ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

# Oncologia, Ematologia e Patologia

Ciclo XXXVI

**Settore Concorsuale:** Oncologia Medica (MED/06)

**Settore Scientifico Disciplinare:** Malattie del sangue, Oncologia e Reumatologia (06/D3)

## A prospective study on the early evaluation of response to androgen receptor-targeted agents with $^{11}$C-Choline, $^{68}$Ga-PSMA and $^{18}$F-FACBC PET in metastatic castration-resistant prostate cancer: a single center experience.

**Presentata da:** Dott.ssa Veronica Mollica

**Coordinatore Dottorato**

Prof.ssa Manuela Ferracin

**Supervisore**

Prof. Andrea Ardizzoni

**Co-Supervisore**

Dott. Francesco Massari

Esame finale anno 2024

**ABSTRACT:**

**Background:** The early identification of responsive and resistant patients to androgen-receptor targeting agents (ARTA) in metastatic castration resistant-prostate cancer (CRPC) is not completely possible with PSA assessment and conventional imaging. Considering its ability to determine metabolic activity of lesions, PET assessment might be a promising tool.

**Materials and methods:** We performed a monocentric prospective study in patients with metastatic CRPC under treatment with ARTA to evaluate the role of different PET radiotracers: 49 patients were randomized to receive $^{11}$C-Choline, $^{18}$F-FACBC or $^{68}$Ga-PSMA PET, one scan before therapy onset and one two months later.

The primary aim was to investigate the performance of three different novel PET radiotracers for the early evaluation of response to ARTA in metastatic CRPC patients; with regards to this aim, the outcome evaluated was biochemical response (PSA reduction ≥50%). The secondary aim was to investigate the prognostic role of several semiquantitative PET parameters and their variations with the different radiotracers in terms of biochemical PFS (bPFS) and overall survival (OS).

The study was promoted by the Italian Department of Health (code RF-2016-02364809).

**Results:** With regards to the primary endpoint, at univariate analysis a statistically significant correlation was found between MTV_VARIATION% (p=0.018) and TLA_VARIATION% (p=0.025) with $^{68}$Ga-PSMA PET and biochemical response. As for the secondary endpoints, significant correlations with bPFS were found for $^{68}$Ga-PSMA PET MTV_TOT_PET1 (p=0.001), TLA_TOT_PET1 (p=0.025), MTV_VARIATION% (p=0.031). For OS, statistically significant correlations were found for: MAJ_SUV_MAX_PET1 with $^{11}$C-Choline PET (p=0.007); MTV_TOT_PET1 (p=0.004), MAJ_SUV_MAX_PET1 (p=0.029), SUVMAX_VARIATION% (p=0.04), MTV_VARIATION% (p=0.015), TLA_VARIATION% (p=0.03) with $^{68}$Ga-PSMA PET,; MTV_TOT_PET1 (p=0.011), TLA_TOT_PET1 (p=0.009), MAJ_SUV_MAX_PET1 (p=0.027), MTV_VARIATION% (p=0.048) with $^{18}$F-FACBC.

**Conclusions:** Our prospective study highlighted that several $^{68}$Ga-PSMA and $^{18}$F-FACBC semiquantitative PET parameters and their variations present a prognostic value in terms of OS and bPFS and a correlation with biochemical response, that could help to assess response to ARTA.

## 1. Introduction:

In the current year, prostate cancer (PCa) still represents the most common malignancy and the second cause of cancer-related death in men worldwide [1]. Fortunately, the therapeutic landscape is constantly evolving in all settings of the disease, including metastatic castration-resistant prostate cancer (mCRPC) [2]. Abiraterone acetate and enzalutamide, two novel androgen-receptor targeting agents (ARTA), continue to play a crucial role in the treatment of mCRPC, regardless of the previous administration of docetaxel [3,4,5,6,7]. Of great impact on clinical practice could be the early identification of patients who develop resistance to these compounds or patients who are primary refractory. Nowadays, the monitoring of prostate-specific antigen (PSA) levels is commonly adopted to evaluate therapy response [8], but its determination could be impaired in case of non-producing tumors [9] (for example, in neuroendocrine prostate cancer or induced by hormonal treatments for mCRPC) [10] and in case of initial and transient increase of PSA levels due to the "flare" phenomenon [11]. Besides, the imaging evaluation with conventional imaging (CIM), consisting in computed tomography (CT), magnetic resonance imaging (MRI) or bone scintigraphy, is not completely able to identify responsive or resistant patients to ARTA. Otherwise, a promising assessment of response to therapy could be performed with positron emission tomography (PET), as already demonstrated in other cancers [12,13,14], also in view of its ability to determine the extent of disease with respect to sites and number of metabolically active lesions. Of note, a systematic review and meta-analysis pointed out the quite relevant discordance (about 25% of cases) between PSA and $^{68}$Ga-PSMA PET response assessments in mCRPC patients undergoing systemic therapies [15].

A single-arm study, that enrolled 16 mCRPC patients treated with abiraterone acetate or enzalutamide, showed that the decrease of the uptake at the PSMA-PET performed after 2-4 months the start of ARTA was strongly correlated to treatment response [16].

Regarding the potential prognostic role of PET in PCa, several studies have begun to investigate this topic. In patients undergoing radiotherapy (RT) for localized disease or as salvage therapy, $^{11}$C-Choline and $^{68}$Ga-PSMA PET/CT were found to play an important prognostic role, as emerged in a

recent systematic review of the literature, while controversial was their utility in course of systemic therapies [17]. An European, multicenter, retrospective analysis highlighted that patients with positive $^{68}$Ga-PSMA PET, who already underwent salvage treatments after radical prostatectomy and PSA relapse, presented worse outcomes if compared to men with no uptake at the PET scan, while the result of the PET scan in patients who have never received salvage therapies did not affect their oncologic outcomes [18]. Another retrospective, observational trial showed that $^{68}$Ga-PSMA PET seems to be a more reliable prognostic factor for progression-free survival (PFS) than PSA levels in mCRPC [19].

In addition to $^{11}$C-Choline, $^{68}$Ga-PSMA and $^{18}$F-FACBC, several other PET radiotracers are under evaluation in PCa [20], but limited data are available on which radiotracer is more effective in predicting patient's outcome and the early response to therapy. Yet to be defined is also which is the most reliable PET-derived parameter in terms of prognostic and predictive value. The possibility to discriminate responder patients from resistant ones could help clinicians in mCRPC management, leading to a more tailored therapeutic approach. According to that, several trials have already revealed that performing $^{68}$Ga-PSMA-PET in metastatic castration-sensitive prostate cancer could lead to management changes [21,22,23].

In this prospective monocentric interventional study, which is part of the research project with code RF-2016-02364809 promoted by the Italian Department of Health, we tried to shed light on these unveiled and controversial topics.

## 2. Patients and Methods:

### *2.1 Study Design:*

We performed a prospective, interventional, monocentric, explorative study that enrolled patients with mCRPC assigned to treatment with abiraterone acetate or enzalutamide (before or after docetaxel chemotherapy). Patients were randomly assigned to receive $^{11}$C-Choline, $^{18}$F-FACBC or

$^{68}$Ga-PSMA PET, one scan before therapy onset (PET1) and one two months later (PET2). PET scans have been evaluated by 3 experienced nuclear medicine physicians visually and semi-quantitatively and maximum Standardized Uptake Value (SUVmax) and SUVmean have been measured in all hot lesions outside the normal tracer distribution. PET scans were achieved in conformity with the Joint European Association of Nuclear Medicine (EANM) and Society of Nuclear Medicine and Molecular Imaging (SNMMI) procedure guidelines for PCa imaging. The study has been conducted according to Good Clinical Practices, after local Ethical Committee and AIFA (Associazione Italiana del Farmaco) approval. The response at PET2 has been evaluated according to the European Organization For Research And Treatment Of Cancer (EORTC) PET response criteria. In Figure 1, a brief representation of the study design is reported.

The study enrolled patients from January 2019 to August 2022. This is the first report of the study results.

