reduce cost, improve outcomes, and enhance quality of life, and these assumptions must be validated in studies assessing the impact across the full disease trajectory. Potentially, patient subgroups will be identified for whom PSMA PET/CT is particularly beneficial, so that accessibility can be prioritised where the greatest clinical impact is expected.

Challenges when introducing a more sensitive imaging modality

The introduction of new imaging modalities (such as PSMA PET/CT) that have superior diagnostic accuracy compared to previous imaging modalities (such as bone scintigraphy and CT), brings several challenges. Firstly, as discussed earlier, this will likely lead to stage migration, where patients are reclassified into more advanced stages. However, stage migration can cause an alteration in prognosis statistics without true changes in patient outcomes, which is called the Will Rogers phenomenon [15]. Because upstaged patients represent a group with a worse

prognosis than the patients that were not upstaged, but with a better prognosis than those with evident metastatic disease, their reclassification improves the average prognosis of both groups (Figure 2). It seems like survival benefit has been "created", however, the overall average prognosis remains the same. Furthermore, a more sensitive imaging modality can introduce uncertainty around treatment decisions. For example, it can potentially lead to overtreatment of upstaged patients which might have less aggressive disease than patients with evident metastatic disease. On the other hand, some patients may be understaged by older modalities, so the new imaging could decrease undertreatment and improve disease control. However, those potential benefits require evidence.

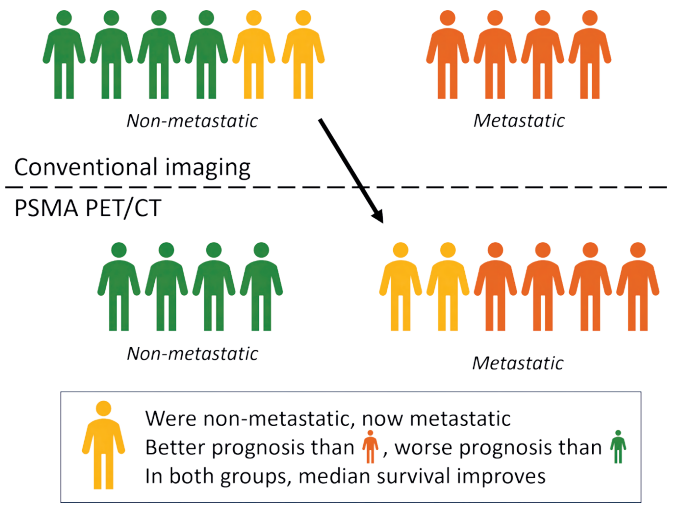


Figure 2. The Will Rogers phenomenon.

The Fryback and Thornbury hierarchy of diagnostic efficacy, which outlines six levels of evaluating imaging modalities, can help navigate the introduction of a new imaging modality (Table 1). After the technical aspects have been developed and are considered feasible, prospective studies such as the proPSMA trial are needed to evaluate diagnostic accuracy efficacy (level 2), diagnostic thinking efficacy (level 3) and therapeutic efficacy (level 4). For example, the phase 3 proPSMA trial demonstrated that PSMA PET/CT had a 27% greater accuracy (92% versus 65%), fewer uncertain results (7% versus 23%) and greater impact on treatment decisions (28% versus 15%) compared to conventional imaging. While levels 1-4 are usually well reported, patient outcome efficacy (level 5) and societal efficacy (level 6) are often more uncertain for a longer period of time. The effect on patient outcomes are harder to prove as long-term randomised studies are needed to show that a

management change triggered by imaging improves survival or quality of life. Furthermore, many downstream factors can affect patient outcomes, making it difficult to isolate the contribution of the imaging modality itself. The highest level of clinical evidence are randomised controlled trials and their meta-analyses. However, when one modality has demonstrated superior diagnostic accuracy over another, it becomes ethically questionable to randomise patients to the inferior modality, particularly as it will likely result in missed metastases and suboptimal treatment in a subset of patients. This creates a paradox in evidence generation: the most robust evidence may be unattainable once clinical guidelines shift toward the newer standard. For data on societal efficacy, robust health economic modelling and real-world data are required, which takes time to gather. Because EMA/FDA approval is often based on levels 1-4 evidence and levels 5-6 need long-term follow-up and system level-data, often uncertainties persists even when the imaging modality is already used in clinical practice.

Table 1. The Fryback and Thornbury hierarchy of diagnostic efficacy [16].

Level	Measures
Level 1: Technical efficacy	Resolution of images
Level 2: Diagnostic accuracy efficacy	Sensitivity and specificity
Level 3: Diagnostic thinking efficacy	Impact on clinician's diagnostic process
Level 4: Therapeutic efficacy	Impact on treatment decisions
Level 5: Patient outcome efficacy	Patient benefit
Level 6: Societal efficacy	Cost-benefit analysis

Although the FDA approved the first PSMA tracer in 2020, robust evidence demonstrating its impact on patient benefit is still lacking. In particular, it remains unclear whether PSMA PET/CT-guided treatment changes translate into improved survival. To address this, prospective trials and large registry studies are needed, preferably including patients that are not only (re)staged with PSMA PET/CT, but also receive PSMA PET/CT for all follow-up imaging. Such frameworks would enable the collection of detailed longitudinal data on treatment decisions, their rationale (what was the decision based on?), survival outcomes, time on treatment and toxicity, thereby allowing evaluation of the entire care pathway. Standardised reporting of PSMA PET/CT findings is essential here, to ensure comparability across studies.

Another unresolved question is the optimal treatment strategy for patients who are upstaged or downstaged by PSMA PET/CT. This requires trials that assess treatment intensification and de-escalation strategies, with stratification and randomisation based on imaging results. Outcome-focused phase 3 studies, such as the THUNDER trial, are expected to provide new insights but results are still pending.

Despite the large number of PSMA PET/CT scans already performed worldwide, many of these questions remain unanswered due to the lack of an infrastructure to systematically collect data. In the Netherlands, for example, the imPRINT consortium (the "Dutch PSMA study group") was initiated in 2020 with the goal of pooling PSMA PET/CT and clinical data across different centres, but resource limitations (funding, time, personnel) hindered its execution. Looking ahead, advances in artificial intelligence and automated extraction of clinical information from electronic health records may greatly facilitate such large-scale analyses in the future.

Challenges in the use of PSMA-RLT in non-prostate cancers

Beyond prostate cancer, there is growing interest in exploring the potential of PSMA-targeted therapies for treating other malignancies. Immunohistochemical studies have revealed the presence of PSMA expression in a wide variety of non-prostate cancers, potentially providing an opportunity to expand the use of PSMA-RLT beyond prostate cancer. Interestingly, PSMA expression generally increases in more aggressive tumours and has been associated with worse survival. For instance, PSMA expression is significantly higher in sarcomas than in benign bone or soft tissue tumours, and in osteosarcomas, the presence of PSMA expression has been linked to a poorer five-year survival rate (*Chapter 6*). Furthermore, published case reports of PSMA-tracer uptake in sarcomas on PET/CT imaging revealed highest uptake values in metastasised disease. This is highly relevant, as patients with aggressive metastatic disease are also in the greatest need of new therapeutic options, such as PSMA-RLT.

