68Ga-NOTA-PRGD2 PET/CT for Integrin Imaging in Patients with Lung Cancer

Kun Zheng1*, Naixin Liang2*, Jingjing Zhang1*, Lixin Lang3, Wei Zhang1, Shanqing Li2, Jun Zhao4, Gang Niu3, Fang Li1, Zhaohui Zhu1#, Xiaoyuan Chen3#

1Department of Nuclear Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2Department of Thoracic Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
3Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland, USA
4Department of Thoracic Surgery, Cancer Hospital of Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

* Kun Zheng, Naixin Liang and Jingjing Zhang contributed equally to this work

Running title: 68Ga-NOTA-PRGD2 PET/CT of Lung Cancer

#Corresponding authors: Zhaohui Zhu (zhuzhh@pumch.cn); Xiaoyuan Chen (shawn.chen@nih.gov)
ABSTRACT

This study was designed to assess the diagnostic value of $^{68}$Ga-NOTA-PRGD2 PET/CT in lung cancer. **Methods:** Ninety-one patients (M 48, F 43, 22–82 y) with suspected lung lesions on CT were enrolled with informed consent. Immediately after intravenous injection of $117.7 \pm 37.7$ MBq of $^{68}$Ga-NOTA-PRGD2, 15 patients underwent dynamic whole-body PET/CT scans for 1–2 h, and the remaining 76 patients underwent whole-body PET/CT scans at $30 \pm 10$ min after bolus injection. Each patient also had standard $^{18}$F-FDG PET/CT for comparison. **Results:** No side effect was found after $^{68}$Ga-NOTA-PRGD2 injection. $^{68}$Ga-NOTA-PRGD2 was rapidly cleared from the blood pool and primarily excreted through the urinary system. The standard uptake values (SUVs) of proven malignancies were significantly higher than those of the benign ones. With SUV$_{avg} > 1.3$ being considered malignant, the sensitivity, specificity and accuracy of $^{68}$Ga-NOTA-PRGD2 PET/CT in diagnosing lung cancer were 83.8% (57/68), 91.3% (21/23) and 85.7% (78/91), respectively. The diagnostic value of $^{68}$Ga-NOTA-PRGD2 for lung cancer is comparable to that of FDG PET/CT. However, $^{68}$Ga-NOTA-PRGD2 PET/CT is more specific than $^{18}$F-FDG PET/CT in assessing lymph node metastasis, with positive and negative predictive values of 90.0% (27/30) and 93.8% (121/129), respectively, while those of $^{18}$F-FDG PET/CT were 30.2% (29/96) and 90.5% (57/63). **Conclusion:** This study indicates the efficacy of $^{68}$Ga-NOTA-PRGD2 PET/CT in lung cancer diagnosis. It shows significant advantage over $^{18}$F-FDG PET/CT in judging metastatic lymph nodes with higher specificity.

**Key Words:** integrin, $^{68}$Ga-NOTA-PRGD2, PET/CT, lung cancer, lymph node metastasis
INTRODUCTION

Integrin family consists of 24 different heterodimerized transmembrane receptors which play important roles in many physiological and pathological processes including cell survival, growth, differentiation, migration, inflammatory responses, platelet aggregation, tissue repair and tumor invasion (1). Among them, integrin receptor $\alpha_v\beta_3$ is one of the key molecules participating in tumor angiogenesis, invasion and metastasis (2-4). Based on the key role it plays in oncology and its easy accessibility as a cell surface receptor, integrin $\alpha_v\beta_3$ has been intensively investigated as a target for both therapeutic and diagnostic uses in various malignancies (5-8).

Several extracellular matrix (ECM) proteins interact with integrin $\alpha_v\beta_3$ through the arginine-glycine-aspartic acid (RGD) tri-peptide sequence and thus cyclic RGD peptides with various modifications have been labeled with $^{99m}$Tc (9) and $^{111}$In (10) for SPECT imaging and with $^{18}$F (11), $^{64}$Cu (12), $^{68}$Ga (13, 14) and $^{89}$Zr (15) for PET imaging. The peptide modifications have included dimerization and polymerization of up to 8 cyclic RGD peptide units to increase binding affinity, and the attachment of polar functional groups, such as sugar and polyethylene glycol (PEG), to increase renal excretion (16, 17).

