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An open-label, single-arm, rater-blinded, multicenter phase 1/2 study to assess safety and diagnostic accuracy and radiotherapeutic implications of pre-operative Ga-68-PSMA-11 PET/CT imaging in comparison to histopathology, in newly-diagnosed prostate cancer (PCA) patients at high risk for metastasis, scheduled for radical prostatectomy (RP) with extended pelvic lymph node dissection (EPLND)

Short Title	Ga-68-PSMA-11 in high-risk prostate cancer				
Test substance	Ga-68-PSMA-11		Phase:	1/2	
Study No.:	Ga-68-PSMA-11	EudraCT No.:	2016-001815-19	IND:	N/A
First patient first visit	09-Oct-2017	Last patient last visit	03-Jul-2020	End of Study	10-Feb-2021
Date:	29-NOV-2021		Version: 1.0		
Sponsor:	Deutsches Krebsforschungszentrum (DKFZ)				

Synopsis

Date of report:	29-Nov-2021
Study title:	An open-label, single-arm, rater-blinded, multicenter phase 1/2 study to assess safety and diagnostic accuracy and radiotherapeutic implications of pre-operative Ga-68-PSMA-11 PET/CT imaging in comparison to histopathology, in newly-diagnosed prostate cancer (PCA) patients at high risk for metastasis, scheduled for radical prostatectomy (RP) with extended pelvic lymph node dissection (EPLND).
Sponsor's study number:	Ga-68-PSMA-11
NCT number:	NCT03362359
EudraCT number:	2016-001815-19
Sponsor:	DKFZ
Clinical phase:	1/2
Investigators:	Austria PI – Prof. Dr. Irene Virgolini (Innsbruck) Germany- PI: Prof. Frederik Giesel (Heidelberg), Coordinating Investigators Prof. Dr. Jörg Kortzerke (Dresden), Prof. Dr. med. Ken Hermann (Essen), Prof. Dr. med. Dr. nat. med. Philipp Tobias Meyer (Freiburg), Prof. Dr. Christian LaFougère (Tübingen), Prof. Dr. Markus Schwaiger changed to Prof. Dr. med. Wolfgang Weber during the study (TU München), Prof. Dr. med. Torsten Kuwert (Erlangen) Switzerland PI – PD Dr. Irene Andrea Burger (Zurich)
Study centres:	Austria (1 centre), Germany (7 centres) and Switzerland (1 centre)
Study objectives:	**Primary objectives** 1. To assess the ability of Ga-68-PSMA-11 PET/CT imaging to detect prostate cancer tissue within a. the prostate gland on level of quadrant (or octant if possible) and b. pelvic lymph node metastases on level of 8 defined sub-regions 2. To assess the clinical safety of Ga-68-PSMA-11.

	Secondary objectives 1. To assess the ability of Ga-68-PSMA-11 PET/CT imaging to detect bone metastases in comparison to ^{99m}Tc bone scintigraphy 2. To compare Ga-68-PSMA-11 uptake in primary tumours with Gleason score in surgical specimens from RP. 3. Determine the percentage of patients in which pre-operative Ga 68-PSMA-11 PET/CT imaging would result into a change in clinical management. 4. To evaluate the impact of pre-operative Ga-68-PSMA-11 PET/CT imaging on target volume definition for radiotherapy. 5. Exploratory analysis of molecular and clinical biomarkers (Circulating tumour cells [CTC], RNA, DNA, proteins) in blood, serum and plasma.
Test drug:	4,6,12,19-Tetraazadocosane-1,3,7-tricarboxylic acid, 22-[3-[[[2-[[[5-(2-carboxy-ethyl)-2-hydroxyphenyl]-methyl]-(carboxy-methyl)amino]ethyl](carboxymethyl)amino]-methyl]-4-hydroxyphenyl]-5,13,20-trioxo-, (3S,7S) (the PSMA-specific pharmacophore Glu-NH-CO-NH-Lys, covalently linked to the chelator HBED-CC); labelled with Gallium-68 (^{68}Ga), a synthetic positron emitting isotope of gallium, third element of the third main group (atomic number 31).
Name of active ingredients:	Ga-68-PSMA-11
Dose:	Single administration of 150 MBq (±50 MBq), corresponding to a mass dose of ≤6 µg. A 2nd administration of 150 MBq (±50 MBq), corresponding to a mass dose of ≤6 µg is possible in the unlikely case of a negative histological result (i.e. no PSMA expression in dissected lymph nodes) to verify if PSMA PET-positive tissue as seen on day 1 has not been removed during RP with EPLND.
Route of administration:	Slow intravenous injection (IV)
Duration of treatment:	Single administration of diagnostic agent. (2nd administration possible)
Reference drug:	Not applicable