However, there are significant challenges in the use of PSMA-RLT in non-prostate cancer. A major obstacle is the heterogeneous expression of PSMA and the lower tracer uptake seen on PET/CT scans compared to prostate cancer. In non-prostate cancer PSMA expression is mainly restricted to tumour-associated neovasculature, and its intensity varies widely between and within tumour types, Although the prospective feasibility study that we conducted to analyse PSMA

expression and PSMA-tracer uptake on PET/CT imaging in metastatic soft tissue sarcoma patients revealed high PSMA expression in 11 of 25 patients (44%) and an SUV_{max} > 8 in 3 out of 5 patients that were imaged with [^{18}F]-JK-PSMA-7 PET/CT, the uptake within these patients was too heterogeneous (median SUV_{max} = 3.8; range 0.5-16.7) for them to be eligible for PSMA-RLT (*Chapter 7*). Also, in our other prospective feasibility study where we used [^{18}F]DCFPyL PET/CT imaging in 10 patients with non-metastatic colon, gastric or pancreatic cancer, only one patient had adequate PSMA-tracer uptake with an SUV_{max} of 9.9 (*Chapter 8*). The [^{18}F]DCFPyL PET/CT was negative in three patients and [^{18}F]FDG PET/CT consistently outperformed [^{18}F]DCFPyL PET/CT. These results, together with the fact that PSMA expression is only observed in a subset of non-prostate cancers, indicate that PSMA-targeted imaging is not suitable for any diagnostic purposes in non-prostate cancers. Furthermore, the observed heterogeneity hampers the potential effectiveness of PSMA-RLT as monotherapy.

Another potential challenge for PSMA-RLT in non-prostate cancer is the greater distance between the radiotracer, which binds to the neovasculature, and the tumour cells itself. No data exists on how this influences the effectiveness of PSMA-RLT. On the other hand, the emitted beta particles from Lutetium-177 have a tissue range of 0.2 – 2mm and radiation doses to the tumour microenvironment may eventually lead to a tumour reduction as well. There is also no insightful data available on tracer binding and retention in the endothelium. No internalisation of the tracer takes place, in contrast to prostate cancer, and there is speculation that tracer washout is quicker in non-prostate cancers. Dosimetry and PET/CT findings show considerable variability in tracer uptake between patients and among lesions within the same patient, highlighting the need for further research into radiotracer pharmacokinetics and absorbed dose distributions.

In parallel, patient selection methods must be developed for non-prostate cancers, with the aim to select patients likely to respond to PSMA-RLT, without excluding those who could have benefited. The identification of predictive biomarkers is necessary for this purpose, to ensure an acceptable costs/benefit ratio. Testing for PSMA expression using immunohistochemistry in biopsy or resection material seems unsuitable due to the heterogeneity between lesions and the lack of correlation between immunohistochemical PSMA expression and SUVs on PSMA PET/CT imaging (*Chapter 7 and Chapter 8*). This makes it difficult to select the right patients. Furthermore, there is currently no established consensus on PET/CT-based (visual or quantitative) criteria defining what constitutes sufficient tracer

uptake to be eligible for PSMA-RLT, also not within prostate cancer. Lastly, as cancer types vary in radiosensitivity, this must also be considered when selecting patients for PSMA-RLT.

Overall, PSMA-RLT can still hold promise as a treatment for non-prostate cancers. Although challenges remain, such as heterogeneous PSMA expression and lower tracer uptake, for some malignancies significant treatment responses may be achieved compared with currently available treatment options [17, 18]. Especially since PSMA expression appears to increase with tumour aggressiveness, in cases of intrapatient heterogeneity PSMA-RLT could target the most aggressive lesions. Combining PSMA-RLT with other systemic treatments could potentially enhance treatment efficacy and lead to more durable responses. Before large-scale trials can be justified, a deeper understanding is needed of the mechanisms driving PSMA expression and PSMA tracer retention in non-prostate cancers. PSMA expression could potentially be induced or enhanced pharmacologically to expand the patient population eligible for PSMA-RLT. Further data on PSMA-RLT dosimetry and predictive biomarkers is needed. Caution is warranted regarding dose-limiting toxicities and long-term effects in non-prostate cancer patients, particularly in younger patients. Meanwhile, developments in PSMA research for prostate cancer must be closely monitored, e.g. on the development of radiotracers with improved retention time or longer half-life, or on improvements in PSMA-RLT efficacy.

From tumour type to tumour characteristics in treatment decision-making

Looking ahead, I believe that the paradigm in oncology is gradually shifting from treatment strategies based on tumour type to treatment strategies based on tumour characteristics, such as targetable genetics or proteins. A good example of this is the currently recruiting DRUP trial (NCT02925234), which is a prospective, pan-cancer clinical trial designed to evaluate the efficacy of off-label, targeted anticancer drugs ("-mabs" and "-nibs"), which are selected based on the results of a molecular profiling test of the patient's tumour. This allows drugs that were originally developed for a specific tumour type to be used for treating other tumours that share the same targetable characteristic.

Following the same principle, radionuclide treatments can be used in all tumours with evident expression of their molecular target. Besides PSMA, there are also other proteins that are expressed in a multitude of tumours, such as fibroblast activation protein (FAP), C-X-C chemokine receptor type 4 (CXCR4) and somatostatin receptors (SSTR) [19–22]. By using similar methods as the

DRUP trial, a panel of molecular targets can be tested for across different tumour types to identify eligible patients for radionuclide treatment. Such use of targeted treatments based on tumour characteristics will enable more personalised treatment strategies and increase available treatment options in a subset of patients, which may increase effectiveness and improve patient outcomes. Potentially, in the case of highly heterogeneous tumours, dual-targeted tracers or combination therapies may offer an even more effective approach. With the discovery of new molecular targets within oncology, the availability of radionuclide treatments can be expanded even more in the future.