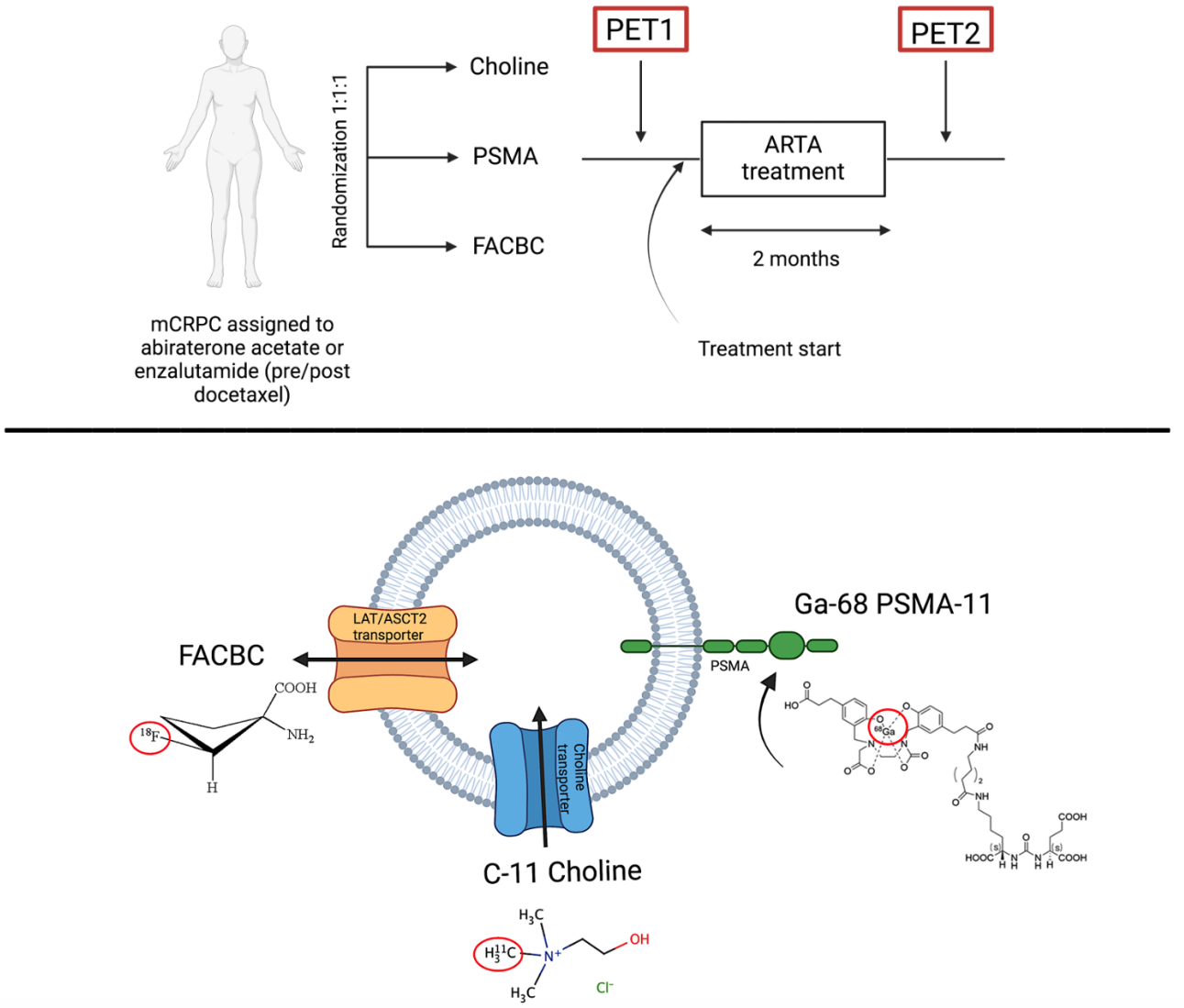


**Figure 1.** Brief graphic scheme of the study design and chemical structure and transporters of the three radiotracers used in this study.

FACBC enters in the cancer cell through the human l-type amino acid transporter-alanine-serine-cysteine transporter2 (LAT/ASCT2) and it is upregulated in several carcinomas, including prostate cancer [24]. Choline is essential for the synthesis of phospholipids in the plasma membrane and consequently for tumor cell proliferation. Three main family of transporters are involved in the uptake of choline: choline transporter 1 (CHT1/SLC5A7), choline transporter-like proteins (CTL1-5/SLC44A1-5) and polyspecific organic cation transporters (OCT1-2/SLC22A1-2) [25]. PSMA is a transmembrane protein highly expressed on the majority of prostate cancer cells, representing an important target for imaging and also therapeutic compounds such as $^{177}$Lu-PSMA-617 [26].

Legend: ARTA: androgen-receptor targeting agents; mCRPC: metastatic castration-resistant prostate cancer.

*2.2 Study Population:*

Inclusion criteria:

1) diagnosis of mCRPC as defined by the European Association of Urology (EAU);

2) radiological evidence of metastatic disease at either computed tomography or bone scintigraphy;

3) eligible for ARTA (abiraterone acetate or enzalutamide), before or after docetaxel treatment (could have received docetaxel for metastatic hormone sensitive or castration resistant setting);

4) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1;

5) abiraterone acetate or enzalutamide naïve;

6) age ≥ 18 years-old;

7) signed informed consent.

Exclusion Criteria:

1) patients not eligible for ARTA;

2) life expectancy ≤ 6 months.

*2.3 Statistical analysis:*

The primary aim was to investigate the performance of PET scan with different novel radiotracers for early therapy assessment in mCRPC patients treated with an ARTA. With regards to this aim, the primary endpoint was biochemical response (PSA response ≥50%), that was correlated with PET parameters difference and percentage variation. Biochemical response was defined as a ≥50% reduction of PSA at the time of PET2 from baseline. PSA values taken into account were PSA at baseline (≤4 weeks before ARTA start) and PSA at the time of PET2 (+ 4 weeks).

The secondary aim was to investigate the prognostic role of PET with different radiotracers. Secondary endpoints were biochemical progression free survival (bPFS) and overall survival (OS). Data resulting from pre-treatment PET parameters and their variations among the two PET scans have been analyzed in relation to bPFS and OS.

bPFS was defined as the time of ARTA start to the time of PSA increase >50% from baseline value. OS was defined from therapy start to death from any cause.

Evaluated semiquantitative PET parameters and variations were:

- Standard Uptake Value (SUVmax);
- Metabolic Tumor Volume (MTV: the volume of the metabolically active areas of the disease);
- Total Lesion Activity (TLA: MTVxSUVmean);
- major value of SUVmax reported in each PET/CT scan (majSUVmax);
- difference of the parameter at PET2 compared to PET1 (DIFF_majSUVmax, DIFF_MTV, DIFF_TLA);
- percentage of changes among the two PET scans (majSUVmax_VARIATION%, MTV_VARIATION%, TLA_VARIATION%).

For the statistical analysis, we used Wilcoxon signed rank test, ROC curves, Kaplan-Meier curve, univariate analysis. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program version 26.0 (IBM, Armonk, NY, USA).

## 3. Results:

From January 2019 to August 2022, we enrolled 49 mCRPC patients treated with abiraterone acetate or enzalutamide, randomized 1:1:1 to receive PET scan with $^{11}$C-Choline (n=16), $^{68}$Ga-PSMA (n=18), or $^{18}$F-FACBC (n=15). The median follow-up was 16 months (range 2-39 months).

Five patients were excluded from the analysis because they did not receive PET2 for death or worsening of ECOG PS.

All the main patients' characteristics are reported in the tables below (Table 1).

| | $^{11}$C-CHOLINE PET (n= 16 pts) n (%) | $^{68}$Ga-PSMA PET (n= 18 pts) n (%) | $^{18}$F-FACBC PET (n= 15 pts) n (%) |
|---|---|---|---|
| **General Characteristics** | | | |
| Median age at PET1 | 76 years | 76 years | 78 years |
| Median PSA at PET1 | 11,3 ng/ml | 19,4 ng/ml | 17,8 ng/ml |
| Median first PSA on ARTA | 26,1 ng/ml | 7,8 ng/ml | 8,0 ng/ml |
| Previous docetaxel | 3 (18,8%) | 2 (11,1%) | 0 |
| Bisphosphonates use | 5 (31,3%) | 4 (22,2%) | 2 (13,3%) |
| Biochemical response (reduction of PSA >50% from baseline) | 6 (33,3%) | 4 (22,2%) | 5 (33,3%) |
| Biochemical progression (increase of PSA >50% from baseline) | 6 (33,3%) | 2 (11,1%) | 7 (46,7%) |
| EORTC response at PET2 | 0 CR, 2 PR, 4 SD | 1 CR, 3 PR, 4 SD | 0 CR, 3 PR, 1 SD |
| EORTC progression at PET2 | 9 PD | 7 PD | 7 PD |
| Median bPFS | 5 months | 2 months | 6 months |
| **PET-derived parameters** | | | |
| Median major SUVmax at PET1 | 9,20 | 30,65 | 11,2 |
| Median major SUVmax at PET2 | 11,50 | 30,40 | 11 |
| Median total MTV at PET1 | 162,10 | 56 | 278,2 |
| Median total MTV at PET2 | 216,55 | 75,5 | 109,7 |
| Median total TLA at PET1 | 467,45 | 515,8 | 969,1 |
| Median total TLA at PET2 | 789,80 | 1403,4 | 470,1 |
| Patients not undergoing PET2 | 1 (6,3%) | 3 (16,7%) | 4 (26,7%) |
| **Sites with PET uptake at PET1** | | | |
| Prostate | 9 (56,3%) | 5 (27,8%) | 6 (40%) |
| Pelvic lymph nodes | 7 (43,8%) | 7 (38,9%) | 2 (13,3%) |
| Extra-pelvic lymph nodes | 11 (68,8%) | 10 (55,6%) | 3 (20%) |
| Bone | 12 (75%) | 12 (66,7%) | 10 (66,7%) |
| Lung | 1 (6,3%) | 4 (22,0%) | 2 (13,3%) |

| | | | |
|---|---|---|---|
| Liver | 0 | 0 | 0 |
| Abdominal nodules | 0 | 0 | 0 |

**Table 1.** Patients' characteristics for each randomized group.
Legend: ARTA: androgen receptor targeted agents; bPFS: biochemical progression-free survival; CR: complete response; PD: progression disease; PR: partial response; SD: stable disease; pts: patients; SUVmax: Standard Uptake Value; MTV: Metabolic Tumor Volume; TLA: Total Lesion Activity.