One clinical study reported the use of $^{68}$Ga-labeled RGD monomer in pediatric patients with moyamoya disease (18). Compared with the monomer, the dimeric RGD peptide binds the receptor in a divalent manner and is thus more preferable in targeting integrin-expressing cells both in vitro and in mouse models. Therefore, dimeric RGD is expected to have more intense uptake and more prolonged retention than the monomeric counterpart in integrin-expressing
Lung cancer is one of the leading causes of cancer mortality worldwide. PET imaging using 2-deoxy-2-$^{18}$F-fluoro-D-glucose ($^{18}$F-FDG) has become the standard of care in the initial management of patients with lung cancer, especially non-small cell lung cancer (21). However, false-positive FDG PET/CT results in nodal staging have been shown in patients with coexistent inflammatory or infectious diseases (22). Therefore, alternative imaging probes for accurate staging studies are necessary to assess the extent of disease and to determine appropriate treatment. In this study, we investigated the diagnostic value of a $^{68}$Ga-labeled RGD dimer, $^{68}$Ga-NOTA-PRGD2, for lung cancer using PET/CT.
MATERIALS AND METHODS

This clinical study was approved by the Institute Review Board (IRB) of the Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (IRB Protocol # S-417). All subjects signed a written informed consent. This study was registered at the NIH ClinicalTrials.gov (NCT01737112).

Volunteers and Patients

To validate the safety of 68Ga-NOTA-PRGD2 PET/CT, 7 healthy volunteers (M 3, F 4, 38–65 y, 48 ± 9 y) were enrolled (see supporting information for more details). To study the diagnostic value of 68Ga-NOTA-PRGD2 PET/CT, 91 patients (M 48, F 43, 22–82 y, 56.5 ± 14.9 y) with suspected lung lesions according to CT were enrolled. The inclusion criteria were age ≥18 years, identified with lung lesion by CT in suspicion of primary lung cancer, and able to provide basic information and sign the written informed consent. The exclusion criteria included claustrophobia, possible pregnancy, lactation, kidney or liver failure, and inability to fulfill the study. Of the 91 patients recruited, 68 patients (M 36, F 32, 30–82 y, 58.7 ± 12.9 y) were diagnosed as lung cancer, based on pathological result of surgical removal or biopsy. Twenty-three benign diagnoses (M 12, F 11, 22–76 y, 50.1 ± 18.6 y) were made on both pathological results (n = 19) and follow-up (n = 4). The follow-up CT and/or PET/CT reexamination in the 4 patients were obviously diminished after anti-inflammatory treatment and disappeared after 6 months to 1 year. The detailed diagnostic information of all patients was listed in Table 1.
68Ga-NOTA-PRGD2 Preparation

68Ga-NOTA-PRGD2 was synthesized in a sterile hot cell. The precursor NOTA-PRGD2 (14) was dissolved in deionized water to 1 μg/μL and stored at 4 °C before use. Fresh 68Ga was eluted into 3 tubes (~1.3 ml each) from the 68Ge/68Ga generator (ITG Co.) using 4 mL 0.05 M HCL (J.T. Baker). A pipette (Thermo Co., USA) was used to draw 1000 μL of 68GaCl₃ eluent from the middle tube and the radioactivity was measured. The solution was then mixed with 50 μL of 1.25 M NaOAc and 40 μL of stored precursor. After shaking, the mixture was kept in a 100 °C metal heater (Gingko) for 10 min. After the product was cooled down to room temperature, a 0.22 μm aseptic filtration membrane was used for purification. Radio-TLC (Bioscan) was used to test the radiochemical purity with CH₃OH:NH₄OAc (v/v 1:1) as the developing solution. The radiochemical purity of the product 68Ga-NOTA-PRGD2 exceeded 97%.