REFERENCES

1. Hofman MS, Lawrentschuk N, Francis RJ, Tang C, Vela I, Thomas P, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. Lancet. 2020;395(10231):1208–16.
2. Hope TA, Benz M, Jiang F, Thompson D, Barbato F, Juarez R, et al. Do Bone Scans Overstage Disease Compared with PSMA PET at Initial Staging? An International Multicenter Retrospective Study with Masked Independent Readers. J Nucl Med. 2023;64(11):1744–7.
3. Ravi P, Xie W, Buyse M, Halabi S, Kantoff PW, Sartor O, et al. Refining Risk Stratification of High-risk and Locoregional Prostate Cancer: A Pooled Analysis of Randomized Trials. Eur Urol. 2025;87(2):217–24.
4. Karpinski MJ, Husing J, Claassen K, Moller L, Kajuter H, Oesterling F, et al. Combining PSMA-PET and PROMISE to re-define disease stage and risk in patients with prostate cancer: a multicentre retrospective study. Lancet Oncol. 2024;25(9):1188–201.
5. Glass TR, Tangen CM, Crawford ED, Thompson I. Metastatic carcinoma of the prostate: identifying prognostic groups using recursive partitioning. J Urol. 2003;169(1):164–9.
6. James ND, Spears MR, Clarke NW, Dearnaley DP, De Bono JS, Gale J, et al. Survival with Newly Diagnosed Metastatic Prostate Cancer in the "Docetaxel Era": Data from 917 Patients in the Control Arm of the STAMPEDE Trial (MRC PR08, CRUK/06/019). Eur Urol. 2015;67(6):1028–38.
7. Freedland SJ, Davis M, Epstein AJ, Arondekar B, Ivanova JI. Real-world treatment patterns and overall survival among men with Metastatic Castration-Resistant Prostate Cancer (mCRPC) in the US Medicare population. Prostate Cancer Prostatic Dis. 2023.
8. Telli TA, Ozguven S, Alan O, Filizoglu N, Ozturk MA, Sariyar N, et al. Role of baseline (68) Ga-PSMA PET/CT-derived whole-body volumetric parameters in predicting survival outcomes of metastatic castration-resistant prostate cancer patients receiving first-line treatment. Ann Nucl Med. 2022;36(11):964–75.
9. Shagera QA, Karfis I, Sideris S, Guiot T, Woff E, Martinez-Chanza N, et al. Tumor Volume on PSMA PET as a Prognostic Biomarker in Prostate Cancer Patients Treated With Cabazitaxel. Clin Nucl Med. 2023;48(9):775–80.
10. Has Simsek D, Kuyumcu S, Karadogan S, Oflas M, Isik EG, Ozkan ZG, et al. Can PSMA-based tumor burden predict response to docetaxel treatment in metastatic castration-resistant prostate cancer? Ann Nucl Med. 2021;35(6):680–90.
11. Mittlmeier LM, Brendel M, Beyer L, Albert NL, Todica A, Zacherl MJ, et al. Feasibility of Different Tumor Delineation Approaches for (18)F-PSMA-1007 PET/CT Imaging in Prostate Cancer Patients. Front Oncol. 2021;11:663631.

12. Fanti S, Goffin K, Hadaschik BA, Herrmann K, Maurer T, MacLennan S, et al. Consensus statements on PSMA PET/CT response assessment criteria in prostate cancer. Eur J Nucl Med Mol Imaging. 2021;48(2):469–76.

13. Fanti S, Hadaschik B, Herrmann K. Proposal for Systemic-Therapy Response-Assessment Criteria at the Time of PSMA PET/CT Imaging: The PSMA PET Progression Criteria. J Nucl Med. 2020;61(5):678–82.

14. Gafita A, Rauscher I, Weber M, Hadaschik B, Wang H, Armstrong WR, et al. Novel Framework for Treatment Response Evaluation Using PSMA PET/CT in Patients with Metastatic Castration-Resistant Prostate Cancer (RECIP 1.0): An International Multicenter Study. J Nucl Med. 2022;63(11):1651–8.

15. Sormani MP. The Will Rogers phenomenon: the effect of different diagnostic criteria. J Neurol Sci. 2009;287 Suppl 1:S46–9.

16. Fryback DG, Thornbury JR. The efficacy of diagnostic imaging. Med Decis Making. 1991;11(2):88–94.

17. Uijen MJM, Derks YHW, Merkx RIJ, Schilham MGM, Roosen J, Prive BM, et al. PSMA radioligand therapy for solid tumors other than prostate cancer: background, opportunities, challenges, and first clinical reports. Eur J Nucl Med Mol Imaging. 2021;48(13):4350–68.

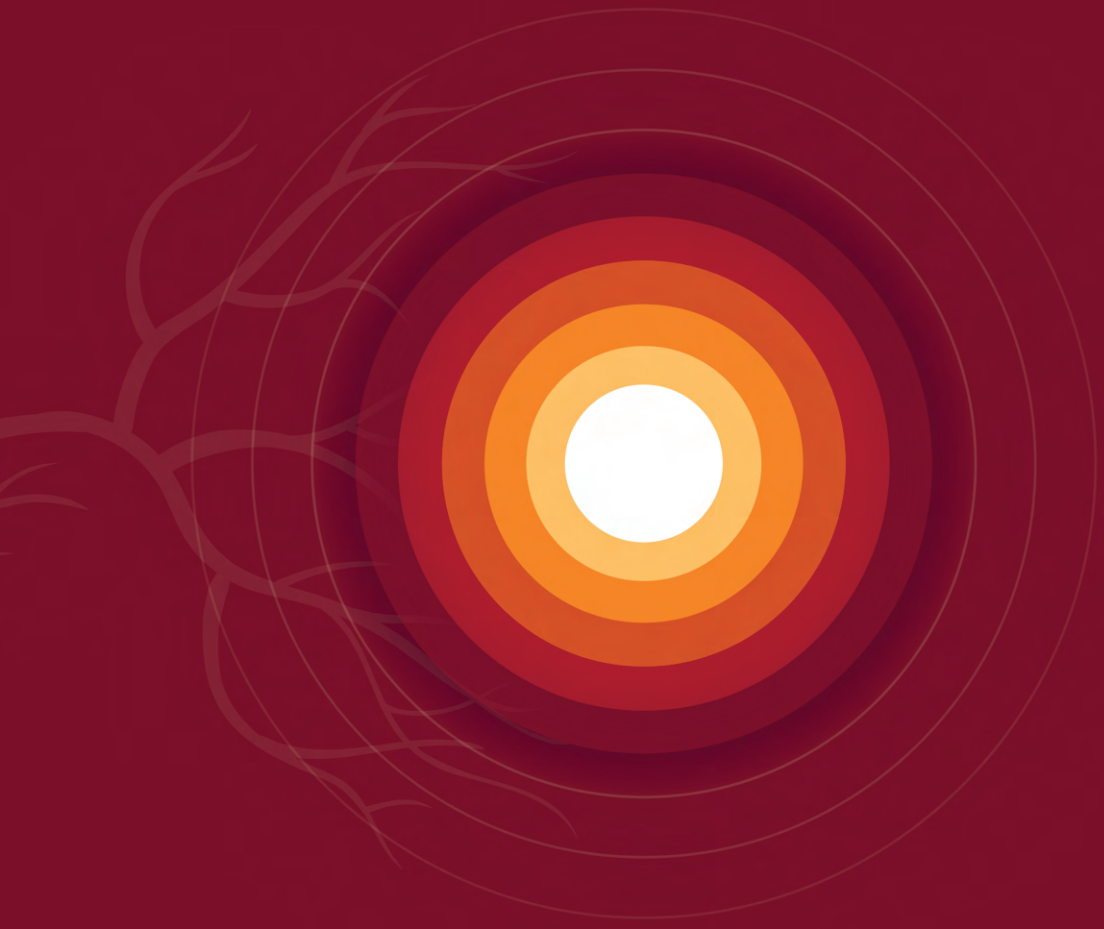
18. Wang JH, Kiess AP. PSMA-targeted therapy for non-prostate cancers. Front Oncol. 2023;13:1220586.