*3.1 Primary aim: correlation of PET parameters at baseline and percentage variation with biochemical response.*

For each radiotracer, we correlated PET parameters at baseline and their variations with biochemical response using the Wilcoxon signed rank test. The only statistically significant correlation was found for MTV_VARIATION% with $^{68}$Ga-PSMA (p=0.043, figure 2).

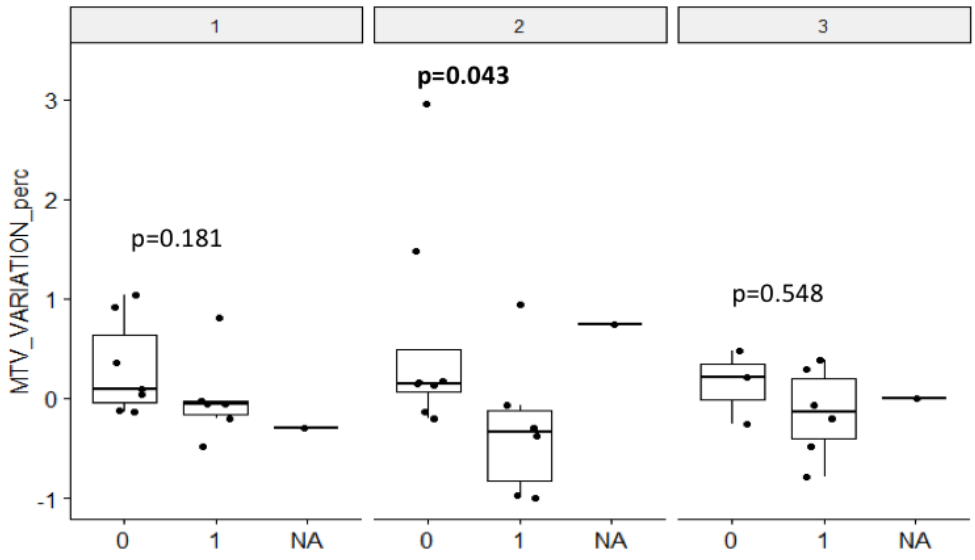


**Figure 2.** Wilcoxon signed rank test correlations of MTV_VARIATION% with $^{11}$C-Choline (1), $^{68}$Ga-PSMA (2), $^{18}$F-FACBC (3) PET and biochemical response. In bold, statistically significant variables.

Cut-off values of PET parameters at baseline and their percentage variation (reported in table 2) were assessed through ROC curve and dichotomized into lower or higher of cut-offs.

| | $^{11}$C-CHOLINE PET | $^{68}$Ga-PSMA PET | $^{18}$F-FACBC PET |
|---|---|---|---|
| MTV_TOT_PET1 | 148.4 | 173.4 | 87.7 |
| TLA_TOT_PET1 | 2020.6 | 60.2 | 815.7 |
| MAJ_SUV_MAX_PET1 | 499 | 156 | 404 |
| SUVMAX_VARIATION% | -0.9744 | -0.8658 | -0.9728 |
| MTV_VARIATION% | 0.035 | -0.1975 | 0.2132 |
| TLA_VARIATION% | 0.045 | 0.3343 | 0.8333 |

**Table 2.** Cut-off of PET parameters and percentage variation for each radiotracer.
Legend: MTV: Metabolic Tumor Volume; Maj: Major; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

The cut-offs for each variable were correlated with biochemical response through log rank test. A statistically significant correlation was found between MTV_VARIATION% (p=0.018, figure 3) and TLA_VARIATION% (p=0.025, figure 4) with $^{68}$Ga-PSMA PET and biochemical response (table 5).

| | $^{11}$C-CHOLINE PET | $^{68}$Ga-PSMA PET | $^{18}$F-FACBC PET |
|---|---|---|---|
| MTV_TOT_PET1 | 0.22 | 0.84 | 0.5 |
| TLA_TOT_PET1 | 0.58 | 0.47 | 0.8 |
| MAJ_SUV_MAX_PET1 | 0.33 | 0.26 | 0.78 |
| SUVMAX_VARIATION% | 0.11 | 0.62 | 0.84 |
| MTV_VARIATION% | 0.63 | **0.018** | 0.96 |
| TLA_VARIATION% | 0.055 | **0.025** | 0.71 |

**Table 3.** Log rank test for the correlation of PET parameters and percentage variation and biochemical response for each radiotracer. In bold, statistically significant variables.
Legend: bPFS: biochemical progression-free survival; MTV: Metabolic Tumor Volume; Maj: Major; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

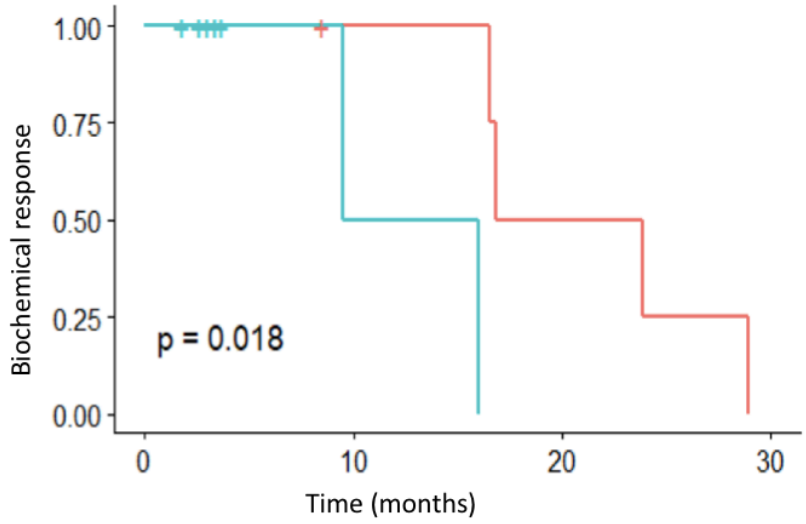


**Figure 3:** Kaplan-Meier estimates of biochemical response according to $^{68}$Ga-PSMA PET MTV_VARIATION%. Blue curve: higher than cut-off. Red curve: lower than cut-off.

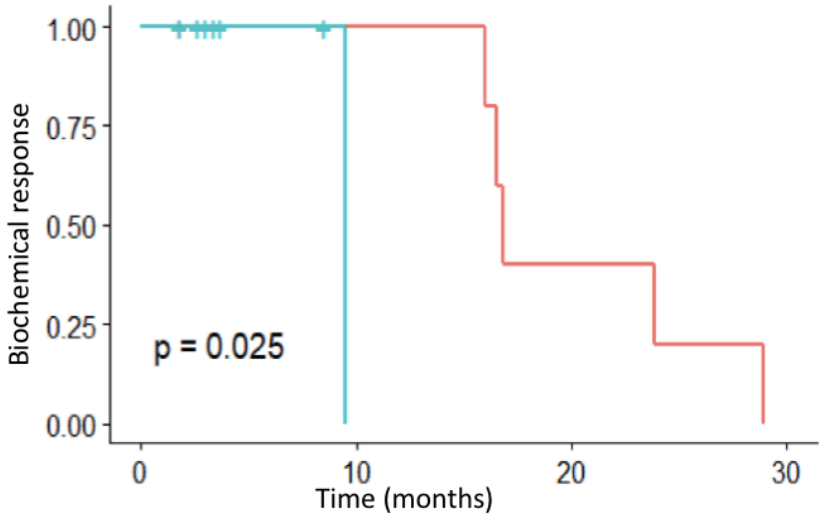


**Figure 4:** Kaplan-Meier estimates of biochemical response according to $^{68}$Ga-PSMA PET TLA_VARIATION%. Blue curve: higher than cut-off. Red curve: lower than cut-off.

*3.2 Secondary aim: correlation of pre-treatment PET parameters and their variations with biochemical PFS.*

PET parameters at baseline and their variations were correlated with bPFS using the Wilcoxon signed rank test for each radiotracer and no statistically significant correlation was found. Using ROC curve,

cut-off values of PET parameters at baseline and their percentage variation were found and subsequently dichotomized according to lower or higher of cut-offs.