Examination Procedures

A Siemens Biograph 128 mCT X was used for 68Ga-NOTA-PRGD2 PET/CT. Volunteers and patients were asked to urinate right before examination. For 15 patients, after the whole-body low-dose CT scan (140 kV, 35 mA, pitch 1:1, layer 5 mm, layer spacing 3 mm, matrix 512 x 512, FOV 70 cm), approximately 111 MBq (3 mCi) of 68Ga-NOTA-PRGD2 was injected intravenously, followed by immediate serial whole-body dynamic PET acquisitions. The duration was 3 sec/bed position for the 1–8 phases, 6 sec/bed position for the 9–14 phases, 60 sec/bed position for the 15–17 phases, 120 sec/bed position for the next several phases and the last phase lasted for 240 sec/bed position. The whole body images were obtained in sequence and
the total scanning lasted for 1 to 2 h based on the patient’s height and number of phases. The other 76 patients underwent $^{68}$Ga-NOTA-PRGD2 PET/CT scans at 30 ± 10 min after injection. Except for one patient with benign lesion, all the other 90 patients had standard routine $^{18}$F-FDG PET/CT within one week. Patients were asked to fast for at least 4 h before the examination and the blood glucose levels were below 6.4 mM. After injection of approximately 7.4 MBq/kg of $^{18}$F-FDG, the patients were asked to rest quietly in a warm and dark room for about 1 h. Then, the patients underwent low dose CT and PET scans from pelvic bottom to skull base. A scan usually included 5 or 6 bed positions according to the patient’s height, and each bed position lasted for 2 min.

**Integrin Immunohistochemical (IHC) Staining**

Representative tumor and lymph node samples were fixed with 10% neutral buffered formalin and embedded in paraffin. Five µm-thick tissue sections were blocked with endogenous peroxidase using 3% H$_2$O$_2$ for 20 min. Sections were then washed three times with PBS and briefly in a buffer containing 1% polymerized bovine serum albumin (BSA) and incubated with mouse anti-human monoclonal antibody against human integrin $\alpha_v\beta_3$ (1:200, sc-7312, Santa Cruz Biotechnology) at 37 °C for 2 h. After washing with PBS, each section was incubated with horseradish peroxidase (HRP)-conjugated anti-goat IgG for 60 min at room temperature. Diaminobenzidine (DAB) was used as the chromogen and HE counterstaining was performed. Six fields were randomly selected from each section and observed using a light microscope (BX41, Olympus).
Data Analysis

The same physician measured all images with the same standard in the final analysis. A Siemens MMWP workstation was used for post-processing. The volume of interest (VOI) of 15 normal organs/tissues and concerned lesions were drawn on the last set of the serial images and the software automatically obtained the radioactivity concentration and standardized uptake value (SUV) in the VOIs. Prism 5.0 software was used for statistical analysis. The same physician compared and measured the $^{68}$Ga-NOTA-PRGD2 and $^{18}$F-FDG uptake in the lesions side by side. Receiver operating characteristic curve (ROC) was used to set the threshold for diagnosis of lung cancer. Student’s t-test was used to compare the SUVs of different groups. Z-test was used to compare the performance of $^{68}$Ga-NOTA-PRGD2 and $^{18}$F-FDG PET/CT in evaluating lung cancer. $P < 0.05$ was considered statistically significant.

RESULTS

Lung Cancer Evaluation

The biodistribution and dosimetry studies in healthy volunteers showed predominant renal clearance of $^{68}$Ga-NOTA-PRGD2 and comparable total body effective dose to that from routine $^{18}$F-FDG (more details see supplementary materials). The low background in the lung region allows the detection of lung lesions with high target-to-background ratio. Moreover, the activity in the heart and mediastinum vasculature faded away quickly, which facilitates easy recognition of abnormal lymph nodes. All positive lesions, including primary tumors, lymphatic and bone
metastases and benign foci could be clearly observed at 30 to 45 min after intravenous injection of $^{68}$Ga-NOTA-PRGD2 (Fig. 1). The lesions with prominent $^{68}$Ga-NOTA-PRGD2 uptake appeared to be integrin $\alpha_v\beta_3$ positive.