19. Filippi L, Ferrari C, Rubini G. Theranostic strategies in sarcoma: preliminary clinical evidence. Expert Opin Investig Drugs. 2024;33(11):1119–27.

20. Buck AK, Serfling SE, Lindner T, Hanscheid H, Schirbel A, Hahner S, et al. CXCR4-targeted theranostics in oncology. Eur J Nucl Med Mol Imaging. 2022;49(12):4133–44.

21. Vag T, Gerngross C, Herhaus P, Eiber M, Philipp-Abbrederis K, Graner FP, et al. First Experience with Chemokine Receptor CXCR4-Targeted PET Imaging of Patients with Solid Cancers. J Nucl Med. 2016;57(5):741–6.

22. Schillaci O, Corleto VD, Annibale B, Scopinaro F, Delle Fave G. Single photon emission computed tomography procedure improves accuracy of somatostatin receptor scintigraphy in gastro-entero pancreatic tumours. Ital J Gastroenterol Hepatol. 1999;31 Suppl 2:S186–9.



APPENDICES

SUMMARY OF THIS THESIS

For patients with prostate cancer, accurate disease staging and risk stratification are crucial for guiding and optimising treatment decisions. In the last decade, prostate-specific membrane antigen (PSMA) targeted PET/CT has emerged as a novel imaging modality that allows for more accurate detection of tumour lesions compared to conventional imaging (CT and bone scintigraphy). PSMA PET/CT can also provide a multitude of quantitative parameters that may reflect tumour burden and aggressiveness of the disease. At the same time, PSMA has been investigated as a target for molecular imaging and therapy in malignancies beyond prostate cancer. The aim of this thesis was to evaluate the clinical value of PSMA PET/CT in different stages of prostate cancer, to explore its prognostic potential, and to investigate the feasibility of PSMA-targeted treatment in non-prostate tumours such as sarcomas and gastrointestinal cancers.

Part 1: PSMA PET/CT in prostate cancer

The first part of this thesis focuses on PSMA-targeted PET/CT imaging in patients with prostate cancer. Chapter 2 describes stage migration on PSMA PET/CT in comparison to conventional imaging in high-risk prostate cancer patients that are referred for radiation therapy. This preplanned substudy of the prospective phase 2/3 THUNDER trial used data collected in the first year of the trial. All patients underwent both conventional imaging and PSMA PET/CT before treatment. PSMA PET/CT led to disease upstaging in 42 of 142 patients (30%). Upstaging from localised to pelvic nodal disease occurred in 23% of patients, while upstaging from non-metastatic to metastatic occurred in 13% of patients. The rate of upstaging was approximately twice as high as in previously published proPSMA trial results, likely due to THUNDER trial cohort having more aggressive disease characteristics than the proPSMA trial cohort. Importantly, the probability of upstaging increased with the number of high-risk features at baseline. These findings highlight the importance of using PSMA PET/CT for staging, especially in patients with multiple high-risk features, and suggest the need for treatment adaptations accordingly. This will be further investigated in the THUNDER trial.

The prognostic value of pre-treatment PSMA PET/CT in patients with synchronous metastatic hormone-sensitive prostate cancer (mHSPC) and metastatic castration-resistant prostate cancer (mCRPC), is evaluated in Chapters 3 and 4, respectively. In both patient groups, PSMA-derived total tumour volume (PSMA-TV) was a robust predictor of overall survival (OS). For patients with mHSPC, this

is particularly relevant as they have traditionally been stratified using conventional imaging into low-volume or high-volume disease, which require different treatment strategies. As PSMA PET/CT is currently used for staging, there is a need for PSMA PET/CT-based stratification criteria to guide treatment decisions. In a retrospective study with 204 synchronous mHSPC patients, multiple PSMA PET/CT-based parameters and clinical parameters were retrieved to assess their prognostic value for OS (Chapter 3). As PSMA-TV was the strongest predictor of OS, independent of WHO performance score and received treatment, we recommend integrating PSMA-TV into clinical decision-making. In our analysed cohort, the optimal threshold for PSMA-TV-based high-volume disease in terms of differentiating short-term and long-term survivors was: PSMA-TV ≥150 mL or any visceral metastasis. However, instead of using a single cut-off value, which results in loss of prognostic information, we propose focusing on the development of a prognostic model incorporating PSMA-TV as a continuous variable. Multicentre validation with a large dataset is needed before it can be implemented in phase 3 trials or clinical practice.

For patients with mCRPC, which is a very heterogeneous disease with varying survival outcomes, prognostic markers can help guide decisions regarding initiating or not initiating treatment. In 60 mCRPC patients that were treated with androgen receptor targeted agents (ARTAs) or chemotherapy, both pretreatment PSMA PET/CT-based parameters and clinical parameters were collected (Chapter 4). PSMA-TV was the best prognostic imaging-based biomarker for OS, followed by total lesion uptake (TL-PSMA) and DmaxVox (distance between the two outermost voxels in cm). In clinical centres where automated total tumour volume segmentation software is not available, DmaxVox may serve as an alternative to PSMA-TV. Notably, PSA levels alone were not prognostic, but when combined with PSMA-TV, lower PSA was associated with worse outcomes, likely reflecting tumour dedifferentiation. Additionally, as all patients had a follow-up PSMA PET/CT after 3 – 4 months, a preliminary lesion-level analysis was performed to assess which lesion characteristics were associated with lesion progression. Results showed that higher lesion SUV_{mean} and lesion location in bone (in comparison to prostate lesions) were associated with higher rates of disease progression after 3 – 4 months.

Chapter 5 describes a study comparing PSMA PET/CT with PSA levels for monitoring treatment response in the same patient populations as described in Chapter 4; 60 mCRPC patients treated with an ARTA or chemotherapy. PSMA

PET/CT scans were performed a predefined time points; at baseline and after three months in case of ARTA treatment, or after completion of chemotherapy (after approximately 4 months). The study found that PSMA PET/CT response was the strongest independent predictor of OS, outperforming PSA response. Discordance between PSA and PSMA PET/CT response was observed in nearly half of patients. Strikingly, 31% of patients who achieved a PSA decline >50% still showed progressive disease on PSMA PET/CT, which was associated with significantly worse survival. These findings support the use of PSMA PET/CT for treatment response evaluation in mCRPC patients, as it can likely detect progression of disease earlier than PSA. This could enable earlier discontinuation of ineffective treatments and earlier initiation of alternative treatments, thereby potentially reducing toxicity and costs. Further research is necessary on the cost-effectiveness and impact on patient outcomes.