Cut-off of PET parameters and their variation for each radiotracer are reported in table 4.

| | **$^{11}$C-CHOLINE PET** | **$^{68}$Ga-PSMA PET** | **$^{18}$F-FACBC PET** |
|---|---|---|---|
| MTV_TOT_PET1 | 111.3 | 266.8 | 169.4 |
| TLA_TOT_PET1 | 472.8 | 662.2 | 465.1 |
| MAJ_SUV_MAX_PET1 | 437 | 171.1 | 400 |
| SUVMAX_VARIATION% | -0.9712 | -0.8481 | -0.9716 |
| MTV_VARIATION% | -0.0905 | -0.0004 | -0.194 |
| TLA_VARIATION% | -0.0022 | 16.2028 | -0.8399 |

**Table 4.** Cut-off of PET parameters and percentage variation for each radiotracer.
Legend: MTV: Metabolic Tumor Volume; Maj: Major; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

Subsequently, these variables were correlated with bPFS through log rank test and a statistically significant correlation was found only for $^{68}$Ga-PSMA PET MTV_TOT_PET1 (p=0.001, figure 5), TLA_TOT_PET1 (p=0.025, figure 6), MTV_VARIATION% (p=0.031, figure 7). Log rank test for the correlation of PET parameters and percentage variation and bPFS for each radiotracer are reported in table 5.

| | **$^{11}$C-CHOLINE PET** | **$^{68}$Ga-PSMA PET** | **$^{18}$F-FACBC PET** |
|---|---|---|---|
| MTV_TOT_PET1 | 0.12 | **0.001** | 0.83 |
| TLA_TOT_PET1 | 0.86 | **0.025** | 0.6 |
| MAJ_SUV_MAX_PET1 | 0.82 | 0.27 | 0.8 |
| SUVMAX_VARIATION% | 0.86 | 0.1 | 0.2 |
| MTV_VARIATION% | 0.81 | **0.031** | 0.18 |
| TLA_VARIATION% | 0.56 | 0.3 | 0.3 |

**Table 5.** Log rank test for the correlation of PET parameters and percentage variation and bPFS for each radiotracer. In bold, statistically significant variables.
Legend: bPFS: biochemical progression-free survival; MTV: Metabolic Tumor Volume; Maj: Major; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

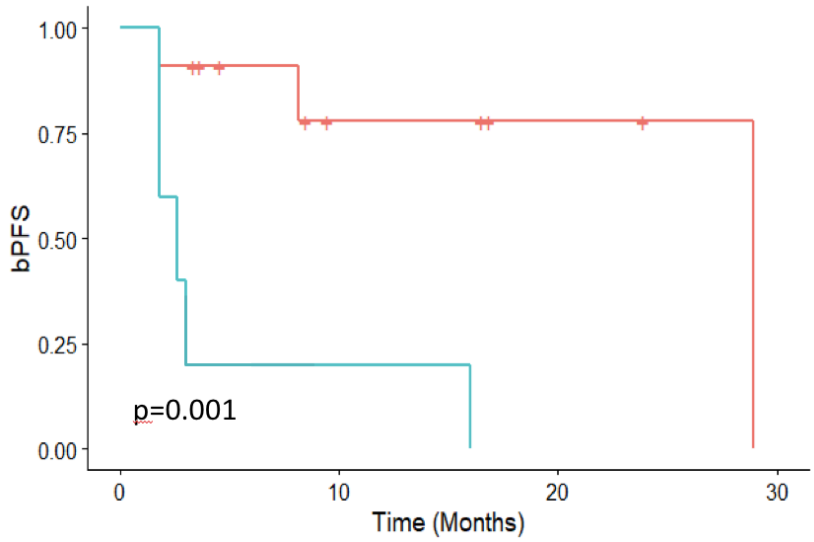


**Figure 5:** Kaplan-Meier estimates of bPFS according to $^{68}$Ga-PSMA PET MTV_TOT_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

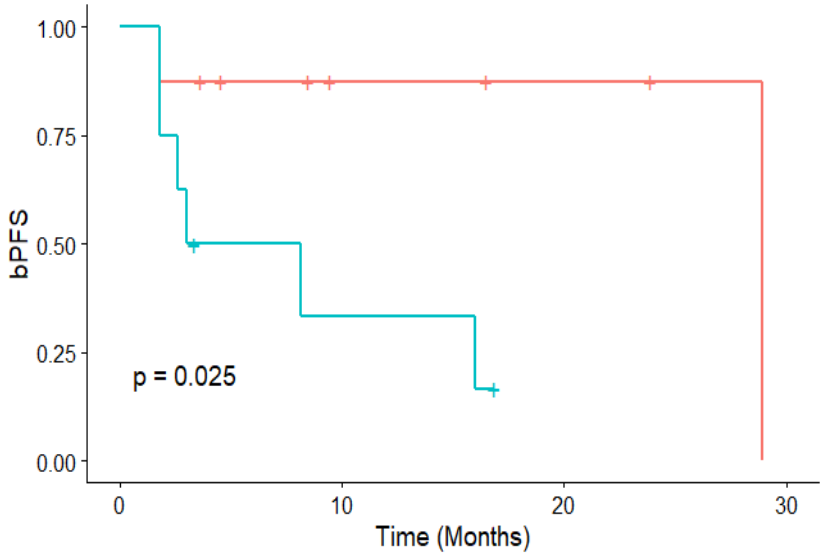


**Figure 6:** Kaplan-Meier estimates of bPFS according to $^{68}$Ga-PSMA PET TLA_TOT_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

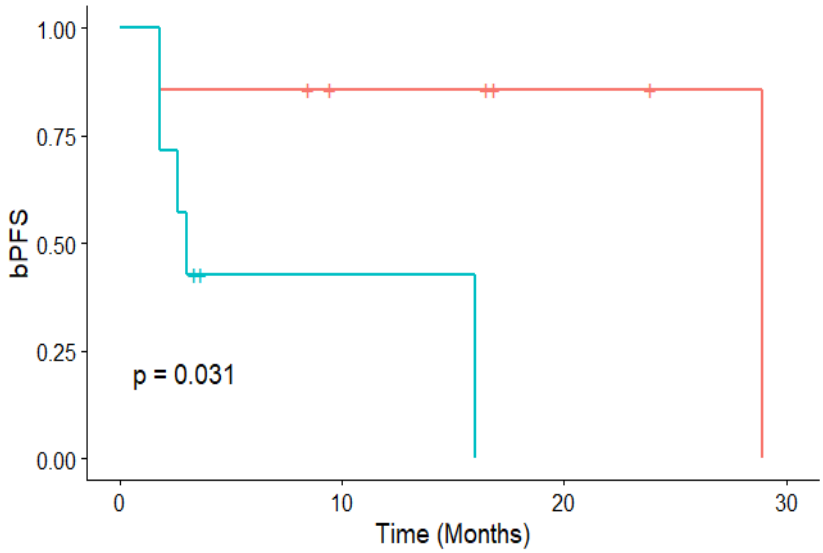


**Figure 7:** Kaplan-Meier estimates of bPFS according to $^{68}$Ga-PSMA PET MTV_VARIATION%. Blue curve: higher than cut-off. Red curve: lower than cut-off.

*3.2 Secondary aim: correlation of pre-treatment PET parameters and their variations with OS.*

PET parameter at baseline and their percentage variation were correlated to OS using the Wilcoxon signed rank test for each radiotracer. Significant correlations with OS were found for MTV_TOT_PET1 of $^{68}$Ga-PSMA (p=0.044) and $^{18}$F-FACBC PET (p=0.025, figure 8), MTV_VARIATION% of $^{68}$Ga-PSMA PET (p=0.04, figure 9), TLA_VARIATION% of $^{18}$F-FACBC PET (p=0.044, figure 10).

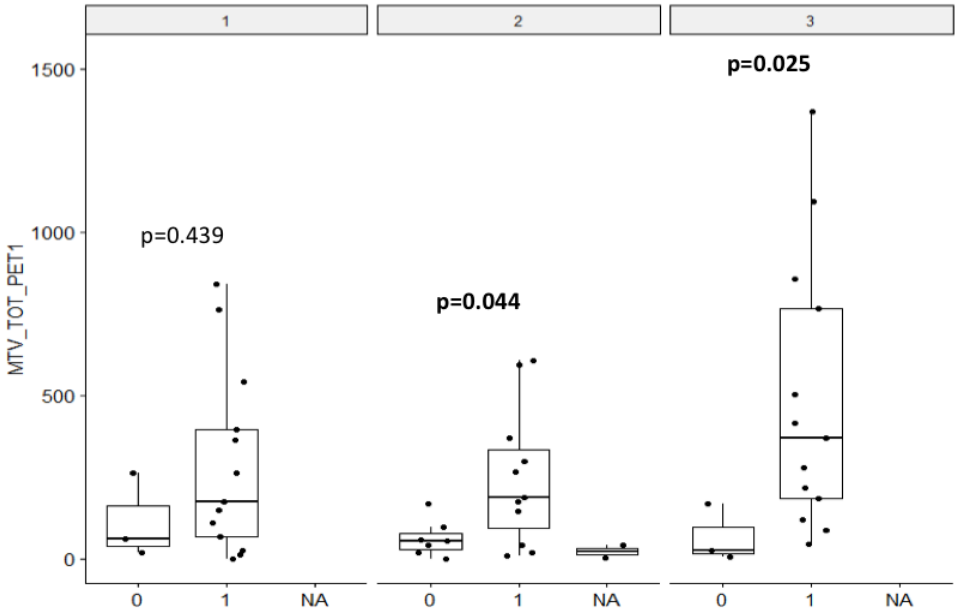

**Figure 8.** Wilcoxon signed rank test correlations of MTV_TOT_PET1 with $^{11}$C-Choline (1), $^{68}$Ga-PSMA (2), $^{18}$F-FACBC (3) PET and OS. In bold, statistically significant variables.