Indication:	Newly diagnosed PCA with high risk of metastasis formation.
Diagnosis and main criteria for inclusion:	All patients had to meet all of the following criteria: 1. Written informed consent. 2. Male ≥18 years of age. 3. Histologically confirmed adenocarcinoma of the prostate. 4. High risk for metastasis, defined by either: a. stadium cT3 according to TNM classification, or b. Gleason Score >7, or c. PSA >20 ng/mL. 5. Patient scheduled for radical prostatectomy (RP) with extended pelvic lymph node dissection (EPLND) according to current guidelines 7–60 days after start of study. 6. Consent to practise contraception until end of study (6 days after Ga-68-PSMA-11 injection). 7. Preoperative PCA staging performed according to guidelines, to include a mandatory ^{99m}Tc bone scintigraphy and an optional pelvic MRI or CT, not older than 56 days prior to inclusion, according to standard of care.
Exclusion criteria	Patients were excluded if one or more of the following criteria were met: 1. Known hypersensitivity to Ga-68-PSMA-11 or its components. 2. Presence of known lymph node metastases outside surgical field. 3. More than 5 bone metastases, as determined by ^{99m}Tc bone scintigraphy. 4. Previous prostate cancer therapy. 5. Administration of any kind of PET tracer within a period corresponding to 8 half-lives of the respective radionuclide. 6. Any other investigational medicinal product within 30 days prior and 7 days after receiving study medication. 7. Evidence of neuroendocrine small cell carcinoma. 8. Patients not able to declare meaningful informed consent on their own (e.g. with legal guardian for mental disorders). 9. Simultaneous participation in other clinical trials

Study design:	This was an open-label, single-arm, rater-blinded, multicenter, diagnostic phase 1/2 study to assess safety and diagnostic performance of Ga-68-PSMA-11 PET/CT imaging to detect tumour tissue in patients with newly diagnosed PCA and a high risk for metastasis. Comprehensive histopathology covering prostate and the tributary pelvic lymph node system, was used as standard of truth. Therefore, only patients scheduled for RP with EPLND (as part of their standard of care) were eligible. Patients were recruited in 9 uro-oncological sites in Germany, Austria, and Switzerland, with access to a radiopharmaceutical laboratory, experienced to prepare ^{68}Ga-labelled compounds, and high-quality PET/CT imaging. Upon histological confirmation of PCA, pre-operative staging was performed according to EAU guideline (Mottet et al., 2015) (included a mandatory ^{99m}Tc-bone scan and an optional pelvic MRI or CT), in order to establish the indication for RP with EPLND. If the indication was confirmed, patients were invited to participate in the present study. After consenting, review of inclusion and exclusion criteria, as well as screening investigations were performed by the uro-oncologist (day 0). Thereafter, patients were referred to the collaborating nuclear medicine department for tracer injection, imaging, and post-dose safety evaluations (day 1). Subsequent investigations (day 2 and at end of study) were made by the uro-oncologist or experienced nuclear medicine physician. Study participation ended on day 7. Routine surgery (RP with EPLND) was performed after end of study, but no later than 60 days after study inclusion. This sequence allowed adequate characterisation of tracer safety, while at the same avoiding unnecessary delay of, or confounding safety signals from therapy. In total, 150 evaluable patients were to be included to receive a single ^{68}Ga dose of 150 MBq (±50 MBq), administered as IV infusion. Due to an assumed dropout rate of 15%, up to 173 patients were to be included in study. <u>Visit schedule</u> Day 0: Upon written informed consent eligible patients underwent screening evaluations (medical history, physical examination, laboratory). This visit could be performed either at urology/oncology department or nuclear medicine department. Day 1: This visit was performed at the nuclear medicine department. After pre-dose baseline safety evaluations (physical exam, vital signs, 12-lead electrocardiogram [ECG], pulse oximetry), Ga-68-PSMA-11 tracer was administered (dosing) followed by PET/CT

	imaging at 1 hour post-dose (low dose, non-enhanced CT) directly followed by a contrast enhanced diagnostic CT, and where available, PET/MRI imaging at 3 hours-post dose. Post-dose safety evaluations (vital signs, laboratory, 12 lead ECG, pulse oximetry) were performed at 1.5 and 6 hours after administration. The first low dose CT was done without contrast agent, to avoid any possible artefacts that could interfere with the attenuation correction. The diagnostic CT with contrast was necessary to allow for state-of-the-art morphological assessment of lymph nodes. Day 2: Safety evaluations (physical exam, vital signs, laboratory, 12 lead ECG) were repeated at 24 hours post-dose either at the uro oncological department or nuclear medicine department. Day 7: End-of-study (EOS) visit was performed either at the uro oncological department or nuclear medicine department, consisting of safety evaluation (physical exam, vital signs, laboratory, 12-lead ECG). After the study, in the period from Day 7 (after EOS visit) to 60, RP with EPLND (radical prostatectomy with extended pelvic lymph node dissection) were performed as part of standard care, outside the study, and blinded to the Ga-68-PSMA-11 scan result. Information about PSMA-positive lymph nodes outside the extended pelvic region and additional bone lesions in comparison to the ^{99m}Tc bone scintigraphy, that were detected by the local nuclear medicine were transmitted to the surgeon (partly unblinding) for dissection respectively change of treatment planning. Histological results (extent and distribution of PSMA expression in prostate and lymph nodes) were collected to serve as standard of truth for comparison with the PET imaging results obtained (primary and secondary objectives). A photographical documentation after completion of RP with EPLND were recorded for quality control, in order to ensure a complete lymph node dissection. Histology analysis were made centrally by a single pathologist blind to imaging results. Conversely, PET/CT (PET/MR) images were analysed by two central readers plus a central adjudication reader blind to histology results. The PET/CT / PET/MRI images were uploaded to a central platform (research picture archiving and communication system [PACS]) for evaluation. In the case of a negative histological result (i.e. no PSMA expression in dissected lymph nodes despite previous positive PSMA-scan) a 2nd Ga-68-PSMA 11 administration and PET/CT imaging including all safety examinations from 1 hour prior to injection to Day 7 as described above was to be conducted not later than 3 month after Day 1. This was to verify, if PSMA PET positive

