Head to head comparison of ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 in patients with metastatic prostate cancer: a prospective study

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[Running Title] Comparison of ^{68}Ga-NGUL and ^{68}Ga-PSMA-11

ABSTRACT

Introduction ^{68}Ga-NOTA Glu-Urea-Lys (NGUL) is a novel prostate-specific membrane antigen (PSMA) targeting tracer used for PET/CT imaging. This study aims to compare the performance in the detection of primary and metastatic lesions, and to compare biodistribution between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 in the same patients with prostate cancer. **Methods** Eleven patients with metastatic prostate cancer were prospectively recruited. The quantitative tracer uptake was obtained in normal organs and, primary and metastatic lesions. **Results** ^{68}Ga-NGUL showed significantly lower normal organ uptake and rapid urinary clearance. The number and sites of detected PSMA positive primary and metastatic lesions were identical and no significant quantitative uptake difference was observed. ^{68}Ga-NGUL showed a relatively lower tumor-to-background ratio than ^{68}Ga-PSMA-11. **Conclusion** In head to head comparison with ^{68}Ga-PSMA-11, ^{68}Ga-NGUL showed lower uptake in normal organs with similar performance to detect PSMA avid primary and metastatic lesions. ^{68}Ga-NGUL could be a valuable option for PSMA imaging.

Keywords: Prostate-specific membrane antigen, ^{68}Ga-NGUL, ^{68}Ga-PSMA-11, biodistribution

INTRODUCTION

Prostate-specific membrane antigen (PSMA), a transmembrane protein overexpressed in prostate cancer, has been one of the most highlighted targets for imaging and therapy of prostate cancer (*1*,*2*). Among many PSMA PET tracers, ^{68}Ga-PSMA-11, is the most extensively investigated and well-established tracer (*3*). ^{68}Ga-PSMA-11 is superior than conventional imaging modalities in staging and detection of biochemical failure in patients with prostate cancer (*4-7*).

We recently have developed a novel PSMA targeting tracer based on Glu-Urea-Lys (GUL) derivatives, conjugated with NOTA chelator via a thiourea-type short linker, named ^{68}Ga-NOTA-GUL (NGUL) (*8*). In our previous study, ^{68}Ga-NGUL showed a higher tumor to background ratio, and substantially lower kidney uptake than ^{68}Ga-PSMA-11 in PSMA positive tumor xenografted mice (*8*).

To further investigate the clinical feasibility of ^{68}Ga-NGUL, we have conducted a prospective head to head comparison study between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 PET/CT. The specific aims of this study are to compare the detection efficacy and biodistribution between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 in the same patients with metastatic prostate cancer.

MATERIALS AND METHODS

Subjects

Patients with metastatic prostate cancer were prospectively recruited in this study. Each patient underwent ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 scans. The quality was assessed before administration, and as a result, ^{68}Ga-NGUL showed high purity and stability (Supplementary Figure 1). This study was approved by the Institutional Review Board. All patients gave written informed consent to have two consecutive PSMA targeted PET/CT scan. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Image acquisition and analysis

The PET/CT scans were performed at 60 minutes after tracer injection. Any focal accumulation of ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 not explained by physiologic uptake were defined as pathologic lesions. Lesion numbers and lesion uptake, as SUV_{max}, were compared (Supplementary Figure 2A). The quantitative tracer uptakes were obtained in normal organs including salivary glands, liver, spleen,

and kidney and blood pool activity was measured in the inferior vena cava (Supplementary Figure 2B). The normal organ distribution of both tracers was quantified as SUV_{mean}. In addition, three patients underwent dynamic PET/CT scanning (60 min) of the pelvic region to evaluate the urinary clearance.

Statistical Analysis

Statistical analyses were performed using PRISM version 5.0 (GraphPad Software, San Diego, CA, USA) and the MedCalc statistical packages version 14.8 (MedCalc Statistical Software, Mariakerke, Belgium). Shapiro-Wilk test was used to evaluate data normality. A comparison between the two tracers was done using Wilcoxon signed-rank test, linear regression, and Bland-Altman analysis.

RESULTS

Eleven patients were prospectively enrolled in the study. The patients' characteristics are summarized in Supplementary Table 1. The time interval between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 PET/CT scan was 1 to 4 days and no patient received any treatment between both scans. Quantitative data are expressed as the median and interquartile range.

Normal organ distribution

Overall, both scans showed similar distribution patterns with the highest uptake in the kidneys (Fig. 1). An intra-patient comparison using quantitative value revealed significantly different organ uptake in both scans. The SUV_{mean} in the kidneys, salivary glands, spleen, and liver, was significantly lower on ^{68}Ga-NGUL compared with ^{68}Ga-PSMA-11 (Supplementary Table 2, Fig. 1). Linear correlation and agreement between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 are demonstrated in Supplementary Table 2 and Supplementary Figure 3.

From the dynamic PET imaging, the time-activity curve of the bladder was obtained for both tracers (Fig. 2). Over time, higher bladder retention was observed with ^{68}Ga-NGUL, reflecting more rapid urinary clearance than ^{68}Ga-PSMA-11.