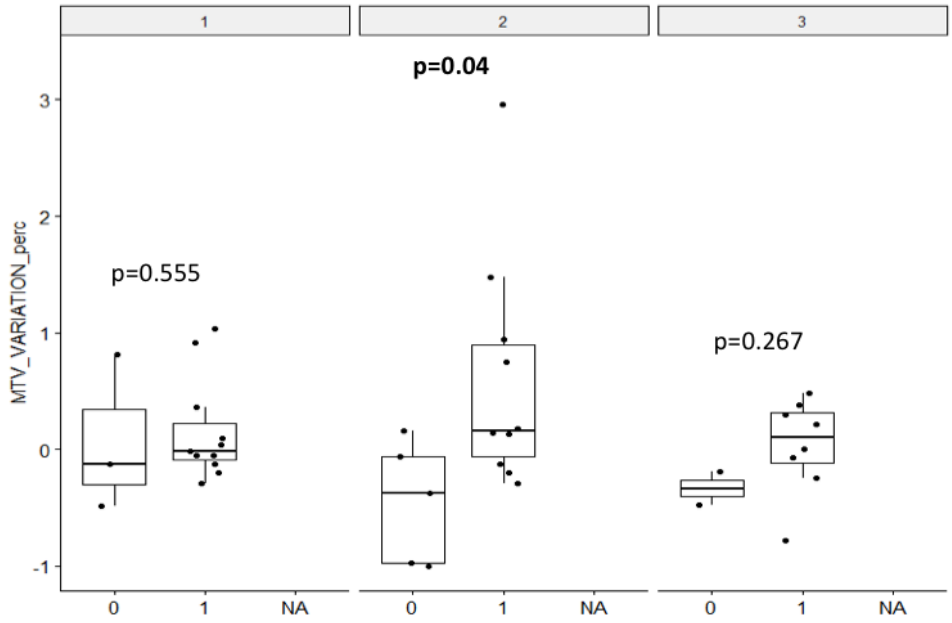


**Figure 9.** Wilcoxon signed rank test correlations of MTV_VARIATION% with $^{11}$C-Choline (1), $^{68}$Ga-PSMA (2), $^{18}$F-FACBC (3) PET and OS. In bold, statistically significant variables.

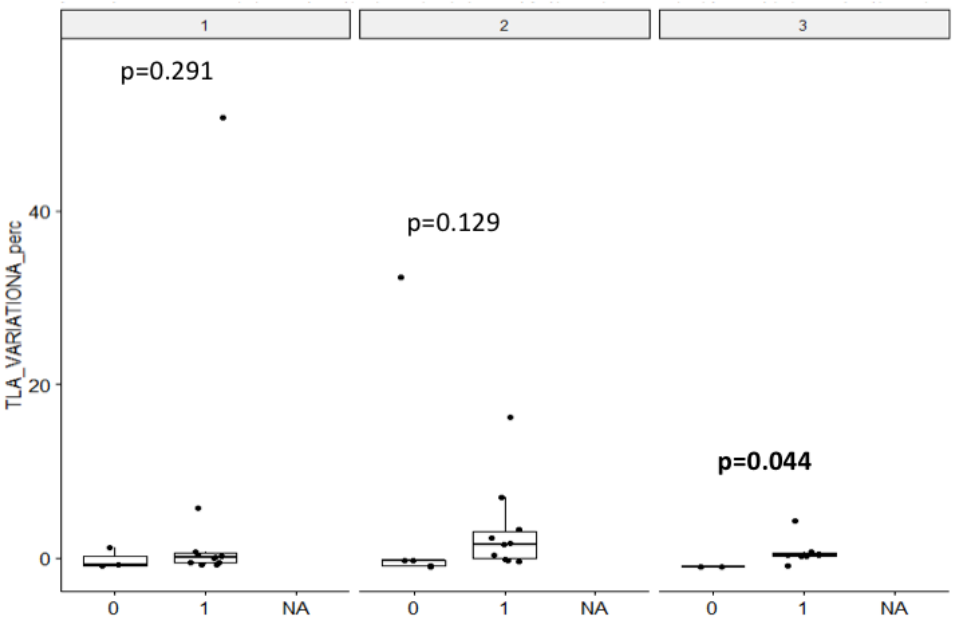


**Figure 10.** Wilcoxon signed rank test correlations of TLA_VARIATION% with $^{11}$C-Choline (1), $^{68}$Ga-PSMA (2), $^{18}$F-FACBC (3) PET and OS. In bold, statistically significant variables.

Cut-off values of each PET parameter were calculated using ROC curves and were subsequently dichotomized into lower or higher than cut-off (table 6). Then, we evaluated the correlation of each PET parameter variable, calculated with ROC curve, and OS with log rank test.

| | **$^{11}$C-CHOLINE PET** | **$^{68}$Ga-PSMA PET** | **$^{18}$F-FACBC PET** |
|---|---|---|---|
| MTV_TOT_PET1 | 67.2 | 173.4 | 184.5 |
| TLA_TOT_PET1 | 462.1 | 963.8 | 871.1 |
| MAJ_SUV_MAX_PET1 | 499 | 160 | 424 |
| SUVMAX_VARIATION% | -0.9792 | -0.8658 | -0.9776 |
| MTV_VARIATION% | -0.0544 | -0.2927 | -0.0703 |
| TLA_VARIATION% | -0.7677 | -0.2695 | -0.8399 |

**Table 6.** Cut-off of PET parameters and percentage variation for each radiotracer.
Legend: MTV: Metabolic Tumor Volume; Maj: Major; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

Then, we evaluated the correlation of each PET parameter variable, calculated with ROC curve, and OS with log rank test (table 7). With regards to $^{11}$C-Choline PET, a significant correlation was found for MAJ_SUV_MAX_PET1 (p=0.007, figure 11). For $^{68}$Ga-PSMA PET, parameters statistically correlated to OS were MTV_TOT_PET1 (p=0.004, figure 12), MAJ_SUV_MAX_PET1 (p=0.029, figure 13), SUVMAX_VARIATION% (p=0.04, figure 14), MTV_VARIATION% (p=0.015, figure 15), TLA_VARIATION% (p=0.03, figure 16). With $^{18}$F-FACBC radiotracer, a statistically significant correlation was found for MTV_TOT_PET1 (p=0.011, figure 17), TLA_TOT_PET1 (p=0.009, figure 18), MAJ_SUV_MAX_PET1 (p=0.027, figure 19), MTV_VARIATION% (p=0.048, figure 20).

| | **$^{11}$C-CHOLINE PET** | **$^{68}$Ga-PSMA PET** | **$^{18}$F-FACBC PET** |
|---|---|---|---|
| MTV_TOT_PET1 | 0.26 | **0.004** | **0.011** |
| TLA_TOT_PET1 | 0.34 | 0.056 | **0.009** |
| MAJ_SUV_MAX_PET1 | **0.007** | **0.029** | **0.027** |
| SUVMAX_VARIATION% | 0.022 | **0.04** | 0.57 |
| MTV_VARIATION% | 0.58 | **0.015** | **0.048** |
| TLA_VARIATION% | 0.27 | **0.03** | 0.29 |

**Table 7.** Log rank test for the correlation of PET parameters and percentage variation and OS for each radiotracer. In bold, statistically significant variables.

Legend: MTV: Metabolic Tumor Volume; Maj: Major; OS: overall survival; SUVmax: Standard Uptake Value; TLA: Total Lesion Activity.