	tissue as seen on day 1 had not been removed during RP with EPLND. Visits could be performed on an outpatient or inpatient basis, at the discretion of the investigator. Repetitive technical evaluations (ECG, pulse oximetry) were to be performed using the same technical device and, where possible, by the same person.
Methodology:	• <u>Safety & tolerability</u> Safety parameters were obtained at screening visit, pre-dose, 1.5, 6 and 24 hours post-dose, and at EOS. They included: ○ Laboratory (haematology, serum chemistry, urine analysis) ○ Pulse oximetry ○ 12-lead ECG ○ Physical examination and vital signs • <u>Imaging</u> ○ PET/CT PET/CT was performed 1 hour after tracer injection (post dose). CT was low dose and non-enhanced and was used to correct PET attenuation. A whole body 3D PET was acquired immediately after low-dose CT, directly followed by a contrast enhanced diagnostic CT. ○ PET/MRI A supplementing PET/MRI (PET with magnetic resonance imaging) could additionally be performed 3 hours after tracer injection, if device was available. Image analysis was done visually and semi-quantitatively. CT (and MRI, where available) and PET were fused and analysed for co-localisation of tumour tissue. The images PET and CT and fused images were uploaded to a central research PACS system for further evaluation. • <u>Histological investigation</u> Histological investigations of specimens obtained from prostate gland and pelvic lymph nodes were done locally and centrally and blinded to results of RP/EPLND by microscopic evaluation. Assessment included Gleason score. • <u>Sensitivity and specificity of Ga-68-PSMA-11 imaging to detect PCA in soft tissue using qualitative criteria</u>.

	Test performance of Ga-68-PSMA-11 PET/CT imaging to detect PCA was assessed, considering sensitivity and specificity (primary variables) of the method, using histology as standard of truth. Analysis of signal in Ga-68-PSMA-11 PET/CT (positive vs. equivocal vs. negative) was done by two independent central readers, blind to the histological results or clinical information. In addition to the global test performance (i.e. positive test if any disease present), the ability to detect prostatic versus. any lymphatic disease was assessed separately: The assignment of individual signal in Ga-68-PSMA-11 PET/CT to 8 individual histologically PCA-positive lymph node sub-regions from EPLND and to the prostate gland was be made locally at each site, and by a central core lab by two central readers. In ambiguous cases the results were confirmed by a central adjudication reader to assure the correct assignment between lymph node fields in imaging and histopathology. The number of pelvic lymph nodes with histologically confirmed PCA as reference lesion number were compared to the number of lesions with positive signal in Ga-68-PSMA-11 PET/CT images of the pelvis. • <u>Sensitivity and specificity of Ga-68-PSMA-11 imaging to detect PCA in soft tissue using quantitative criteria</u> Tracer uptake on PET/CT (PET/MRI) images were semi quantitatively analysed, using standardised uptake values (SUV_{max}, SUV_{mean} using a 70% isocontour VOI) and tumour background-ratios. Tracer uptake was assessed for each segment (quadrant, if possible octant) of the prostate as well as for each of 8 lymph node regions by placing the VOI on the lesion/lymph node with the highest uptake. Other than originally planned, receiver operator characteristic (ROC) assessment to find an objective cut-off for positive patients was not performed due to the study design of including only patients with a primary tumour. • <u>Correlation of Ga-68-PSMA-11 uptake and Gleason score of prostate gland after RP</u> Semi-quantitative analysis of tracer uptake on PET/CT (PET/MRI) images in primary tumours in the prostate gland was evaluated for correlation to histological grading of PCA by Gleason score after RP.

	• Sensitivity of Ga-68-PSMA-11 imaging to detect PCA bone lesions The number of bone lesions with Ga-68-PSMA-11 uptake on whole body images were compared to the number of lesions identified with ^{99m}Tc bone scintigraphy from standard work-up. Results obtained with both imaging methods were analysed locally at each site, and by a central core lab by two central readers. In ambiguous cases the results were confirmed by a central adjudication reader. • Impact of Ga-68-PSMA-11 imaging on target volume definition in radiotherapy was evaluated (no additional procedure necessary for the patient). The evaluation of Ga-68-PSMA-11 imaging on target volume definition was performed in a three step double blind design. First the target volume for radiotherapy (Gross tumour volume [GTV] plus clinical target volume [CTV]) was segmented in a treatment planning system based on clinical and CT / MR information. A photon intensity-modulated radiation therapy (IMRT) and a proton plan was generated. In the second step, (GTV plus CTV) was segmented based on Ga-68-PSMA-11 imaging. A photon IMRT and a proton plan was generated. The third step compared the results of step 1 and 2 to quantify the potential change in clinical management of the patients. • Molecular and clinical biomarkers. Blood samples were taken on day 0 and shipped to a central laboratory for analysis: Whole blood: Count of circulating tumour cells (CTC) after immunofluorescence staining in whole blood. Serum: MicroRNAs were extracted using commercial systems from serum. Protein determination was carried out directly in serum. The aim was to quantify the miRNA miR375 and the soluble urokinase-type plasminogen activator receptor (uPAR) protein in serum. Plasma: Circulating cell-free DNA was extracted using commercial systems from the plasma. The aim was to sequence a representative, predefined, panel of prostate cancer relevant genes in free circulating DNA, including tumour DNA.
Publications based on the study (references):	None