Analysis of primary and metastatic lesions

^{68}Ga-NGUL and ^{68}Ga-PSMA-11 could detect primary lesions in all patients (n =11). There was no

significant difference between the SUVmax of primary tumor (Fig. 3A, Supplementary Table 2).

In a total of 11 patients, 161 nodal and 59 bone PSMA avid metastases were identified. All lesions were detected identically by both tracers and there was no lesion detected only by either ^{68}Ga-NGUL or ^{68}Ga-PSMA-11 (Supplementary Table 3). Quantitative uptake was evaluated in a total of 36 lesions (20 lymph nodes, and 16 bone metastases), which were selected up to a maximum of five lesions (and a maximum of two lesions per organ) in each patient. No significant difference of lymph node and bone metastases uptake were observed between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 (Fig. 3A, Supplementary Table 2). Linear correlation and agreement between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 are demonstrated in Supplementary Table 2 and Supplementary Figure 4. The tumor-to-background ratio of ^{68}Ga-NGUL tended to be lower than that of ^{68}Ga-PSMA-11 in primary tumors (37.5 (26.8 – 62.8) vs 58.3 (33.5 – 90.4); p = 0.067) and lymph node metastases (29.7 (18.5 – 55.9) vs 48.1 (12.5 – 99.1); p = 0.114), and the difference was statistically significant in case of bone metastases (48.7 (29.1 – 61.9) vs 81.0 (25.7 – 97.8); p = 0.007) (Fig. 3B).

DISCUSSION

We found that ^{68}Ga-NGUL showed lower uptake in the normal organs including the kidneys, salivary glands, spleen, and liver. ^{68}Ga-NGUL also showed more rapid clearance through the urinary system than ^{68}Ga-PSMA-11. There was no significant difference for absolute lesion uptake, however, ^{68}Ga-NGUL tended to show a lower tumor-to-background ratio compared to ^{68}Ga-PSMA-11. Still, the ability to detect primary and metastatic lesions between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 was identical.

Several biodistribution studies of ^{68}Ga-PSMA-11 have well demonstrated the cellular expression of PSMA throughout the body, in parts of the lacrimal glands and major salivary glands, liver, spleen, kidneys, and intestines (*9*,*10*). In this study, ^{68}Ga-NGUL showed a visually similar distribution pattern compared with ^{68}Ga-PSMA-11. However, clearance via the urinary tract was more rapid in ^{68}Ga-NGUL than ^{68}Ga-PSMA-11. Also, normal organ uptake of ^{68}Ga-NGUL in the kidney, liver, salivary glands, and spleen were significantly lower compared to ^{68}Ga-PSMA-11. Several factors, including hydrophilicity, small molecular size, and low protein binding property, could explain the rapid clearance of ^{68}Ga-NGUL (*11*,*12*). NGUL has a lower molecular weight (769.82 vs. 947 g/mol) and higher hydrophilicity (log P = -3.3 vs. -3.9) than PSMA-11 (Supplementary Figure 5). Indeed, as a diagnostic imaging agent, early clearance of the ^{68}Ga-NGUL through the kidney to the bladder may interfere with the detection of lesions adjacent to the urinary tract. In order to overcome this limitation, proper hydration and post-void delayed scan should be considered in future imaging protocol for

^{68}Ga-NGUL.

Despite the faster clearance of ^{68}Ga-NGUL, there was a trend of a lower tumor-to-background ratio. In our previous study, the binding affinity of ^{68}Ga-NGUL was 18.3nM (*8*), which is relatively lower than that of ^{68}Ga-PSMA-11, reported to be 24.3nM (*13*). Thus, it is speculated that the fraction of the unbound ^{68}Ga-NGUL is relatively higher and ^{68}Ga-NGUL taken up by normal organs or tumor is relatively lower compared to the ^{68}Ga-PSMA-11. As a result, the difference in the tumor-to-background ratio becomes more pronounced.

Some limitations should be noted. Firstly, due to a small number of patients, we cannot allow a generalized conclusion. However, as a head to head comparison study, the difference between the distribution of the two compounds seems to be solid. Nonetheless, further studies with a larger number of patients are needed to validate our findings. Secondly, our cohort does not have whole-body PET data of multiple time points. As a result, we were unable to assess the clinical dose difference between the two agents. However, the effective dose measured from the animal experiments was 0.019 mSv/MBq (Supplementary Table 4), which is similar to the dosimetry data provided by ^{68}Ga PSMA-11 clinical studies. Lastly, the PSA level was not considered comprehensively. As PSMA-avid tumor burden significantly correlates to PSA levels, it is considered to be a good indicator to reflect the tumor status at each scan time points (*4*,*14*). However, since the term between two scans was short, within 4 days, we speculate that the difference of tumor status in each imaging point is negligible.

CONCLUSION

Head to head comparison of ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 revealed that ^{68}Ga-NGUL showed lower uptake in the normal organs including the kidneys, salivary glands, spleen, and liver, and more rapid clearance through the urinary system. Although, ^{68}Ga-NGUL showed a trend of low tumor-to-background ratio, its ability to detect primary and metastatic lesions was the same as that of ^{68}Ga-PSMA-11. Therefore, ^{68}Ga-NGUL could be a valuable option for PSMA PET/CT imaging.