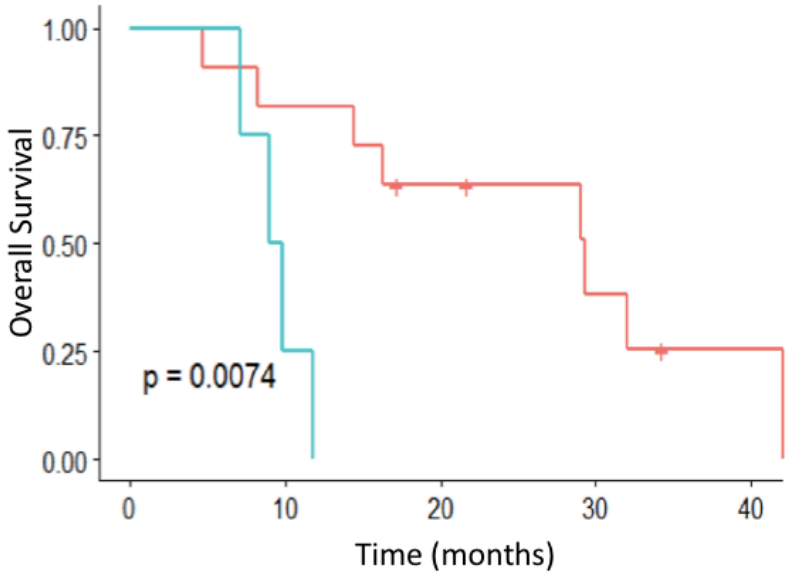


**Figure 11:** Kaplan-Meier estimates of OS according to $^{11}$C-Choline PET MAJ_SUV_MAX_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

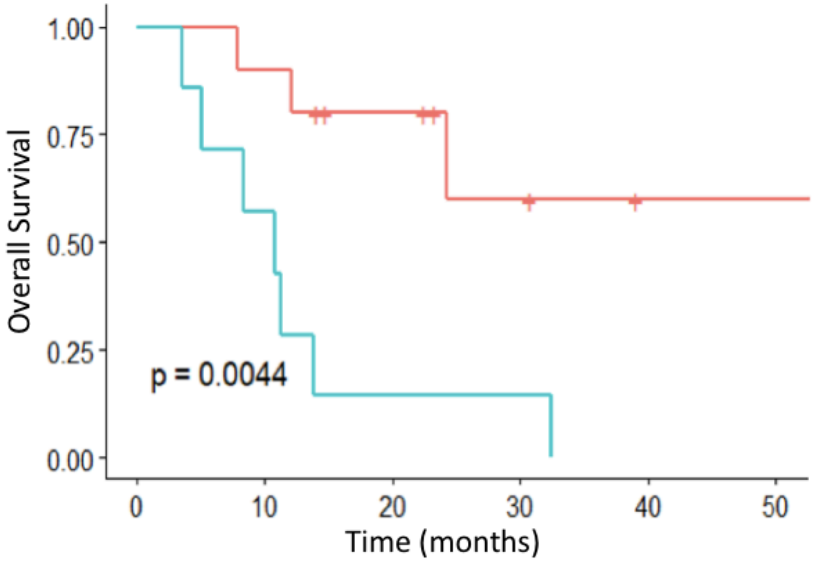


**Figure 12:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET MTV_TOT_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

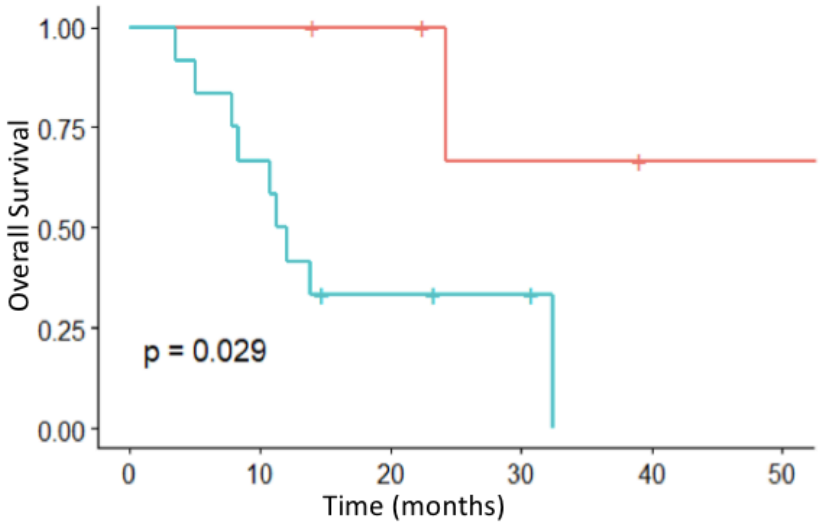


**Figure 13:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET MAJ_SUV_MAX_PET1.

Blue curve: higher than cut-off. Red curve: lower than cut-off.

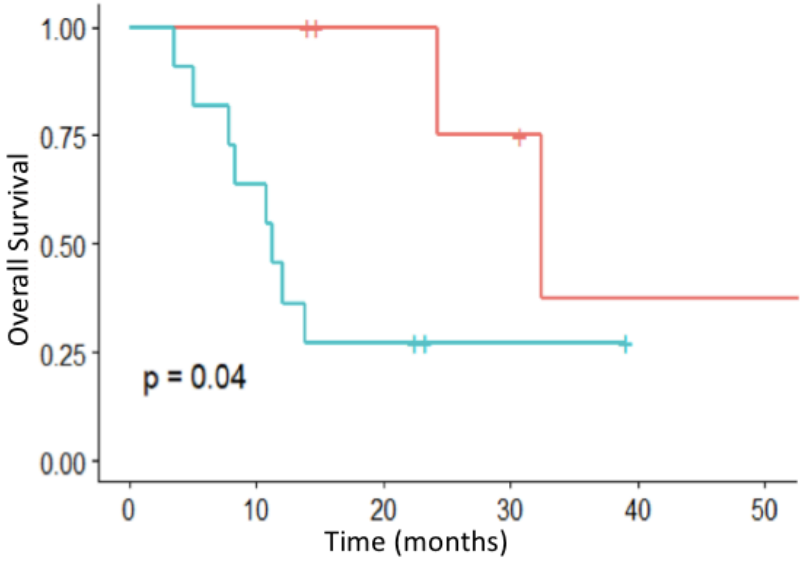


**Figure 14:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET

SUVMAX_VARIATION%. Blue curve: higher than cut-off. Red curve: lower than cut-off.

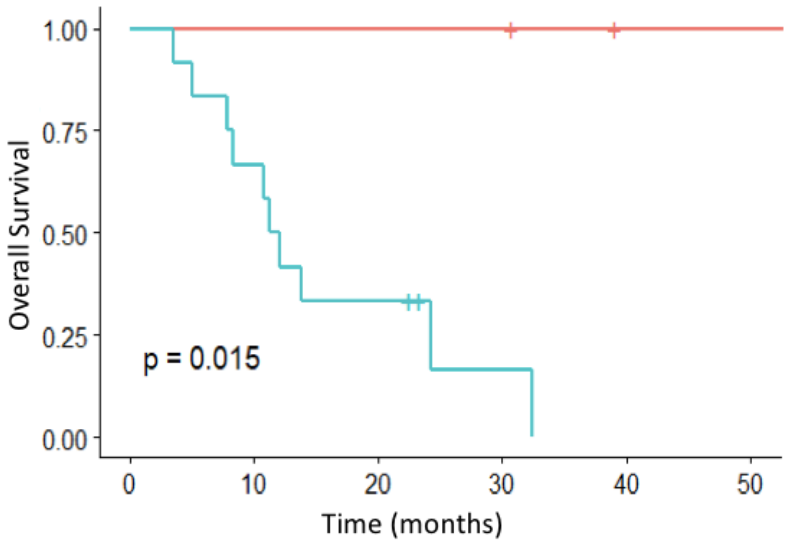


**Figure 15:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET MTV_VARIATION%.

Blue curve: higher than cut-off. Red curve: lower than cut-off.

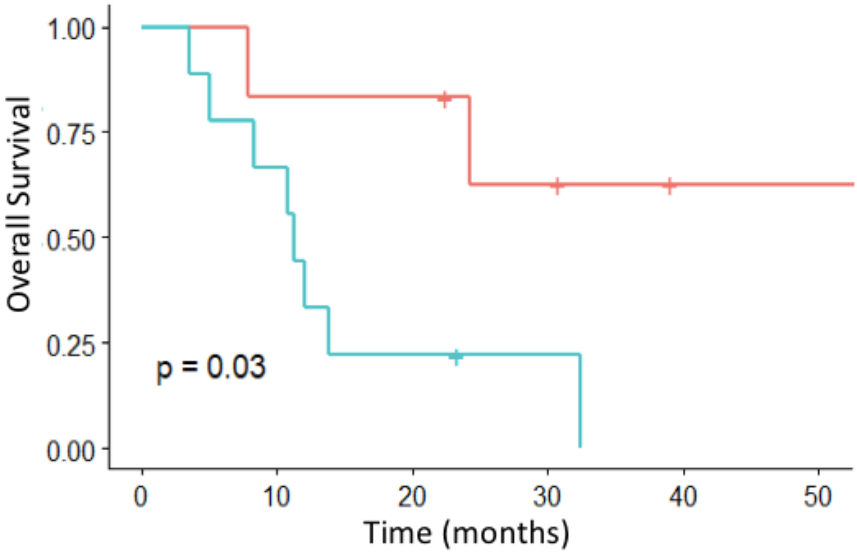


**Figure 16:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET TLA_VARIATION%.

Blue curve: higher than cut-off. Red curve: lower than cut-off.