Study period:	First patient, first visit: 09-Oct-2017 Last patient, last visit: 03-Jul-2020
Early termination:	No
Number of patients:	The study planned to have 150 evaluable patients with histologically verified PCA. A total of 173 patients were enrolled and 163 patients completed the study (for more details, see results section below).
Criteria for evaluation: **Primary variables**	**Sensitivity and specificity** True positive fraction (TPF) and false positive fraction (FPF) of identified tumour tissue in soft tissue, analysed separately for prostate gland and pelvic lymph nodes, using histopathology as standard of truth, as follows: a) Sensitivity= TP / (TP + FN) b) Specificity= TN / (TN + FP) c) PPV= TP / (TP + FP) d) NPV= TN / (TN + FN) e) Accuracy = (TP + TN) / (TP + TN + FP + FN) Negative predictive value (NPV), positive predictive value (PPV), true negative (TN), true positive (TP), false negative (FN), false positive (FP) Analysis for prostate gland were done on 3 levels of precision: i. per-patient, ii. per-quadrant (n=4) iii. per-octant (n=8), if possible Analysis for lymph nodes were done on 3 levels of precision: iv. per-patient, v. per-gross-region (n=2): pelvic left/right, vi. per-sub-region (n=8): common iliac left and right, external iliac left and right, obturatoria left and right, internal iliac left and right. **Safety and tolerability** Frequency of occurrence and severity of abnormal findings in safety investigations (physical examination, vital signs, 12-lead ECG, pulse oximetry, clinical laboratory, adverse events, concomitant medication).

Criteria for evaluation: **Secondary variables**	**Sensitivity to detect bone lesions** a) Number of identified bone lesions, per patient. b) Proportion of bone lesions detected per patient with Ga 68 PSMA-11, relative to those detected by conventional bone imaging (N_{tracer} / N_{99mTc}) **Correlation of Ga-68-PSMA-11 signal with Gleason score** a) Correlation coefficient of recovery-corrected SUV values plotted against Gleason score in primaries after RP **Fraction of patients with change in clinical management** a) Percentage of patients with additional dissection of lymph nodes outside the defined 8 pelvic regions due to Ga-68-PSMA-11 uptake b) Percentage of patients for whom the RP and EPLND was not be conducted e.g. because of previously unknown (^{99m}Tc bone scan negative) bone lesions and/or distant lymph node due to Ga-68-PSMA-11 uptake **Fraction of patients with change of (potential) radiotherapeutic management** a) Expected benefit of 10% for Tumour Control Probability (TCP) by using PSMA-PET-CT planning b) Expected benefit of 10% for Normal Tissue Complication Probability (NTCP) by using PSMA-PET-CT planning **Exploratory analysis of molecular and clinical biomarkers** a) Quantity of circulating tumour cells in blood b) Quantity of miRNA-miR375 and uPAR-protein in serum, c) Identification of tumour specific mutations of circulating cell-free tumour DNA (cfDNA).
Safety endpoints:	**Safety and tolerability** Safety and tolerability of Ga-68-PSMA-11 was assessed by means of frequency of occurrence and severity of abnormal findings in safety investigations: • physical examination • vital signs • 12-lead ECG • pulse oximetry

	• clinical laboratory • adverse events • concomitant medication
Efficacy endpoints:	Efficacy endpoints were: a) Detection of tracer uptake in prostate gland and lymph nodes of the pelvis after Ga-68-PSMA-11 injection, b) Standardised uptake values (SUV) of tracer uptake in prostate gland and lymph nodes of the pelvis, c) Detection of tracer uptake outside the pelvis.
Other endpoints:	**Fraction of patients with change in clinical management** a) Percentage of patients with additional dissection of lymph nodes outside the defined 8 pelvic regions due to Ga-68-PSMA-11 uptake b) Percentage of patients for whom the RP and EPLND was not be conducted e.g. because of previously unknown (^{99m}Tc bone scan negative) bone lesions and/or distant lymph node due to Ga-68-PSMA-11 uptake **Fraction of patients with change of (potential) radiotherapeutic management** a) Expected benefit of 10% for Tumour Control Probability (TCP) by using PSMA-PET-CT planning b) Expected benefit of 10% for Normal Tissue Complication Probability (NTCP) by using PSMA-PET-CT planning **Exploratory analysis of molecular and clinical biomarkers** a) Quantity of circulating tumour cells in blood b) Quantity of miRNA-miR375 and uPAR-protein in serum c) Identification of tumour specific mutations of circulating cell-free tumour DNA (cfDNA).
Statistical analysis	All variables were analysed descriptively. Test performance parameters were analysed descriptively, separately for primary tumours and lymph nodes, giving 95% confidence intervals
Results:	
Study patients:	Disposition

The study was conducted at multiple sites in Austria (1 centre), Germany (7 centres) and Switzerland (1 centre). The site at TU München had the highest number of patients screened (N =84) and highest number of patients treated (N =81).