DISCLOSURE

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2020R1A2C2011428), Korea Health Technology R&D Project through the Korea Health Industry Development Institute (HI18C1916, HI19C0339), and Creative-Pioneering Researchers Program through Seoul National University (SNU).

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NGUL kit vial was provided by Cellbion Co., Ltd. (Seoul, Korea).

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No other potential conflicts of interest relevant to this article exist.

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None

KEY POINTS

Questions How does ^{68}Ga-NGUL PET/CT perform in comparison to ^{68}Ga-PSMA-11 in patients with metastatic prostate cancer?

Pertinent Findings We found that the ^{68}Ga-NGUL showed lower uptake in the normal organs and more rapid clearance than ^{68}Ga-PSMA-11. ^{68}Ga-NGUL tended to show lower tumor-to-background ratio compared to ^{68}Ga-PSMA-11. Still the ability to detect primary and metastatic lesions between ^{68}Ga-NGUL and ^{68}Ga-PSMA-11 was identical and no significant difference with respect to lesion uptake was observed.

Implication for patient care ^{68}Ga-NGUL can be a valuable option for metastatic prostate cancer patient imaging and theranostics.

REFERENCES

1. Iravani A, Violet J, Azad A, Hofman MS. Lutetium-177 prostate-specific membrane antigen (PSMA) theranostics: practical nuances and intricacies. *Prostate Cancer Prostatic Dis.* 2020;23:38-52.

2. Siva S, Udovicich C, Tran B, Zargar H, Murphy DG, Hofman MS. Expanding the role of small-molecule PSMA ligands beyond PET staging of prostate cancer. *Nat Rev Urol.* 2020;17:107-118.

3. Eder M, Schafer M, Bauder-Wust U, et al. ^{68}Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjug Chem.* 2012;23:688-697.

4. Ceci F, Uprimny C, Nilica B, et al. ^{68}Ga-PSMA PET/CT for restaging recurrent prostate cancer: which factors are associated with PET/CT detection rate? *Eur J Nucl Med Mol Imaging.* 2015;42:1284-1294.

5. Fendler WP, Eiber M, Beheshti M, et al. ^{68}Ga-PSMA PET/CT: Joint EANM and SNMMI procedure guideline for prostate cancer imaging: version 1.0. *Eur J Nucl Med Mol Imaging.* 2017;44:1014-1024.

6. Eiber M, Maurer T, Souvatzoglou M, et al. Evaluation of hybrid ^{68}Ga-PSMA ligand PET/CT in 248 patients with biochemical recurrence after radical prostatectomy. *J Nucl Med.* 2015;56:668-674.

7. 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. *The Lancet.* 2020;395:1208-1216.

8. Moon SH, Hong MK, Kim YJ, et al. Development of a Ga-68 labeled PET tracer with short linker for prostate-specific membrane antigen (PSMA) targeting. *Bioorg Med Chem.* 2018;26:2501-2507.

9. Afshar-Oromieh A, Malcher A, Eder M, et al. PET imaging with a [^{68}Ga]gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. *Eur J Nucl Med Mol Imaging.* 2013;40:486-495.

10. Prasad V, Steffen IG, Diederichs G, Makowski MR, Wust P, Brenner W. Biodistribution of [^{68}Ga]PSMA-HBED-CC in Patients with Prostate Cancer: Characterization of Uptake in Normal Organs and Tumour Lesions. *Mol Imaging Biol.* 2016;18:428-436.

11. Di L. Strategic approaches to optimizing peptide ADME properties. *AAPS J.* 2015;17:134-143.

12. Varma MV, Feng B, Obach RS, et al. Physicochemical determinants of human renal clearance. *J Med Chem.* 2009;52:4844-4852.

13. Kelly J, Amor-Coarasa A, Nikolopoulou A, et al. Synthesis and pre-clinical evaluation of a new class of high-affinity ^{18}F-labeled PSMA ligands for detection of prostate cancer by PET imaging. *Eur J Nucl Med Mol Imaging.* 2017;44:647-661.

14. Schmidkonz C, Cordes M, Schmidt D, et al. ^{68}Ga-PSMA-11 PET/CT-derived metabolic parameters for determination of whole-body tumor burden and treatment response in prostate cancer. *Eur J Nucl Med Mol Imaging.* 2018;45:1862-1872.