In the $^{68}$Ga-NOTA-PRGD2 PET/CT images of the patients, the SUV$_{\text{avg}}$ and SUV$_{\text{max}}$ of malignances at 30 min after injection were 2.12 ± 1.30 and 3.66 ± 2.87, respectively, which were significantly higher than those of the benign ones (SUV$_{\text{avg}} = 0.94 ± 0.43$ and SUV$_{\text{max}} = 1.57 ± 0.71$, $P < 0.05$). The SUV$_{\text{mean}}$ of normal lung tissue was 0.31 ± 0.19 and SUV$_{\text{mean}}$ of normal aortic arch was 0.85 ± 0.44. By using receiver operating characteristic (ROC) curve, and area under the curve (AUC) of SUV data of $^{68}$Ga-NOTA-PRGD2 PET/CT, we determined a threshold of 1.3 for SUV$_{\text{avg}}$ and 2.0 for SUV$_{\text{max}}$. By using a cut-off value of 1.3 for SUV$_{\text{avg}}$, the sensitivity, specificity and accuracy of $^{68}$Ga-NOTA-PRGD2 PET/CT in diagnosis of lung cancer were 83.8% (57/68), 91.3% (21/23) and 85.7% (78/91), respectively. By using a cut-off value of 1.3 for SUV$_{\text{avg}}$, the minimal size of detected tumor was 7.5 mm as measured on the CT images. When using SUV$_{\text{max}}$ with a cut-off value of 2.0, the sensitivity, specificity and accuracy of $^{68}$Ga-NOTA-PRGD2 PET/CT in the diagnosis of lung cancer were 80.9% (55/68), 82.6% (19/23) and 81.3% (74/91), respectively. For FDG PET/CT, with a cut-off SUV$_{\text{avg}}$ value of 2.0, the sensitivity, specificity and accuracy were 86.8% (59/68), 69.6% (16/23) and 82.4 % (75/91), respectively. With SUV$_{\text{max}}$ cut-off value of 3.0, the sensitivity, specificity and accuracy of FDG PET/CT in the diagnosis of lung cancer were 85.3% (58/68), 69.6% (16/23) and 81.3% (74/91), respectively. The critical ratio $z$ for comparing $^{68}$Ga-NOTA-PRGD2 and $^{18}$F-FDG PET/CT in
evaluating lung cancer was 1.033 for SUV_{ave} (P = 0.30) and 0.077 for SUV_{max} (P = 0.94).

Both SUV_{avg} and SUV_{max} of $^{18}$F-FDG were significantly higher than those of $^{68}$Ga-NOTA-PRGD2 ($P < 0.0001$). There was no significant correlation between the $^{18}$F-FDG uptake and the $^{68}$Ga-NOTA-PRGD2 accumulation. No significant difference in $^{68}$Ga-NOTA-PRGD2 accumulation and $^{18}$F-FDG uptake in primary lung lesions with or without metastatic lymphonodus ($P = 0.87$ and $P = 0.86$). The SUV_{avg} and SUV_{max} of adenocarcinoma ($n = 43$) at 30 min after injection of $^{68}$Ga-NOTA-PRGD2 were $1.78 \pm 1.81$ and $3.36 \pm 3.42$ respectively, and those of squamous cell carcinoma ($n = 15$) were $2.12 \pm 1.05$ and $4.43 \pm 2.31$, respectively. There was no significant difference between the two types of tumors ($P = 0.63$ for SUV_{avg} and $P = 0.49$ for SUV_{max}). When all the tumors were graded as high, moderate or low differentiation, no significant correlation was found between SUV values and grade of tumor (the correlation coefficient $r = 0.208$, $P > 0.05$).