Part 2: PSMA PET/CT in non-prostate cancer

In contrast to what its name suggests, PSMA is also expressed in a wide variety of other malignancies, mainly in the tumour-associated neovasculature. In this thesis, PSMA expression and PSMA PET/CT imaging in sarcomas and gastrointestinal cancers are evaluated. Chapter 6 provides an overview of available literature on PSMA expression and PSMA PET/CT in sarcomas. In immunohistochemistry studies analysing sarcoma tissue samples, PSMA expression was more frequent in sarcomas compared to soft tissue and bone tumours with benign or intermediate biological potential. Furthermore, a multitude of case reports was published on PSMA-tracer accumulation in sarcomas, reaching SUV_{max} values of up to 35. Strikingly, strong PSMA expression and high PSMA uptake on PET/CT imaging seemed to be most prevalent in aggressive and advanced sarcomas. Together, this means that potentially a subgroup of sarcoma patients can be eligible for PSMA-targeted radioligand therapy (PSMA-RLT). However, no prospective data existed on PSMA expression and PSMA PET/CT in sarcoma patients, and insights into patient selection methods were lacking.

Therefore, we performed a prospective feasibility study to assess the feasibility of PSMA-RLT in patients with metastatic soft tissue sarcoma (Chapter 7). Twenty-five patients across seven histological subtypes were included. Immunohistochemistry was performed on tumour samples to assess PSMA expression, and patients with high PSMA expression underwent PSMA PET/CT imaging. PSMA expression was observed in 72% of patients, with high expression in 44%. However, PET/CT imaging revealed heterogeneous tracer uptake across lesions, with SUV_{max}

values ranging widely between sites. Although some lesions demonstrated sufficient uptake, heterogeneity limited the feasibility of PSMA-RLT in this cohort, leading to early study termination. This study underscored both the promise and the challenges of extending PSMA-RLT to soft tissue sarcoma patients. While high PSMA-tracer accumulation was seen in several metastatic sites, variability in tracer uptake complicate potential treatment with PSMA-RLT. Future research should focus on understanding the underlying biology of this heterogeneous PSMA expression in sarcomas and on refining patient selection criteria.

Chapter 8 provides the results of another prospective feasibility study, in which PSMA PET/CT imaging was performed in ten patients with colon, gastric or pancreatic cancer. PSMA PET/CT detected the primary tumour in seven out of ten patients, but uptake was generally low and heterogeneous, with poor tumour-to-background ratios. No correlation was found between PSMA expression in resection specimens and PSMA-tracer uptake on PET/CT imaging. [^{18}F]FDG PET/CT was superior in all cases, highlighting that PSMA PET/CT has no role for diagnostic purposes.

In conclusion, the studies presented in this thesis demonstrate that PSMA PET/CT provides added value across multiple stages of prostate cancer. It improves staging in high-risk patients, provides prognostic biomarkers in both hormone-sensitive and castration-resistant metastatic disease, and offers superior treatment response monitoring compared to PSA values in castration-resistant prostate cancer. These findings support the further evaluation of integrating PSMA PET/CT into routine clinical practice for these purposes. Beyond prostate cancer, exploratory work in sarcomas and gastrointestinal tumours revealed that although adequate PSMA-tracer uptake could be achieved, uptake patterns were too heterogeneous across lesions for patients to be eligible for PSMA-targeted treatment. Further research is required to address challenges related to treatment efficacy and patient selection. Once these challenges have been overcome, PSMA-targeted treatment could become a valuable treatment option for selected non-prostate cancer patients, potentially in combination with other treatments.

NEDERLANDSE SAMENVATTING

Voor patiënten met prostaatkanker zijn een nauwkeurige stadiëring van de ziekte en risicostratificatie cruciaal voor het maken en optimaliseren van behandelingsbeslissingen. In het afgelopen decennium is gebleken dat beeldvorming met prostaat-specifiek membraan antigeen (PSMA) PET/CT een nauwkeurigere detectie van tumorlaesies mogelijk maakt in vergelijking met conventionele beeldvorming (CT en botscintigrafie). PSMA PET/CT scans kunnen ook meerdere kwantitatieve parameters aanleveren die een indicatie kunnen geven van de tumorload en de agressiviteit van de ziekte. Tegelijkertijd is PSMA onderzocht als doelwit voor moleculaire beeldvorming en therapie bij andere maligniteiten buiten prostaatkanker. Het doel van dit proefschrift was om de klinische waarde van PSMA PET/CT in verschillende stadia van prostaatkanker te evalueren, het prognostische potentieel ervan te onderzoeken en de haalbaarheid van PSMA-gerichte behandeling bij niet-prostaattumoren, zoals sarcomen en gastro-intestinale kankers, te onderzoeken.

Deel 1: PSMA PET/CT bij prostaatkanker

Het eerste deel van dit proefschrift richt zich op beeldvorming met PSMA-gerichte PET/CT bij patiënten met prostaatkanker. Hoofdstuk 2 beschrijft verschuiving van stadiëring op basis van PSMA PET/CT in vergelijking met conventionele beeldvorming bij hoog-risicopatiënten met prostaatkanker die worden doorverwezen voor radiotherapie. Deze vooraf geplande substudie van de prospectieve fase 2/3 THUNDER studie maakte gebruik van gegevens die in het eerste jaar van de studie waren verzameld. Alle patiënten ondergingen zowel conventionele beeldvorming als PSMA PET/CT voor start van de behandeling. PSMA PET/CT leidde tot een hogere stadiëring van de ziekte bij 42 van de 142 patiënten (30%). Bij 23% van de patiënten vond een verschuiving plaats van gelokaliseerde ziekte naar pelviene lymfeklieren, terwijl bij 13% van de patiënten een verschuiving plaatsvond van niet-gemetastaseerde naar gemetastaseerde ziekte. Deze percentages waren ongeveer twee keer zo hoog als in eerder gepubliceerde resultaten van de proPSMA studie, waarschijnlijk omdat de patiënten in de THUNDER studie agressievere ziektekenmerken hadden dan de patiënten in de proPSMA studie. Belangrijk is dat de kans op verschuiving in stadiëring toenam met het aantal hoog-risicofactoren bij diagnose. Deze bevindingen benadrukken het belang van het gebruik van PSMA PET/CT voor stadiëring, vooral bij patiënten met meerdere hoog-risicofactoren, en wijzen op de noodzaak om de behandeling hierop aan te passen. Dit zal verder worden onderzocht in de THUNDER studie.