A total of 182 patients were enrolled and pre-screened for possible inclusion into the study. Nine of the pre-screened patients were screening failures. A total of 173 patients were included in the study and received the diagnostic tracer; of these, 163 (94.2%) patients completed the study. Of the 19 (11.0%) patients who discontinued from the study, the most frequent reason for study discontinuation was “Other” (15 [8.7%]). Failure of patients to appear to visits was the most frequently reported issue among “Other” reasons. Screening took place from 19-Sep-2017 to 25-Jun-2020.

The summary of treated patients is provided below.

Disposition of patients

	Total (N=173) n (%)
Patients screened	182
Enrolled	173 (100.0%)
Full Analysis Set	173 (100.0%)
Safety Analysis Set	173 (100.0%)
Completed study	163 (94.2%)
Early discontinuation	19 (11.0%)
Reasons for discontinuation	
Other	15 (8.7%)
Protocol violation	3 (1.7%)
Incorrect enrolment	1 (0.6%)

Analysis sets

As both the FAS population (i.e., all patients who were enrolled and were given a treatment number, irrespective of whether or not they received Ga-68-PSMA-11) and Safety Analysis Population (i.e., all patients of the FAS who received Ga-68-PSMA-1) had same patients in them, the tables were presented for the SAF population only. The patients in the different analysis populations were as shown in the following table.

	Data sets analysed in the study **Analysis population** / **Ga-68-PSMA-11 (N)** Full Analysis Set (FAS) / 173 Safety Analysis Set (SAF) / 173 Per protocol (PP) / 139 Image Data Set (IDS) / 139 Demographics / baseline characteristics As per the SAP, the SAF population was only used as the FAS population if it had the same patients as the FAS population. The patients had a mean ± standard deviation (SD) age of 65.8±8.0 years (Range 45–82 years) and all patients were male. A total of 172 (99.4%) patients were of white ethnicity and the 1 (0.6%) were of Asian ethnicity.
Efficacy:	**Primary efficacy variable results** Sensitivity and specificity of ^{68}Ga-PSMA-11 PET/CT imaging in detecting prostate cancer tissue within the prostate gland on level of quadrant. The ability of Ga-68-PSMA-11 PET/CT imagining to detect prostate cancer tissue within the prostate gland on level of quadrant (or octant if possible) at prostate gland was performed per patient level and per quadrant. Histopathology results were the reference for assessment. Analysis of the data per patient resulted in a sensitivity of 0.971 (95% CI 0.928–0.992), PPV, 1.000 (95% CI 0.973–1.000) and accuracy, 0.971 (95% CI 0.943–0.999). Specificity, and NPV could not be calculated because there were no true negatives and false positives. Evaluation of the ability of Ga-68-PSMA-11 PET/CT imaging to detect prostate cancer tissue at octant level using image data was not performed. This is because it was not possible to properly assign uptake to the octants; hence no statistical calculations were done at octant level. Analysis of the data at quadrant level showed a sensitivity of 0.710 (95% CI 0.670–0.750), specificity of 0.600 (95% CI 0.485–0.715), PPV, 0.925 (95% CI 0.898–0.952), NPV 0.230 (95% CI 0.169–0.290) and an accuracy of 0.696 (95% CI 0.658–0.734).

	ROC could not be calculated for prostate evaluation as there were no histological negative patients in the study. Tumour to background ratio (TBR) was calculated from SUV peak measured in the prostate and the SUV mean from the background region in the gluteal muscles. A mean TBR of 24.0±24.7 (Range: 1.6–284.5) was obtained from all measurements performed by both readers. <u>Sensitivity and specificity of Ga-68-PSMA-11 PET/CT imaging in detecting prostate cancer tissue within the pelvic lymph node metastases at the level of 8 defined sub-regions</u> At patient level, sensitivity was 0.400 (95% CI 0.271–0.529) and specificity of 0.988 (95% CI 0.965–1.000). An additional exploratory sensitivity assessment on per-patient basis was performed for larger lymph nodes. Based on the pathological measurements of metastatic lymph node size, the sensitivity was calculated against the size of the metastases. This assessment was performed to show that by excluding smaller lymph node metastasis sensitivity increases. Recalculating the sensitivity for the two categories, the sensitivity increased to 0.564 for lymph nodes above 3 mm and to 0.690 for lymph nodes above 5 mm. For the smallest metastatic dimension, the sensitivity for metastasis above 3 mm was 0.630 and above 5 mm was 0.824. Sensitivity and specificity of Ga-68-PSMA-11 PET/CT imaging in detecting prostate cancer tissue within the pelvic lymph node metastases at the level of 8 defined sub-regions were evaluated per gross region. A sensitivity of 0.345 (95% CI 0.244–0.447) and specificity of 0.995 (95% CI 0.985–1.000) were obtained. Analyses of the data per of the lymph node per sub-region resulted in a sensitivity of 0.155 (95% CI 0.085–0.225) and a specificity of 0.982 (95% CI 0.973–0.990). Finding an SUV cut-off to identify positive patients based on SUV measurements was not possible since no histopathology negative lymph nodes were identified as positive on PET and therefore no SUV measurements for negative patients were available. All identified lymph nodes outside the specified 8 sub-regions were reported as “LN-Other” as well as the regions from histopathology which were not amongst the 8 pre-specified sub-regions. In cases where regions could not be assigned to a sub-region such as in case the histology only reported pelvic left and pelvic right, the sub-region evaluation of such patients was considered not evaluable and