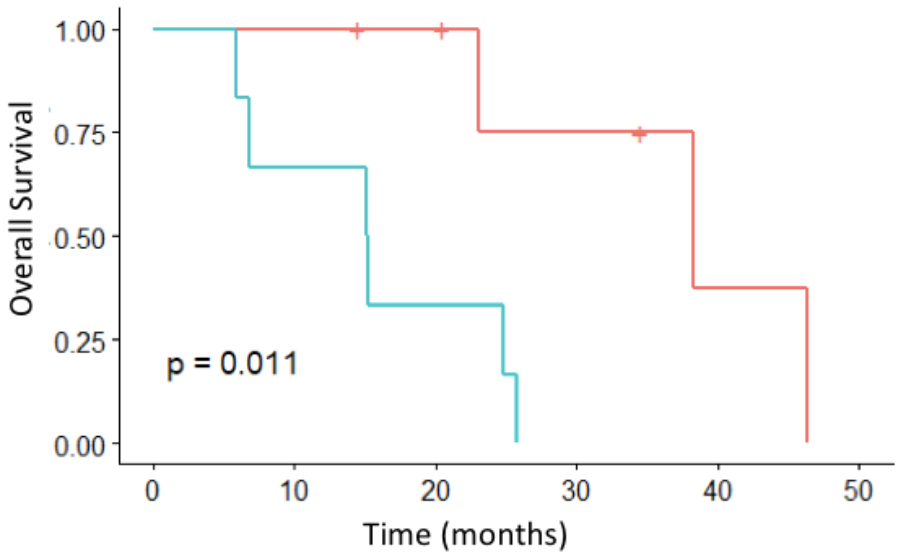


**Figure 17:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET MTV_TOT_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

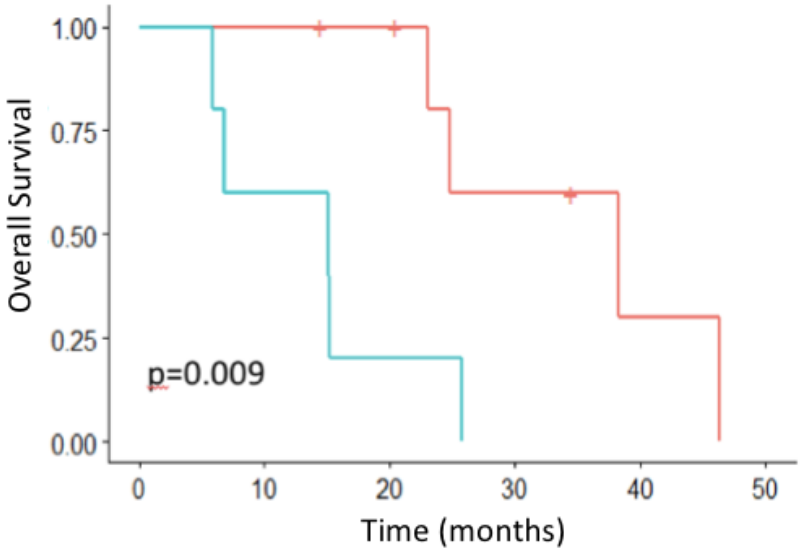


**Figure 18:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA PET TLA_TOT_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

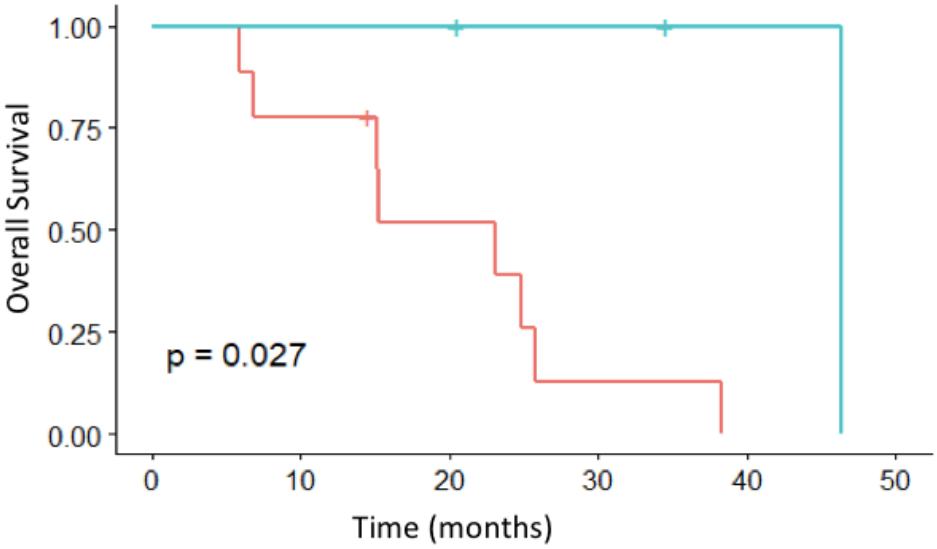


**Figure 19:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA MAJ_SUV_MAX_PET1. Blue curve: higher than cut-off. Red curve: lower than cut-off.

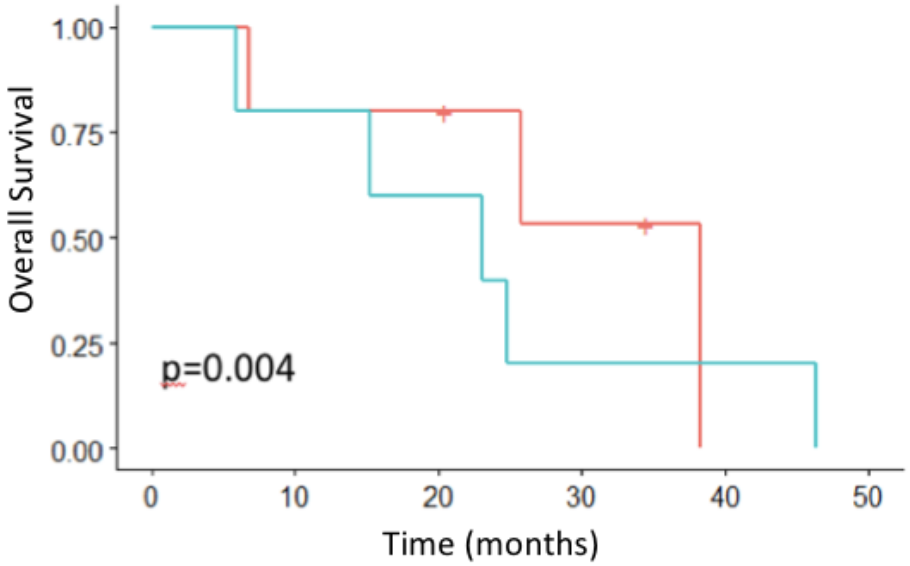


**Figure 20:** Kaplan-Meier estimates of OS according to $^{68}$Ga-PSMA MTV_VARIATION%. Blue curve: higher than cut-off. Red curve: lower than cut-off.

## 4. Discussion:

A crucial aspect about the management of cancer patients is represented by the early identification of responders and resistant subjects, thus enabling replacement of the ongoing oncologic treatment if

indicated, and by the identification of patients with a more aggressive disease and a worse expected outcome, for whom an intensified therapy program may be considered. CIM or PSA assessment are not completely able to fulfill this task, while PET assessment could represent a promising option. Our study was designed to explore the role and the utility of PET scan with three different radiotracers in the early therapy response assessment of mCRPC patients and to determine its prognostic value.

In this study, MTV and TLA variations with $^{68}$Ga-PSMA PET resulted to be associated with biochemical response, thus appearing to be valuable parameters to include in the assessment of response. Moreover, several PET parameters presented a correlation with bPFS and OS, underlining their prognostic role. The results of our study suggest that, along with SUVmax, other semiquantitative parameters, such as TLA and MTV, should be included routinely in the PET/CT reports.

Regarding the prognostic role, $^{11}$C-Choline or $^{68}$Ga-PSMA PET are known to be associated with prognosis in patients undergoing RT, but no sufficient data are available about their role during systemic therapy [17]. Currently, a minor role is played by $^{11}$C-Choline and $^{18}$F-FACBC PET and their use in routine clinical practice in CRPC is not strongly supported, especially in the early therapy assessment. It must be stressed that the limited sample size of these two groups of patients in our study may have influenced the results of the analysis regarding these tracers. Nonetheless, our analysis adds to previous preliminary data in literature supporting the use of $^{68}$Ga-PSMA PET for the precocious assessment of therapy with ARTA [17,19,27,28].