Figure legends

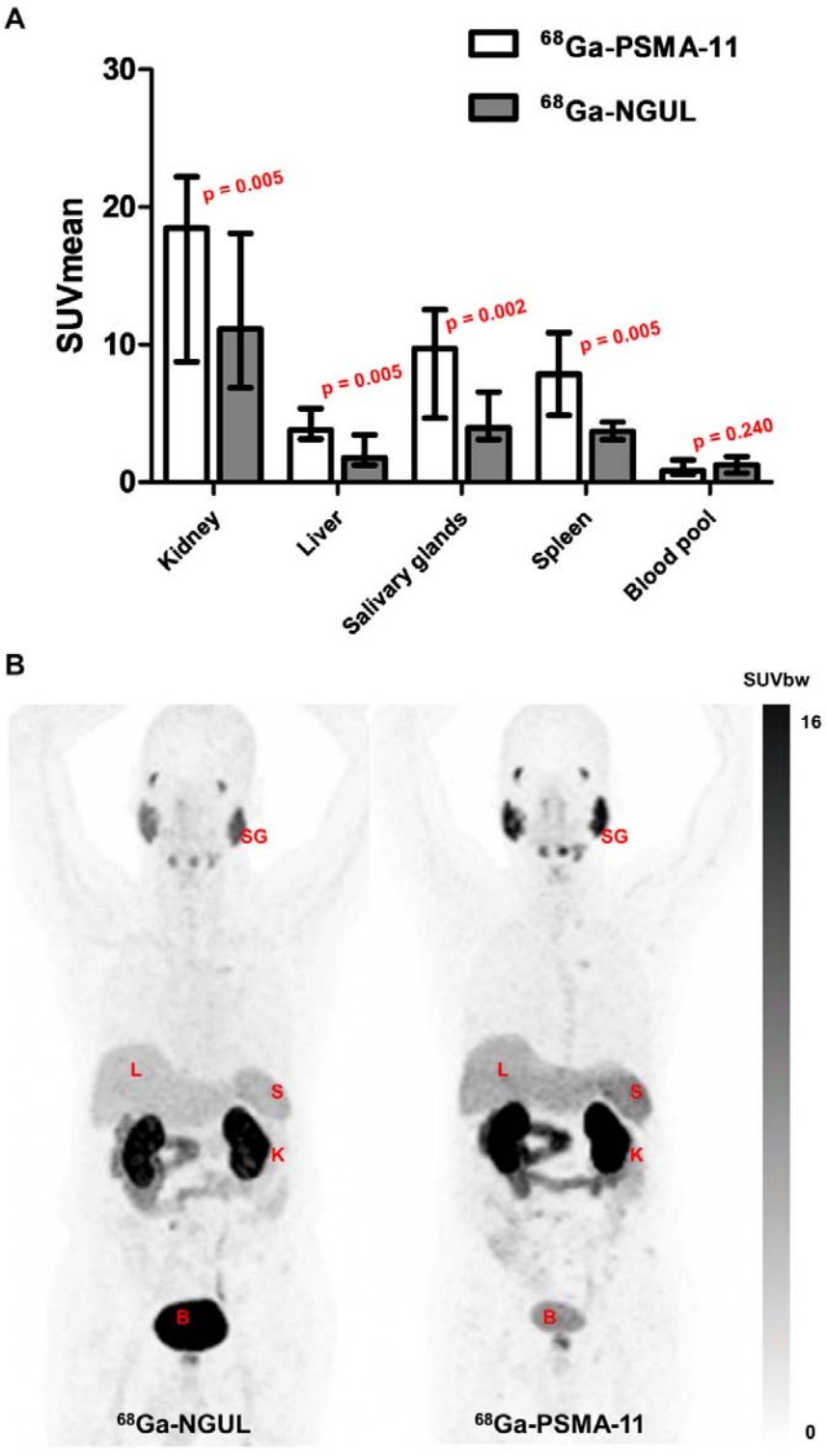


Figure 1

(A) SUV_{mean} value of normal organs for ^{68}Ga-PSMA-11 and ^{68}Ga-NGUL. Median with the interquartile range as an error bar was plotted on the bar chart. Wilcoxon signed-rank test for paired data was used for statistical comparison. (B) Representative image showing normal organ distribution of ^{68}Ga-PSMA-11 and ^{68}Ga-NGUL. (SG, salivary glands; L, liver; S, spleen; K, kidney; B, bladder)

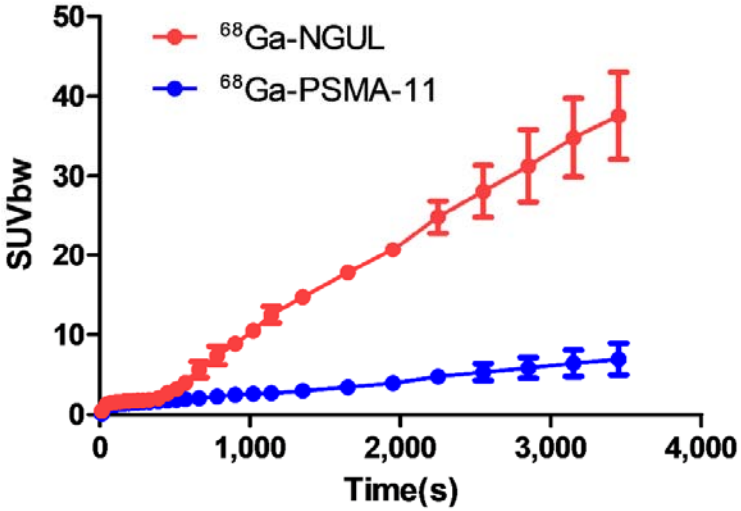


Figure 2

Time-activity curve of both ^{68}Ga-PSMA-11 and ^{68}Ga-NGUL derived from bladder region of interest.