	were excluded from the sub-region sensitivity and specificity evaluation. TBR was calculated from SUV peak measured in the prostate and the SUV mean from the background region in the gluteal muscles. A mean TBR of 11.9±15.7 (Range: 2.0–94.5) was obtained from all measurements performed by both readers. **Secondary efficacy variable results** <u>Ability of Ga-68-PSMA-11 PET/CT imaging to detect bone metastases in comparison to ^{99m}Tc bone scintigraphy</u> At least one positive bone lesion was detected in 12 patients. Seven patients with positive bone lesions on ^{99m}Tc scans had no bone lesions detected on ^{68}Ga scans. Bone lesions were detected in both scans in only one patient (04-041). In two patients (08-049 and 08-077), equivocal bone lesions from ^{99m}Tc scans were reported as positive on ^{68}Ga scans. In one of the two patients (Patient 08-049), ^{68}Ga scan detected 5 bone lesions and ^{99m}Tc reported only one equivocal. Equivocal regions were counted as negative for the assessment in the study. <u>Correlation of Ga-68-PSMA-11 signal with Gleason score</u> The maximum measured SUV peak value from the prostate was measured in the main foci of the primary tumour (dominant lesion) and was plotted against Gleason score categories. Only Gleason scores from 7a to 9 were observed in the study patients. The SUV (mean ± SD) increased from 6.79±3.23 (Range 1.70–13.6) in Gleason score category 7a to 16.97±11.85 (Range 3.8–56.90) in Gleason score category 9. The lower and upper quartiles differences were highest in Gleason score 7a and 9 which were also the groups with the most patients (57 and 45, respectively). The values showed a statistically significant correlation ($p < 0.001$) between SUV peak and Gleason score category. <u>Fraction of Patients with Change in Clinical Management</u> RP and EPLND were not conducted in a total of 23 patients. In this cohort, some of the reasons included; 5 (21.7%) patients refused / declined surgery, and 4 (17.4%) patients received other therapies. The following reasons for not conducting RP and EPLND were given for 3 (13%) patients, each: positive Ga-68-PSMA-11 uptake in distant lymph nodes, previously unknown bone lesions, multiple metastases in pelvis and lost to follow – up.

Fraction of patients with change of (potential) radiotherapeutic management

The fraction of patients with change of (potential) radiotherapeutic management was evaluated. Of the initially 150 patients included in the study, 13 patients were excluded due to technical criteria (e.g., wrong slice thickness of the CT). For the remaining 137 men, the following organs at risk were contoured: rectum, bladder, sigmoid colon, (small) bowel, kidneys, skeleton, penile bulb, femoral heads, spinal cord/ cauda, liver, spleen, abdominal region, external.

Four board-certified radiologists/ radiooncologists divided into two teams – the conventional imaging planning team (CIP) and the PSMA imaging planning team (PIP) – were blinded with regard to the other imaging technique of each patient.

For the CIP, CT imaging of all patients was evaluated in consensus. For bone scans, the results of both central PET/CT readers were available. In total, 23 of 137 patients were classified as cN1, five patients were defined as cM1.

After clinical evaluation, GTV and CTV were contoured according to ESTRO consensus guidelines (prostate) and RTOG consensus guidelines (pelvic nodes). For each patient, two different cases were created according to in-house guidelines. Dose prescription and virtual irradiation was done according to the following in-house guidelines.

cN0: **[CASE 1a]**	34 x 2.25 Gy (total dose: 76.5 Gy)
cN0: **[CASE 1a]**	34 x 1.5 Gy (total dose: 51 Gy) with simultaneous integrated boost (SIB) to the prostate with 34 x 2.25 Gy (total dose 76.5 Gy)
cN1: **[CASE 1a]**	34 x 1.8 Gy (total dose 61.2 Gy) to the positive node(s) with SIB to the prostate with 34 x 2.25 Gy (total dose 76.5 Gy)
cN1: **[CASE 1b]**	34 x 1.5 Gy (total dose 51 Gy) with SIB to the positive node(s) with 34 x 1.8 Gy (total dose 61.2 Gy) and SIB to the prostate with 34 x 2.25 Gy (total dose: 76.5 Gy)
cM1a/b: **[CASE 1a]**	34 x 1.8 Gy (total dose 61.2 Gy) to the positive node(s) with SIB to the prostate with 34 x 2.25 Gy (total dose 76.5 Gy); for bone lesions a stereotactic body radiotherapy (SBRT) of 3x 9 Gy @ 80%-isodose was performed
cM1a/b **[CASE 1b]**	34 x 1.5 Gy (total dose 51 Gy) with SIB to the positive node(s) with 34 x 1.8 Gy (total dose 61.2 Gy) and SIB to the prostate with 34 x 2.25 Gy (total dose: 76.5 Gy); for bone lesions a stereotactic body radiotherapy (SBRT) of 3x 9 Gy @ 80%-isodose was performed