**Lymph Node Evaluation**

For lymph node staging, 38 patients, including 3 cases of chronic inflammation and 35 cases of lung cancer, went through surgery had confirmed multi-region lymphonodus results. A total of 209 regions of lymphonodus were recognized from surgery with varied sizes from 0.1 to 2.0 cm. Among them, 50 were too small (less than 0.5 cm as measured by histopathology) to be identified with the images. In the remaining 159 regions, 35 were metastatic and 124 were normal. As summarized in Table 2, we graded the FDG uptake and RGD accumulation in 3 degrees: high, moderate and low. The representative PET images and immunohistochemical
staining of lymph nodes are shown in Fig. 2. Among the 124 negative lymph nodes, only 3 had low-to-moderate RGD accumulation, but for FDG, 29 had moderate and 38 had high uptake. In 35 malignant lymph nodes, 8 had very low RGD accumulation, 6 had low-to-moderate accumulation and 21 had moderate-to-high uptake. If the latter two are defined as a sign for metastasis, the positive and negative predictive values of RGD for the assessment of lymph node metastasis were 90.0% (27/30) and 93.8% (121/129), while those of FDG were 30.2% (29/96) and 90.5% (57/63). Quantitative analysis showed the RGD SUV<sub>max</sub> of non-metastatic and metastatic lymphonodi were 0.75 ± 0.75 and 1.93 ± 1.03, respectively \( (P < 0.05) \), while the FDG SUV<sub>max</sub> of non-metastatic and metastatic lymphonodi were 2.30 ± 2.31 and 3.91 ± 2.37, respectively \( (P = 0.48) \). No apparent correlation between the \(^{18}\text{F}\)-FDG uptake or \(^{68}\text{Ga}\)-NOTA-PRGD2 accumulation in lymph nodes (Table 2) and the final staging of the 38 patients was found.

**DISCUSSION**

As a diagnostic study, only trace amount of NOTA-PRGD2 was used to target the integrin receptor \( \alpha_v\beta_3 \), so no biological effect was expected. Indeed, no side effect was found according to the safety data. The results are in accordance with other RGD peptide based PET tracers such as \(^{18}\text{F}\)-Galacto-RGD, \(^{18}\text{F}\)-AH111585, \(^{18}\text{F}\)-RGD-K5, \(^{68}\text{Ga}\)-NOTA-RGD, \(^{18}\text{F}\)-Alfatide and \(^{18}\text{F}\)-FP-PRGD2 (23-29).

\(^{18}\text{F}\)-FDG PET has been intensively applied for diagnosis and staging of lung cancer.
However, large variations in sensitivity, specificity and accuracy in lung cancer diagnosis have been reported (30, 31). The accuracy of FDG-PET for diagnosing lung nodules was extremely heterogeneous (32). In this study, the sensitivity of FDG PET was 86.8%, which is within the range of literature reports. Compared with FGD PET, $^{68}$Ga-NOTA-PRGD2 PET showed lower sensitivity but higher specificity. However, there is no significant difference between the diagnostic values of these two tracers. Whether the combination of $^{68}$Ga-NOTA-PRGD2 and $^{18}$F-FDG will have added value to increase the specificity will need further investigation with larger patient population.

$^{68}$Ga-NOTA-PRGD2 PET appears to merit lymph node metastasis assessment, which is very important for clinical decision-making and surgical planning for lung cancer patients. Although $^{18}$F-FDG PET/CT improves the accuracy of N-staging, it still cannot replace invasive staging methods, such as mediastinoscopy, mainly because of its relatively low specificity and high uptake in the inflammatory lymph nodes (33). In this study, a remarkable improvement of positive predictive value was demonstrated from 30.2% (29/96) in $^{18}$F-FDG PET/CT to 90% (27/30) in $^{68}$Ga-NOTA-PRGD2 PET/CT. Thirty-five regions of malignant lymphonodi, including the one as small as 0.6 cm, were detected by $^{68}$Ga-NOTA-PRGD2 PET/CT. Active inflammatory lymphonodi are main reasons of the false-positive results in $^{18}$F-FDG PET/CT (31). Most $^{18}$F-FDG-avid lymph nodes in acute or chronic inflammation are characterized by lymphoid follicular hyperplasia that cause high FDG uptake but not necessarily express integrin $\alpha_v\beta_3$.

$^{68}$Ga-NOTA-PRGD2 PET/CT show advantages over $^{18}$F-FDG PET/CT in the differentiation of
malignant and inflammatory lymphonodi. It is also of note that we performed both visual analysis and semi-quantitative SUV analysis and visual analysis is preferred and SUV would be a secondary aid (34).

As RGD peptide tracers bind specifically with integrin $\alpha_v\beta_3$, there have been numerous reports of positive correlation of tracer uptake with the receptor density (35, 36). We also observed similar correlation between $^{68}$Ga-NOTA-PRGD2 SUVs with immunohistochemical staining results. As we did not have access to all the tumor tissues, it is thus not possible for us to perform a systematic analysis to answer the question of whether a metastatic lymph node is always integrin positive. There is also concern about the lymph node lesion size. In this study, 50 out of a total of 209 regions of lymphonodus that are smaller than 0.5 cm were directly excluded from the analysis, which may have caused negative diagnosis in both $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 scans.