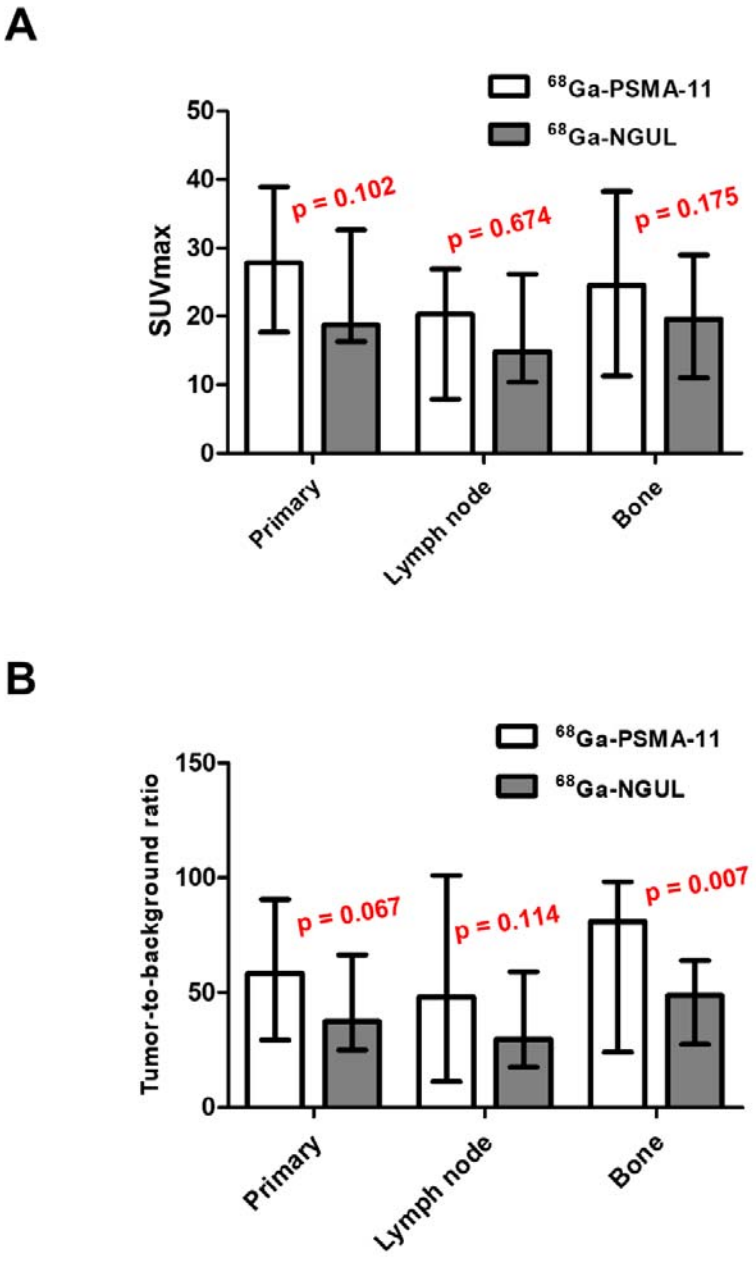
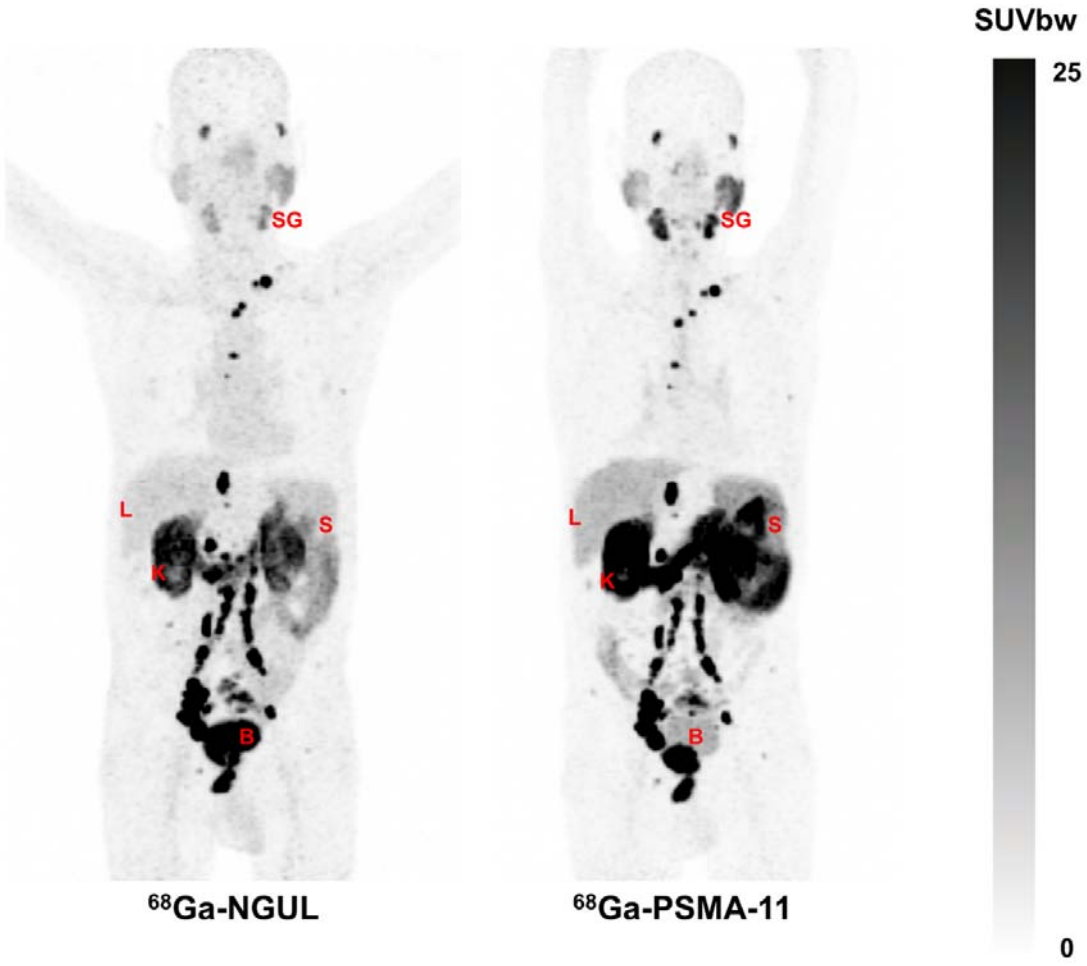