De prognostische waarde van de PSMA PET/CT voor start van de behandeling bij patiënten met synchroon gemetastaseerd hormoongevoelig prostaatcarcinoom (mHSPC) en gemetastaseerd castratieresistente prostaatcarcinoom (mCRPC) wordt respectievelijk in hoofdstuk 3 en 4 geëvalueerd. In beide patiëntengroepen was het totale tumorvolume (PSMA-TV) een sterke voorspeller van de totale overleving (overall survival, OS). Voor patiënten met mHSPC is dit bijzonder relevant, aangezien deze patiënten traditioneel met behulp van conventionele beeldvorming werden gestratificeerd in laag-volume of hoog-volume ziekte, waarvoor verschillende behandelingsstrategieën gelden. Aangezien PSMA PET/CT momenteel wordt gebruikt voor stadiëring, zijn op PSMA PET/CT gebaseerde stratificatiecriteria nodig om behandelingsbeslissingen te maken. In een retrospectieve studie met 204 synchroon mHSPC patiënten werden meerdere PSMA PET/CT parameters en klinische parameters verzameld om hun prognostische waarde voor OS te beoordelen (hoofdstuk 3). Aangezien PSMA-TV de sterkste voorspeller van OS was, onafhankelijk van de WHO performance status en de ontvangen behandeling, raden wij aan om PSMA-TV te integreren in de klinische besluitvorming. In ons geanalyseerde cohort was de optimale grenswaarde voor het onderscheiden van korte- en lange-termijn overlevers: hoog-volume ziekte = PSMA-TV ≥150 ml en/of viscerale metastasen. In plaats van een grenswaarde te gebruiken, wat leidt tot verlies van prognostische informatie, stellen we echter voor om ons te richten op de ontwikkeling van een prognostisch model waarin PSMA-TV als continue variabele wordt opgenomen. Multicenter validatie met een grote dataset is nodig voordat dit kan worden geïmplementeerd in fase 3 onderzoeken of de klinische praktijk.

Voor patiënten met mCRPC, een zeer heterogene ziekte met uiteenlopende overlevingskansen, kunnen prognostische markers helpen bij het nemen van beslissingen over het al dan niet starten van een behandeling. Bij 60 mCRPC patiënten die werden behandeld met androgeenreceptor gerichte middelen (ARTA's) of chemotherapie, werden zowel PSMA PET/CT parameters als klinische parameters van voor start van de behandeling verzameld (hoofdstuk 4). PSMA-TV was de beste prognostische beeldvorming-gebaseerde biomarker voor OS, gevolgd door totale tracer accumulatie (TL-PSMA) en DmaxVox (afstand tussen de twee verst gelegen voxels in cm). In klinische centra waar geen geautomatiseerde software voor segmentatie van het totale tumorvolume beschikbaar is, kan DmaxVox dienen als alternatief voor PSMA-TV. Opvallend was dat PSA-waarden op zichzelf geen prognostische waarde hadden, maar in combinatie met PSMA-TV ging een lagere PSA-waarde gepaard met slechtere

uitkomsten, wat waarschijnlijk een weerspiegeling was van tumor dedifferentiatie. Verder, aangezien alle patiënten na 3-4 maanden een follow-up PSMA PET/CT ondergingen, werd een oriënterende analyse op laesieniveau uitgevoerd om te beoordelen welke laesiekenmerken geassocieerd zijn met laesieprogressie. De resultaten toonden aan dat een hogere SUV_{mean} van de laesie en botlaesies (in vergelijking met prostaatlaesies) geassocieerd waren met hogere percentages van ziekteprogressie na 3-4 maanden.

Hoofdstuk 5 beschrijft het onderzoek waarin PSMA PET/CT wordt vergeleken met PSA-waarden voor het monitoren van therapierespons bij dezelfde patiëntenpopulatie als beschreven in hoofdstuk 4: 60 mCRPC patiënten die werden behandeld met een ARTA of chemotherapie. PSMA PET/CT scans werden uitgevoerd op vooraf bepaalde tijdstippen: bij aanvang en na drie maanden in het geval van ARTA behandeling, of na voltooiing van de chemotherapie (na ongeveer 4 maanden). Uit het onderzoek bleek dat de therapierespons op basis van PSMA PET/CT de sterkste onafhankelijke voorspeller van OS was, beter dan de PSA respons. Bij bijna de helft van de patiënten werd een discrepantie tussen de PSA en PSMA PET/CT respons waargenomen. Opvallend was dat bij 31% van de patiënten die een PSA daling van >50% bereikten, de PSMA PET/CT progressieve ziekte vertoonde, wat geassocieerd was met een significant slechtere overleving. Deze bevindingen ondersteunen het gebruik van PSMA PET/CT voor de evaluatie van therapierespons bij mCRPC patiënten, aangezien PSMA PET/CT de ziekteprogressie waarschijnlijk eerder kan detecteren dan PSA. Dit zou een vroegere beëindiging van ineffectieve behandelingen en een vroegere start van alternatieve behandelingen mogelijk maken, waardoor de toxiciteit en de kosten mogelijk kunnen worden verminderd. Hiervoor is verder onderzoek nodig naar de kosteneffectiviteit en de impact op de klinische uitkomsten van patiënten.

Deel 2: PSMA PET/CT bij niet-prostaatkanker

In tegenstelling tot wat de naam doet vermoeden, komt PSMA ook voor in een groot aantal andere maligniteiten, voornamelijk in de tumor-geassocieerde neovasculatuur. In dit proefschrift worden PSMA expressie en PSMA PET/CT beeldvorming bij sarcomen en gastro-intestinale kankers geëvalueerd. Hoofdstuk 6 geeft een overzicht van de beschikbare literatuur over PSMA expressie en PSMA PET/CT bij sarcomen. In immunohistochemische studies waarin weefsels van sarcomen werden geanalyseerd, kwam PSMA expressie vaker voor bij sarcomen dan bij wekedelen tumoren en bottumoren met een goedaardig of intermediair biologisch potentieel. Verder bestaan er vele casus rapporten over PSMA-tracer

accumulatie in sarcomen, waarbij SUV_{max}-waarden tot 35 werden bereikt. Opvallend was dat sterke PSMA expressie en hoge PSMA-tracer accumulatie op PET/CT het meest voorkwamen bij agressieve en gevorderde sarcomen. Samen betekent dit dat er mogelijk een subgroep van sarcoompatiënten bestaat die in aanmerking kan komen voor PSMA-gerichte radioligandtherapie (PSMA-RLT). Er waren echter geen prospectieve gegevens beschikbaar over PSMA expressie en PSMA PET/CT bij sarcoompatiënten, en er ontbrak inzicht in methoden voor patiëntselectie.