	For the PIP, the results of the central PET/CT reading showed that 9 of 137 patients had no PSMA imaging. For the remaining patients (n = 128), GTV, CTV and PTV delineation as well as radiotherapy planning were done in the same way as performed by the CIP – without the knowledge of conventional imaging [CASE 2a + b]. After completion of all radiotherapy plans, both teams were unblinded and all plans were re-evaluated with regard to the localisation of the metastases and differences of the target volume. PSMA-PET/CT detected 10 lesions outside of the guideline-based target volume (pararectal nodes, M1a nodes and M1b) and led to a significant change of the target volume in 16.4% of cases, for example due to new findings like a PSMA-positive pararectal node or a bone lesion. For 3.9% of all patients, a minor change occurred (e.g., additional SIB within the target volume). No reliable comparison was possible for two of 128 patients with bone lesions in PSMA imaging and no available bone scan. Histopathological results obtained from surgery were then evaluated for all patients and the information was added to the planning module. Moreover, data were compared to PSMA imaging. A total of 32 additional patients were observed with small nodal metastases after surgery (pN1), and lymphadenectomy detected more positive nodes than PET/CT for almost all cN1 patients. In total, 173 SIBs to nodal metastases were missing; one nodal SIB was done without avail. While for two patients' positive nodes outside of the target volume (pararectal + paraaortic) were confirmed after surgery, the situation remained unclear (infield vs. outfield) for 11 patients due to missing pathological information. Radiotherapy planning was then performed on "real life data" [CASE 3] and calculations showed that for the normal tissue complication probability (NTCP) gastrointestinal toxicity (obstruction/perforation, necrosis/stenosis, late rectal bleeding grade ≥2, late effects grade ≥3) as well as side effects with regard to the bladder (contracture, late effects grade ≥3) were obtained. PSMA-guided radiotherapy led to a small reduction (1–2%) of the risk of an obstruction/perforation of the small bowel and late rectal bleeding grade ≥2 which was not significant. In contrast, there was a higher risk (1–14%) for late effects grade ≥3 for the bladder when radiotherapy planning was performed on the basis of PSMA-PET/CT compared to conventional treatment plans. Tumour control probability (TCP) calculations were not feasible due to inaccurate information obtained from histopathology with regard to the precise location of the positive nodes.

	Exploratory Efficacy Results Quantity of Circulating Tumour Cells in Blood Of a total of 167 samples delivered at the investigational centre, only 86 blood samples could be processed within 96 hours as per the guidelines provided for processing blood samples for CTCs detection. In blood samples from 11 of 86 patients (12.8%), ≥1 CTC/7.5 mL blood were detected. More than 1 CTC and ≥5 CTCs/7.5 mL were found in 5/86 (5.8%) and 2/86 patients (2.3%), respectively. Twenty-four CTCs were analysed for PSMA expression. In 8/24 (33%) CTCs, weak to strong intensity of PSMA expression was detected. Biomarker analyses: uPAR and miR-375 Of a total of 143 samples collected for biomarker analyses from seven sites, 125 samples were evaluable. The uPAR protein level in serum (n=125) had a mean value of 0.313 ng/mL (Range 0.000–1.510 ng/mL). The miR-375 levels (40-dCT) measured in serum had a mean value of 30.372 (Range 26.565–33.164). From the correlation of the expression levels of uPAR and miR-375 with the clinical pathological parameters (age, T-Stadium, N-Stadium, M-Stadium, Gleason core, ISUP-Grade Groups, PSA-values, and radiological data), a positive correlation between uPAR protein expression level with the age of the patients (r_s= 0.230; p=0.010) was obtained. Additionally, a negative correlation between uPAR and miR-375 levels was detected (r_s= -0.239; p=0.007). The rest of the parameters did not have any correlation with either uPAR or miR-375.
Safety results:	Exposure A total of 173 patients received the diagnostic tracer (IMP) within the stipulated dose range. Adverse events Overall, 20 treatment-emergent adverse events (TEAEs) were reported in 173 patients. A total of 14 (8.1%) patients had at least one TEAE. There were no patients with treatment-related TEAEs and none of the TEAEs led to death or study discontinuation. None of the TEAEs were reported as serious. TEAEs graded by severity showed that 19 (95%) TEAEs were graded as mild and 1 (5%). Clinical laboratory evaluation

	Abnormal laboratory values flagged as clinically significant by the investigator were reported in five patients. None resulted in any SAE. For haematological parameters, abnormal clinically significant values were recorded in Patient 4-012 for monocytes, monocytes (Absolute), neutrophils, neutrophils (Absolute) and WBC (Total). For biochemical parameters, four patients had abnormal values which were clinically significant as follows: Patient 1-008 for creatinine and for urea; in Patient 1-011, creatinine values were also clinically significant; in Patient, 8-059, abnormal values with clinical significance were recorded for ALT, AST, GGT and total bilirubin; in Patient, 8-073, ALT, AST and GGT had abnormal clinically significant values. No abnormal parameter values with clinical significance were recorded for urinalysis. Haematological parameters showed statistically significant changes from baseline at various time points (Wilcoxon test). The parameters included basophils, haematocrit, leukocytes, lymphocytes, monocytes, platelets, RBC and WBC total. None of the abnormal laboratory parameters with clinical significance were rated as SAEs. No significant changes were recorded for MCH and MCV. Biochemical parameters showed statistically significant changes from baseline at various time points for the following analytes; ALP, ALT, AST, albumin, calcium, chloride, GGT, potassium, sodium, total bilirubin and urea. None of the abnormal laboratory parameters with clinical significance were rated as SAEs. No significant changes were recorded for creatinine and glucose. From urinalysis laboratory values, changes from baseline with significant changes were recorded for the measured parameters which included specific gravity and pH. <u>Vital Signs, physical findings and other observations related to safety</u> No abnormal observations were observed when evaluating parameters of physical examination, vital signs, and pulse oximetry. The only two patients (0.2%) with abnormal ECG results with clinical significance were reported in Patient 6-004 with new tachyrrhythmia absoluta (SOC/PT; Cardiac disorders / Atrial fibrillation and Patient 8-006 with atrial fibrillation (SOC/PT; Cardiac disorders / Atrial fibrillation) both which resolved during the study.
Overall conclusions:	In conclusion, efficacy results showed that; • At patient level, Ga-68-PSMA-11 PET/CT imaging detected prostate cancer tissue within the prostate gland with a higher sensitivity and a slightly reduced sensitivity at quadrant level.