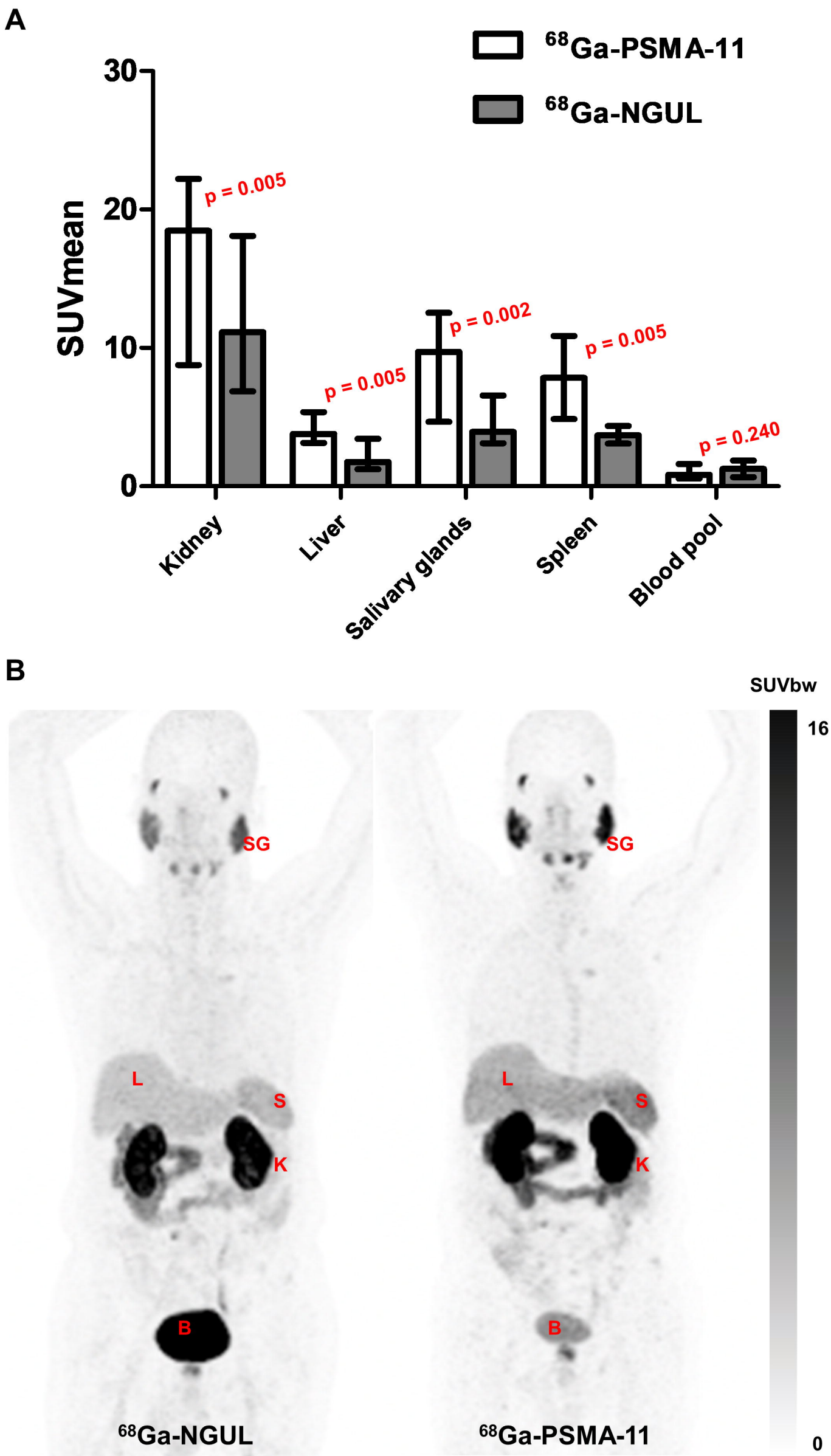