CONCLUSION

$^{68}$Ga-NOTA-PRGD2 is a safe PET agent that offers good human tolerance and clear images. $^{68}$Ga-NOTA-PRGD2 PET/CT has a similar sensitivity and higher specificity value as $^{18}$F-FDG PET/CT in the detection and differentiation of lung lesions. Moreover, $^{68}$Ga-NOTA-PRGD2 PET/CT shows significant advantage over $^{18}$F-FDG PET/CT for N-staging of lung cancer, with a remarkable improvement of positive predictive value in the assessment of lymph node metastasis.
Acknowledgment

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References


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Figure 1. CT, $^{18}$F-FDG PET/CT, $^{68}$Ga-NOTA-PRGD2 PET/CT images and immunohistochemical (IHC) staining of primary lung cancer. The tumor was pointed out by arrows. A. M, 76y, moderately differentiated adenocarcinoma in the inferior lobe of the left lung. The lesion can be visualized by both $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 PET, with a $\text{SUV}_{\text{avg}}/\text{SUV}_{\text{max}}$ of 8.9/14.5 and 4.3/6.1, respectively. The tumor sections showed positive integrin $\alpha_v\beta_3$ staining. B. F, 37y, highly differentiated adenocarcinoma in the superior lobe of right lung. The lesion can only be visualized on $^{68}$Ga-NOTA-PRGD2 PET with a $\text{SUV}_{\text{avg}}/\text{SUV}_{\text{max}}$ of 1.7/2.3. The tumor sections showed positive integrin $\alpha_v\beta_3$ staining. C. F, 61y, high differentiated adenocarcinoma in the superior lobe of right lung. The $\text{SUV}_{\text{avg}}/\text{SUV}_{\text{max}}$ of $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 PET were 2.8/4.7 and 0.8/1.3, respectively. The tumor sections showed negative integrin $\alpha_v\beta_3$ staining. D. F, 61y, high differentiated adenocarcinoma. CT showed a lesion with ground-glass opacity in the inferior lobe of the left lung. Both $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 PET showed low tracer uptake within the lesion with $\text{SUV}_{\text{avg}}/\text{SUV}_{\text{max}}$ of 0.7/0.9 and 0.1/0.2, respectively. The tumor sections showed sparsely positive integrin $\alpha_v\beta_3$ staining.
Figure 2. $^{18}$F-FDG PET/CT, $^{68}$Ga-NOTA-PRGD2 PET/CT and immunohistochemical (IHC) staining of lymph nodes within the lung region. The lymph nodes were pointed out by arrows. **A.** F, 69y, moderately differentiated adenocarcinoma with lymph node metastasis. Both $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 PET showed positive lymph nodes with positive integrin $\alpha_v\beta_3$ staining. **B.** F, 44y, highly differentiated adenocarcinoma with lymph node metastasis. The lymph node is negative on $^{18}$F-FDG PET and positive on $^{68}$Ga-NOTA-PRGD2 PET with positive integrin $\alpha_v\beta_3$ staining. **C.** F, 58y, adenocarcinoma with no lymph node metastasis. However, $^{18}$F-FDG PET showed positive lymph nodes while $^{68}$Ga-NOTA-PRGD2 PET showed negative result with negative integrin $\alpha_v\beta_3$ staining. **D.** F, 62y, highly differentiated adenocarcinoma with no lymph node metastasis. Both $^{18}$F-FDG and $^{68}$Ga-NOTA-PRGD2 PET showed negative lymph nodes with negative integrin $\alpha_v\beta_3$ staining.
Table 1. Patient information and diagnosis

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Table 2. $^{18}$F-FDG uptake and $^{68}$Ga-NOTA-PRGD2 accumulation in lymph nodes

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<td>F2R2</td>
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F stands for $^{18}$F-FDG uptake and 0 to 2 stands for low, moderate and high, respectively. R stands for extremely $^{68}$Ga-NOTA-PRGD2 uptake and 0 to 2 stands for extremely low, low-to-moderate and moderate-to-high, respectively.