	Specificity of prostate gland could not be calculated at patient level and was low at quadrant level.
	• Ga-68-PSMA-11-Study sensitivity and specificity assessment based on lymph nodes showed a high specificity and PPV, and a moderate sensitivity. The moderate sensitivity was within the range of published data.
	  ○ Sensitivity slightly decreased for the gross-region (pelvic left/right) assessment
	  ○ Low sensitivity for the sub-region assessment was obtained
	  ○ Exploratory analysis of lymph nodes with a bigger diameter resulted in increased sensitivity
	• Comparison of Ga-68-PSMA-11 PET/CT imaging and ^{99m}Tc bone scintigraphy in detecting bone metastases did not give a clear pattern.
	• The SUV peak measured in prostate correlated with the Gleason score categories. It may therefore be an indicator of Gleason score but may not reliably predict the exact Gleason score.
	• Change in clinical management due to RP and EPLND not being conducted was observed in a small fraction of patients.
	• The impact of pre-operative Ga-68-PSMA-11 PET/CT imaging on target volume definition of radiotherapy was also investigated but did not yield clear results. PSMA-guided radiotherapy led to a small reduction (1–2%) of the risk of obstruction/ perforation of the small bowel and late rectal bleeding grade ≥2 which was not significant. In contrast, there was a higher risk (1–14%) for late effects grade ≥3 for the bladder compared to conventional treatment plans.
	• Exploratory analyses of molecular and clinical biomarkers showed that;
	  ○ Detection rate of CTC was within the expected range. Weak to strong intensity of PSMA expression was also detected in part of the analysed patient samples.
	  ○ uPAR expression level correlated positively with the age of the patients (i.e uPAR levels increased with age of the patients) and negatively with miR-375 expression.

	The safety data showed that; • Ga-68-PSMA-11 was safe and well-tolerated in the study population and may be used safely in further studies.

Table 1 Schedule of procedures

Study Flow Chart : DKFZ- ^{68}Ga-PSMA-HBED-CC

Flow chart version: Final 4.0, dated 27-Sep-2019

Examination / Evaluation		Data to be obtained from SC*	Study Phase Scr/ BL								EoS	Follow-up (SC*)
Time point	Day	-56 to -1	0	1						2	7	7 to 60
	Hour			Pre-Dose	0	1 (± 10 min)	1.5 (± 30 min)	3 (± 10 min)	6 (± 30 min)	24 (± 4 h)		
Informed Consent			X									
Review Inclusion / Exclusion Criteria			X	X								
General & safety												
Histological confirmed diagnosis [1]		X										
Tumour Staging[1]		X										
^{99m}Tc bone scintigraphy [1]		X										
MRT or CT[2]		X										
Medical History / Interim History			X									
Physical Examination			X	X						X	X	
Vital Signs			X	X			X		X	X	X	
12-Lead ECG			X	X			X		X	X	X	
Puls oxymetry				X			X		X			
Haematology			X				X		X	X	X	
Serum Chemistry			X				X		X	X	X	
Prostate specific antigen (PSA)			X									
Biomarker sample/ CTC samples (central lab Erlangen)			X									
Urine analysis			X				X		X	X	X	
Concomitant medication			X	X →	→	→	→	→	X	X	X	
Baseline Findings / Adverse Events			X	X →	→	→	→	→	X	X	X	
Imaging & outcome												
^{68}Ga-PSMA-HBED-CC administration					X							
^{68}Ga-PET / CT[5]						X						
^{68}Ga-PET / MRT								X[3]				
Tumour Histology [4]												X[6]

* Standard of care: No part of study.

(1) Necessary for inclusion.
(2) Was obtained during study, if not previously available (Optional).
(3) Performed in subgroup (where PET/MRI was available at site).
(4) Central tumour histology, included:
> conventional work-up,
> determination of presence of PSMA expressing tumour tissue at individual lymph node level,
> Gelason score, where possible, semiquantitative grading of PSMA expression in primary tumour and affected lymph nodes.
Surgery was SoC but done according to harmonised central guidelines.
(5) Whole body 3D PET was acquired immediately after low-dose CT, directly followed by a contrast enhanced diagnostic CT.
(6) If tumour histology was PSMA-negative Ga-68-PSMA-11 administration and PET/CT imaging including all safety examinations from 1 hour to injection to Day 7 could be repeated within 3 months.