Figure 3

(A) SUV_{max} value of primary tumor, lymph node, and bone metastases for ^{68}Ga-PSMA-11 and ^{68}Ga-NGUL. (B) Tumor-to-background ratio of the primary tumor, lymph node, and bone metastases for ^{68}Ga-PSMA-11 and ^{68}Ga-NGUL. Median with the interquartile range as an error bar was plotted on the bar chart. Wilcoxon signed-rank test for paired data was used for statistical comparison.

Graphical abstracts

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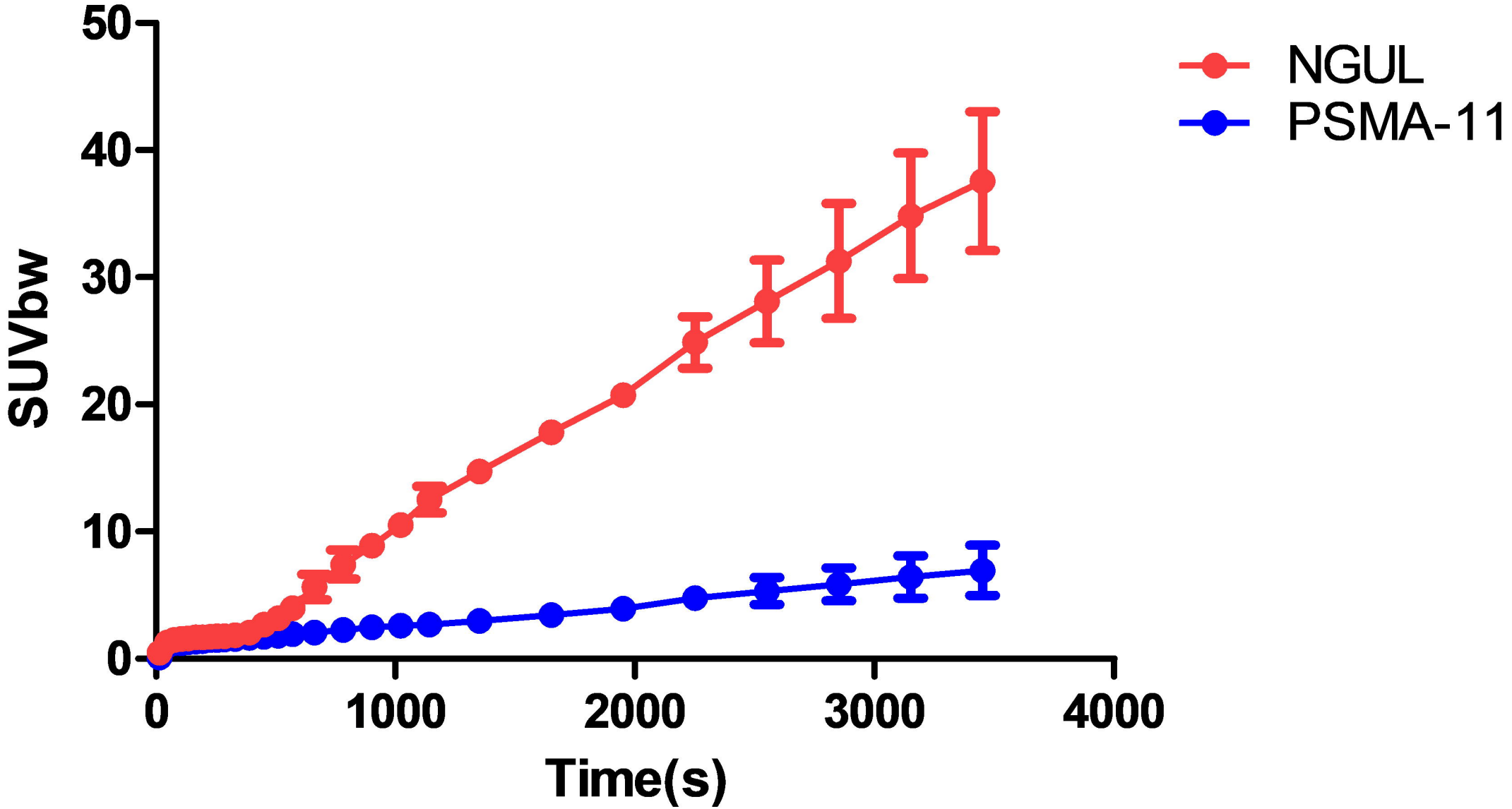
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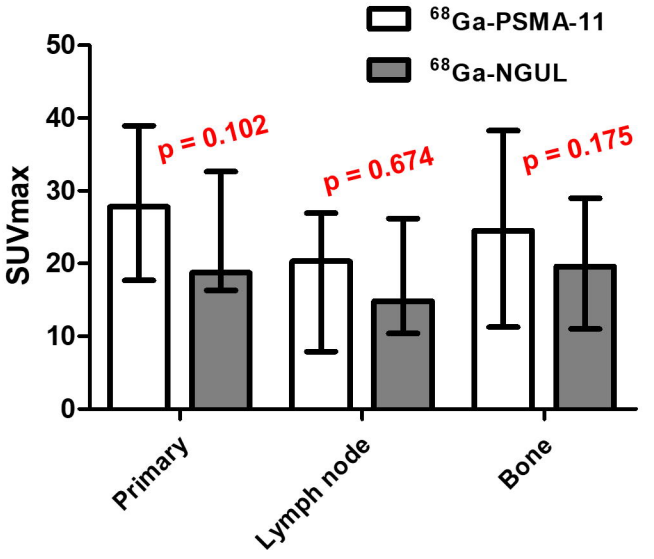
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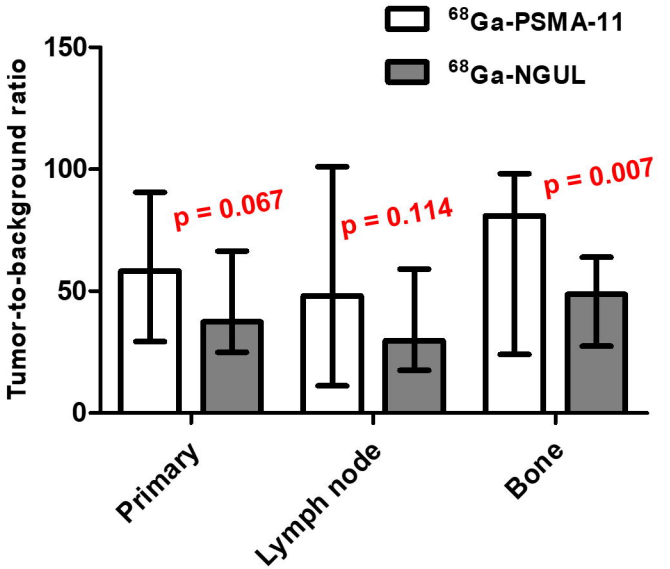
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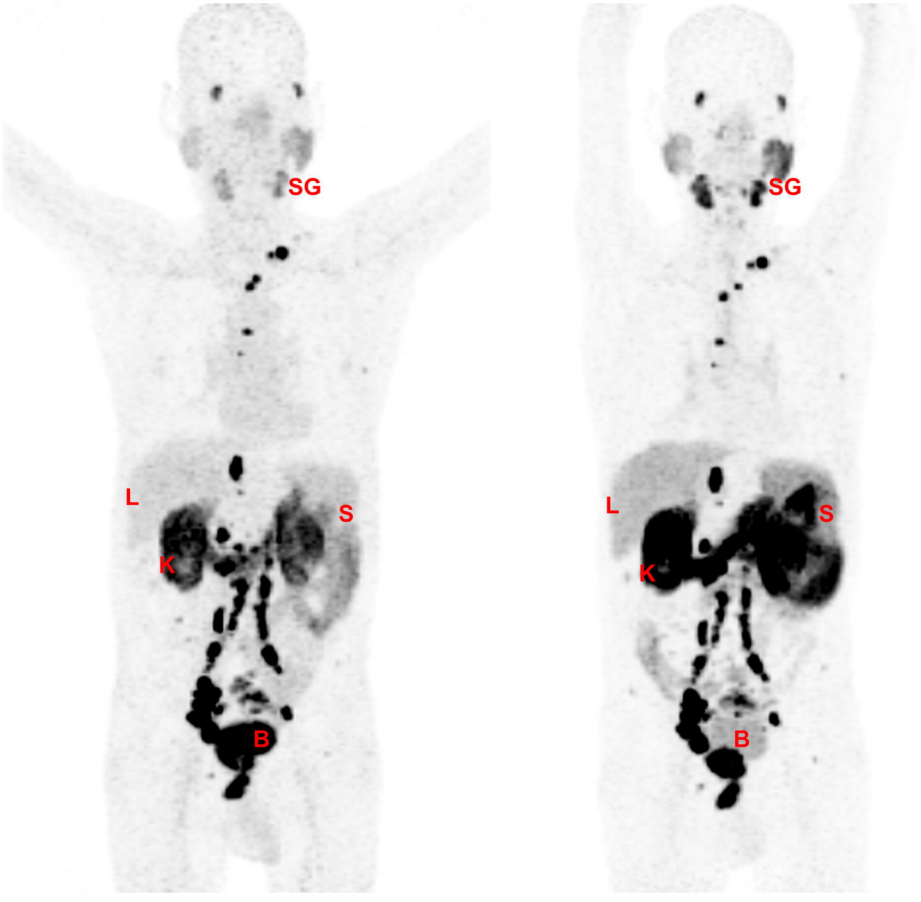
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