Daarom hebben we een prospectieve haalbaarheidsstudie uitgevoerd om de haalbaarheid voor PSMA-RLT bij patiënten met gemetastaseerd wekedelensarcoom te beoordelen (hoofdstuk 7). Er werden 25 patiënten met zeven histologische subtypes in de studie geïncludeerd. Er werd immuunhistochemie uitgevoerd op tumorweefsel om de PSMA expressie te beoordelen, en patiënten met een hoge PSMA expressie ondergingen een PSMA PET/CT. PSMA expressie werd waargenomen bij 72% van de patiënten, met een hoge expressie bij 44%. De PET/CT scans lieten echter heterogene tracer accumulatie zien, met SUV_{max}-waarden die sterk varieerden tussen de verschillende laesies. Hoewel sommige laesies voldoende tracer accumulatie vertoonden, beperkte de heterogeniteit tussen laesies de haalbaarheid van PSMA-RLT in dit cohort, wat leidde tot vroegtijdige beëindiging van het onderzoek. Deze studie liet zowel de mogelijkheden als de uitdagingen van het uitbreiden van PSMA-RLT naar patiënten met wekedelensarcomen zien. Hoewel hoge PSMA-tracer accumulatie werd gedetecteerd in verschillende metastasen, bemoeilijkt de variabiliteit in tracer accumulatie een mogelijke behandeling met PSMA-RLT. Toekomstig onderzoek moet zich richten op het begrijpen van de onderliggende biologie van deze heterogene PSMA expressie in sarcomen en op het verbeteren van de selectiecriteria voor patiënten.

Hoofdstuk 8 bevat de resultaten van een ander prospectief haalbaarheidsonderzoek, waarbij PSMA PET/CT scans zijn uitgevoerd bij tien patiënten met colon-, maag- of pancreascarcinoom. PSMA PET/CT detecteerde de primaire tumor bij zeven van de tien patiënten, maar de tracer accumulatie was laag en heterogeen, met weinig contrast tussen de tracer accumulatie in de tumor en de achtergrond. Er werd geen correlatie gevonden tussen PSMA expressie in resectiemateriaal en PSMA-tracer accumulatie op PET/CT. [^{18}F]FDG PET/CT was in alle gevallen superieur, wat benadrukt dat PSMA PET/CT niet geschikt is voor diagnostische doeleinden.

Concluderend tonen de studies in dit proefschrift aan dat PSMA PET/CT toegevoegde waarde biedt in meerdere stadia van prostaatkanker. Het verbetert de stadiëring bij hoog-risicopatiënten, het biedt prognostische biomarkers bij zowel hormoongevoelige als castratieresistente gemetastaseerde ziekte en biedt superieure monitoring van de therapierespons in vergelijking met PSA-waarden bij castratieresistent prostaatcarcinoom. Deze bevindingen ondersteunen de verdere evaluatie van de integratie van PSMA PET/CT in de klinische praktijk voor deze doeleinden. Naast prostaatkanker heeft verkennend onderzoek bij sarcomen en gastro-intestinale tumoren aangetoond dat, hoewel een adequate accumulatie van PSMA-tracer kon worden bereikt in sommige laesies, het accumulatie patroon te heterogeen was tussen de laesies om patiënten in aanmerking te laten komen voor een PSMA-gerichte behandeling. Er is verder onderzoek nodig om de uitdagingen rondom de effectiviteit van behandeling en patiëntselectie aan te pakken. Zodra deze uitdagingen zijn overwonnen, zou PSMA-gerichte behandeling een waardevolle behandelingsoptie kunnen worden voor geselecteerde niet-prostaatkanker patiënten, mogelijk in combinatie met andere behandelingen.

LIST OF PUBLICATIONS

1. Kleiburg F, Dirix P, Fonteyne V, et al. Stage Migration on Prostate-specific Membrane Antigen Positron Emission Tomography/Computed Tomography in Comparison to Conventional Imaging in Patients with High-risk Prostate Cancer Referred for Radiation Therapy: Results from the Phase 2/3 THUNDER Trial. *Eur Urol Oncol*. Published online September 24, 2025. doi:10.1016/j.euo.2025.08.005
2. Kleiburg F, van der Hulle T, Gelderblom H, et al. PSMA expression and PSMA PET/CT imaging in metastatic soft tissue sarcoma patients, results of a prospective study. *Eur J Nucl Med Mol Imaging.* 2025;52(10):3690-3699. doi:10.1007/s00259-025-07224-z
3. Kleiburg F, de Geus-Oei LF, Spijkerman R, et al. Baseline PSMA PET/CT parameters predict overall survival and treatment response in metastatic castration-resistant prostate cancer patients. *Eur Radiol*. 2025;35(7):4223-4232. doi:10.1007/s00330-025-11360-3
4. Kleiburg F, de Geus-Oei LF, Luelmo SAC, et al. PSMA PET/CT for treatment response evaluation at predefined time points is superior to PSA response for predicting survival in metastatic castration-resistant prostate cancer patients. *Eur J Radiol*. 2024;181:111774. doi:10.1016/j.ejrad.2024.111774
5. Kleiburg F, Heijmen L, Gelderblom H, Kielbasa SM, Bovée JV, De Geus-Oei LF. Prostate-specific membrane antigen (PSMA) as a potential target for molecular imaging and treatment in bone and soft tissue sarcomas. *Br J Radiol*. 2023;96(1145):20220886. doi:10.1259/bjr.20220886
6. Vuijk FA, Kleiburg F, Noortman WA, et al. Prostate-Specific Membrane Antigen Targeted Pet/CT Imaging in Patients with Colon, Gastric and Pancreatic Cancer. *Cancers (Basel)*. 2022;14(24):6209. doi:10.3390/cancers14246209
7. van Eyk HJ, Paiman EHM, Bizino MB, et al. Liraglutide decreases energy expenditure and does not affect the fat fraction of supraclavicular brown adipose tissue in patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis*. 2020;30(4):616-624. doi:10.1016/j.numecd.2019.12.005

CURRICULUM VITAE

Fleur Kleiburg werd op 18 augustus 1994 geboren in Amstelveen. In 2012 behaalde zij cum laude haar tweetalig gymnasium diploma aan het Rijnlands Lyceum Oegstgeest. Aansluitend begon zij met de bachelor Geneeskunde aan de Universiteit Leiden, die zij in 2015 afrondde. Daarna nam zij een tussenjaar, waarin zij door Australië en Nieuw-Zeeland reisde en bestuurslid was van studentenroeivereniging Asopos de Vliet. In deze periode gaf zij ook regelmatig bijles in wiskunde aan middelbare scholieren.

Van 2017 tot 2020 volgde zij de master Geneeskunde aan de Universiteit Leiden. Voor haar coschap kindergeneeskunde werkte zij in het Academisch Ziekenhuis Paramaribo in Suriname, en haar wetenschappelijke stage deed zij op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum. Gedurende haar master was zij tevens vicevoorzitter van de Nederlandse Studenten Roeifederatie, met als portefeuille acquisitie en externe relaties.

In september 2020 startte zij haar promotieonderzoek naar PSMA PET/CT beeldvorming bij prostaat- en niet-prostaatkankers aan de afdeling Nucleaire Geneeskunde van het Leids Universitair Medisch Centrum en de Biomedical Photonic Imaging Group van de Universiteit Twente. Van januari tot maart 2025 werkte zij in Antwerpen samen met radiotherapeut-oncoloog Piet Ost aan de eerstejaars resultaten van de THUNDER studie. Deze resultaten presenteerde zij als Late Breaking Abstract tijdens ESTRO 2025 in Wenen.