A strength of our study is clearly its prospective and randomized design. Moreover, the adherence to international protocols for PET imaging evaluation guarantees high reliability in terms of diagnostic results. Thirdly, another merit of the study was the investigation of three different novel radiotracers. It also has to be underlined that patients' characteristics of each randomized group of the study are quite homogenous, also regarding the burden of the disease, baseline PSA levels and previous therapies received.

Conversely, several limitations have to be pointed out. The first limitation is the relatively restricted number of patients enrolled in each randomized group of the study and the percentage of patients lost to follow-up. These characteristics suggest that the results emerged in this study should be interpreted with caution and need further investigations to confirm them. Second, the short follow-up time did not allow the evaluation of long-term outcomes. Third, this study did not include an imaging re-assessment of disease under treatment with CIM and, consequently, a direct comparison with PET in terms of prognostic value and early response evaluation could not be performed.

The importance of a valid imaging to evaluate treatment response can be reflected in the potential savings from unnecessary collateral effects of ARTA if progressive disease could be detected early. Additionally, an early evaluation of disease progression can eventually lead to a benefit in oncologic outcome and in the effectiveness of subsequent treatment, in view of the possibility to replace ARTA with other active therapies when the burden of the disease is still restricted.

## 5. Conclusion:

Our prospective study highlighted that several semiquantitative PET parameters and their variations present a prognostic value in terms of OS and bPFS, that could help to identify responsive or resistant patients to ARTA. Furthermore, MTV and TLA variations with $^{68}$Ga-PSMA PET appeared to be correlated with biochemical response. The ability of these radiotracers to give information on prognostically worse disease at baseline or throughout novel antiandrogen therapy, is extremely relevant in order to define the best therapeutic strategy, considering the wide plethora of treatment options currently at disposal for prostate cancer patients.

**Reference**


1) Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17-48. doi:10.3322/caac.21763
2) Marchetti A, Tassinari E, Rosellini M, Rizzo A, Massari F, Mollica V. Prostate cancer and novel pharmacological treatment options-what's new for 2022?. Expert Rev Clin Pharmacol. 2023;16(3):231-244. doi:10.1080/17512433.2023.2181783
3) Fizazi K, Scher HI, Molina A, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study [published correction appears in Lancet Oncol. 2012 Nov;13(11):e464] [published correction appears in Lancet Oncol. 2014 Aug;15(9):e365]. *Lancet Oncol*. 2012;13(10):983-992. doi:10.1016/S1470-2045(12)70379-0
4) Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol*. 2015;16(2):152-160. doi:10.1016/S1470-2045(14)71205-7
5) Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol*. 2015;16(2):152-160. doi:10.1016/S1470-2045(14)71205-7
6) Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012;367(13):1187-1197. doi:10.1056/NEJMoa1207506
7) Nigro MC, Mollica V, Marchetti A, et al. Current androgen receptor antagonists under investigation for resistant prostate cancer. *Expert Rev Anticancer Ther*. 2022;22(2):191-202. doi:10.1080/14737140.2022.2020651

8) Scher HI, Morris MJ, Stadler WM, 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*. 2016;34(12):1402-1418. doi:10.1200/JCO.2015.64.2702

9) Pezaro C, Omlin A, Lorente D, et al. Visceral disease in castration-resistant prostate cancer. *Eur Urol*. 2014;65(2):270-273. doi:10.1016/j.eururo.2013.10.055

10) Wang Y, Wang Y, Ci X, et al. Molecular events in neuroendocrine prostate cancer development. *Nat Rev Urol*. 2021;18(10):581-596. doi:10.1038/s41585-021-00490-0

11) Burgio SL, Conteduca V, Rudnas B, et al. PSA flare with abiraterone in patients with metastatic castration-resistant prostate cancer. *Clin Genitourin Cancer*. 2015;13(1):39-43. doi:10.1016/j.clgc.2014.06.010

12) Farag S, Geus-Oei LF, van der Graaf WT, et al. Early Evaluation of Response Using $^{18}$F-FDG PET Influences Management in Gastrointestinal Stromal Tumor Patients Treated with Neoadjuvant Imatinib. *J Nucl Med*. 2018;59(2):194-196. doi:10.2967/jnumed.117.196642

13) Foley KG, Jeffries J, Hannon C, Coles B, Bradley KM, Smyth E. Response rate and diagnostic accuracy of early PET-CT during neo-adjuvant therapies in oesophageal adenocarcinoma: A systematic review and meta-analysis. *Int J Clin Pract*. 2021;75(6):e13906. doi:10.1111/ijcp.13906

14) Vos JL, Zuur CL, Smit LA, et al. [$^{18}$F]FDG-PET accurately identifies pathological response early upon neoadjuvant immune checkpoint blockade in head and neck squamous cell carcinoma. *Eur J Nucl Med Mol Imaging*. 2022;49(6):2010-2022. doi:10.1007/s00259-021-05610-x

15) Han S, Woo S, Kim YI, 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)*. 2021;11(4):663. doi:10.3390/diagnostics11040663

16) Zukotynski KA, Emmenegger U, Hotte S, 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*. 2021;62(10):1430-1437. doi:10.2967/jnumed.120.259069

17) Alongi P, Laudicella R, Lanzafame H, et al. PSMA and Choline PET for the Assessment of Response to Therapy and Survival Outcomes in Prostate Cancer Patients: A Systematic Review from the Literature. *Cancers (Basel)*. 2022;14(7):1770. doi:10.3390/cancers14071770

18) Bianchi L, Ceci F, Costa F, et al. The Impact of PSMA-PET on Oncologic Control in Prostate Cancer Patients Who Experienced PSA Persistence or Recurrence. *Cancers (Basel)*. 2022;15(1):247. doi:10.3390/cancers15010247

19) Calderoni L, Maietti E, Farolfi A, et al. Prostate-Specific Membrane Antigen Expression on Positron Emission Tomography/Computed Tomography in Patients with Metastatic Castration-Resistant Prostate Cancer: A Retrospective Observational Study [published online ahead of print, 2023 Jan 12]. *J Nucl Med*. 2023;jnumed.122.264964. doi:10.2967/jnumed.122.264964

20) Niaz MJ, Sun M, Skafida M, et al. Review of commonly used prostate specific PET tracers used in prostate cancer imaging in current clinical practice. *Clin Imaging*. 2021;79:278-288. doi:10.1016/j.clinimag.2021.06.006

21) Hope TA, Aggarwal R, Chee B, et al. Impact of $^{68}$Ga-PSMA-11 PET on Management in Patients with Biochemically Recurrent Prostate Cancer. *J Nucl Med*. 2017;58(12):1956-1961. doi:10.2967/jnumed.117.192476

22) Donswijk ML, van Leeuwen PJ, Vegt E, et al. Clinical impact of PSMA PET/CT in primary prostate cancer compared to conventional nodal and distant staging: a retrospective single center study. *BMC Cancer*. 2020;20(1):723. doi:10.1186/s12885-020-07192-7

23) Hussain M, Carducci MA, Clarke N, et al. Evolving Role of Prostate-Specific Membrane Antigen-Positron Emission Tomography in Metastatic Hormone-Sensitive Prostate Cancer: More Questions than Answers?. *J Clin Oncol*. 2022;40(26):3011-3014. doi:10.1200/JCO.22.00208

24) Tulipan AJ, Salberg UB, Hole KH, Vlatkovic L, Aarnes EK, Revheim ME, Lyng H, Seierstad T. Amino acid transporter expression and 18F-FACBC uptake at PET in primary prostate cancer. Am J Nucl Med Mol Imaging. 2021;11(4):250-259. PMID: 34513278.

25) Saiki I, Yara M, Yamanaka T, Uchino H, Inazu M. Functional Expression of Choline Transporter-Like Protein 1 in LNCaP Prostate Cancer Cells: A Novel Molecular Target. *Biomol Ther (Seoul)*. 2020;28(2):195-201. doi:10.4062/biomolther.2019.097

26) Maurer T, Eiber M, Schwaiger M, Gschwend JE. Current use of PSMA-PET in prostate cancer management. *Nat Rev Urol*. 2016;13(4):226-235. doi:10.1038/nrurol.2016.26

27) Esen B, Seymen H, Tarim K, et al. Diagnostic Performance of $^{68}$Ga-PSMA-11 Positron Emission Tomography/Computed Tomography to Monitor Treatment Response in Patients with Metastatic Prostate Cancer: The Concordance Between Biochemical Response and Prostate-specific Membrane Antigen Results [published online ahead of print, 2023 Apr 7]. *Eur Urol Focus*. 2023;S2405-4569(23)00093-7. doi:10.1016/j.euf.2023.03.023

28) Plouznikoff N, Artigas C, Sideris S, et al. Evaluation of PSMA expression changes on PET/CT before and after initiation of novel antiandrogen drugs (enzalutamide or abiraterone) in metastatic castration-resistant prostate cancer patients. *Ann Nucl Med*. 2019;33(12):945-954. doi:10.1007/s12149-019-01404-2