Parallel aan haar promotietraject was zij van 2020 tot 2023 voorzitter van JongLUMC, met als doel om alle jonge professionals binnen het LUMC met elkaar in verbinding te brengen. Daarnaast werkte zij parttime bij de GGD Hollands Midden als medisch supervisor op COVID-19 vaccinatielocaties.

In mei 2025 begon zij als ANIOS Interne Geneeskunde in het Antoni van Leeuwenhoek. In maart 2026 start zij met de opleiding tot radiotherapeut-oncoloog aan het Amsterdam UMC.

DANKWOORD

Een promotieonderzoek doe je gelukkig niet alleen. Ik heb ontzettend genoten van mijn onderzoekstijd de afgelopen jaren en dat had nooit gekund zonder alle samenwerkingen, steun en gezelligheid die ik heb ervaren.

Beste Lioe-Fee, dankjewel voor je vertrouwen toen je me de PhD plek aanbood zonder eerder met mij te hebben samengewerkt. Je enthousiasme en warmte, maar ook je scherpe blik en ideeën hebben me erg geholpen. Zonder jou was ik bijvoorbeeld niet op het idee gekomen om naar Antwerpen te gaan, wat mij ontzettend veel gebracht heeft. Ik hoop nog lang met je te mogen samenwerken, we hebben ideeën genoeg!

Beste Linda, je bent een eindeloze bron van kennis op het gebied van PSMA, daar heb ik heel veel aan gehad. Ook mocht ik altijd bij je aankloppen als ik ergens vastliep of input nodig had. Beste Tom, jouw medisch oncologische blik binnen dit nucleair geneeskundig onderzoek was erg waardevol. Je bent altijd gemotiveerd om na te denken over hoe we het beter kunnen doen voor onze patiënten. Beste Srirang, dank voor de tips die je tijdens onze evaluatie momenten hebt gegeven om mijn promotietraject zo soepel mogelijk te laten verlopen.

Graag wil ik alle co-auteurs bedanken voor de fijne samenwerking, en alle collega's van de Nucleaire Geneeskunde afdeling voor hun hulp bij het uitvoeren van de onderzoeken. Aan de leden van de promotiecommissie; dank voor de tijd en moeite die jullie hebben gestoken in het beoordelen van dit proefschrift. Ik hoop leuke discussies met jullie te kunnen voeren de 20e!

PSMA Forum Nederland, dank voor het organiseren van de inspirerende netwerk- en kennisdelingsbijeenkomsten, het podium dat jullie mij meermaals hebben gegeven om over mijn eigen onderzoek te vertellen en de financiële bijdrage die jullie hebben gegeven voor het onderzoeksproject in Antwerpen. Daniela en Jacqueline, het is altijd gezellig om jullie te zien bij evenementen en congressen.

Beste Piet, ik vond het een hele leuke en leerzame ervaring om met jou drie maanden te mogen samenwerken in Antwerpen. Je hebt me laten zien hoe mooi de radiotherapie als vakgebied is en me geïnspireerd om mijn carrière in die richting voort te zetten. Dank voor de kansen die je me hebt gegeven, zoals het

presenteren van de THUNDER data op het hoofdpodium van ESTRO, dat was een hele bijzondere ervaring.

Dank aan alle collega PhD'ers met wie ik eindeloos veel uren heb doorgebracht in het LUMC; achter de computer, in het LUMC restaurant of achter de bar in Hepatho. Pim, Timo, Wyanne, Maaike, Alina, Lisanne, Sietse, Gonnie, Ylva, Coosje, Sarah, Linde, Vesna, Faeze en alle studenten die bij ons een stage hebben gedaan, vooral door jullie heb ik een ontzettend leuke en gezellige tijd gehad! Met als hoogtepunten natuurlijk de weekendjes weg, schrijfweken, borrels in Hepatho met een Hangende Harry en een Disco Daisy, de karaoke avonden bij Van Melzen en de congressen in Barcelona, Hamburg, Wenen en Berlijn.

Lieve vriendinnen, ik heb zo veel geluk met jullie. Roos, Nadia, Leoni, Ilse, ik ben erg blij dat we nog regelmatig etentjes inplannen en kunnen bijkletsen over de leuke (en soms ook minder leuke) dingen in het leven. Suus, Myrna en Annet, ik word altijd vrolijk van jullie en ik weet dat ik altijd bij jullie terecht kan. Maartje en Valerie, we kennen elkaar inmiddels al bijna 20 jaar (!) en dat vind ik toch heel bijzonder. We hebben weinig woorden nodig om aan elkaar uit te leggen hoe het met ons gaat. Ik hoop jullie allemaal nog lang in mijn leven te hebben.

Aan mijn mede bestuursleden van het 55e verenigingsbestuur van Asopos de Vliet, het NSRF bestuur en het JongLUMC bestuur: ik ben heel blij dat ik jullie heb leren kennen en ik heb veel goede herinneringen aan de vele uren die we samen hebben doorgebracht. Ook wil ik graag mijn roeiploeggenootjes van Pink Lady, mijn mede Asopos fanatiekelingen van de Asoposhoek, mijn gildegenootjes van PS en de trailrun vriendengroep bedanken voor al hun gezelligheid.

Lieve AvL collega's, ik heb enorm veel steun van jullie ervaren in het turbulente jaar dat 2025 voor mij was en daar ben ik jullie erg dankbaar voor. Ik heb hele fijne en blije herinneringen aan het AvL, ondanks dat er ook moeilijke momenten waren met mijn moeder, en dat komt door jullie.

Mijn lieve familie en schoonfamilie, ik wil jullie heel erg bedanken voor jullie betrokkenheid en interesse, zowel in mijn promotieonderzoek als alles daarbuiten!

Lieve mam, helaas hebben we op 27 juni afscheid van je moeten nemen en ben je er niet meer om deze mijlpaal mee te maken. Ik zal nooit vergeten hoe trots je was als ik bijvoorbeeld vertelde over een presentatie op een congres of een nieuwe

publicatie. Jouw positieve energie en jouw levensmotto “Embrace Life” zullen me voor altijd bijblijven.

Lieve pap, thuis is voor mij altijd een warme plek geweest, daar heb jij samen met mam voor gezorgd. Dankjewel voor de onvoorwaardelijke steun en liefde, en voor hoe ontzettend trots je altijd op me bent. Ik had me geen betere vader kunnen wensen.

Lieve Sacha, je hebt tijdens iedere stap van deze onderzoekstijd, zowel de successen als de tegenslagen, aan mijn zijde gestaan. Dankjewel voor al je steun en voor de motivatie die je me gaf op momenten dat ik die even nodig had. Ik kijk uit naar alle volgende hoofdstukken van ons leven!