European Journal of Nuclear Medicine and Molecular Imaging

Treatment response evaluation with 18F-FDG PET/CT and 18F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation

--Manuscript Draft--

Manuscript Number:	EJNM-D-16-00467R2
Full Title:	Treatment response evaluation with 18F-FDG PET/CT and 18F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation
Article Type:	Original Article
Corresponding Author:	Christos Sachpekidis Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg Heidelberg, Baden-Württemberg GERMANY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg
Corresponding Author's Secondary Institution:	
First Author:	Christos Sachpekidis
First Author Secondary Information:	
Order of Authors:	Christos Sachpekidis
	Jens Hillengass
	Hartmut Goldschmidt
	Barbara Wagner
	Uwe Haberkorn
	Klaus Kopka
	Antonia Dimitrakopoulou-Strauss
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	The aim of this study was to assess the combined use of the radiotracers 18F-FDG and 18F-NaF in treatment response evaluation of a group of multiple myeloma (MM) patients undergoing high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) by means of static (whole-body) and dynamic PET/CT (dPET/CT). Patients and methods: 34 patients with primary, previously untreated MM scheduled for treatment with HDT followed by ASCT were enrolled in the study. All patients underwent PET/CT scanning with 18F-FDG and 18F-NaF before and after therapy. Treatment response by means of PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria. The evaluation of dPET/CT studies was based on qualitative evaluation, semi-quantitative (SUV) calculation, and quantitative analysis based on 2-tissue compartment modelling and a non-compartmental approach leading to the extraction of fractal dimension (FD). Results: An analysis was possible in 29 patients: 3 with clinical complete response (CR) and 26 with non-CR (13 patients near complete response-nCR, 4 patients very good partial response-VGPR, 9 patients partial response-PR). After treatment, 18F-FDG PET/CT was negative in 14/29 patients and positive in 15/29 patients, showing a sensitivity of 57.5% and a specificity of 100%. According to the EORTC 1999 criteria, 18F-FDG PET/CT-based treatment response revealed CR in 14 patients (18F-FDG PET/CT CR), PR in 11 patients (18F-FDG PET/CT PR) and

	progressive disease in 4 patients (18F-FDG PET/CT PD). In terms of 18F-NaF PET/CT, 4/29 pts (13.8%) had a negative baseline scan, thus failed to depict MM. Regarding the patients, for which a direct lesion-to-lesion comparison was feasible, 18F-NaF PET/CT depicted 56 of the 129 18F-FDG positive lesions (43%). Follow-up 18F-NaF PET/CT showed persistence of 81.5% of the baseline 18F-NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned to 18F-FDG negative. Treatment response according to 18F-NaF PET/CT revealed CR in 1 patient (18F-NaF PET/CT CR), PR in 5 patients (18F-NaF PET/CT PR), SD in 12 patients (18F-NaF PET/CT SD), and PD in 7 patients (18F-NaF PET/CT PD). Dynamic 18F-FDG and 18F-NaF PET/CT studies showed that SUVaverage, SUVmax, as well as the kinetic parameters K1, influx and FD from reference bone marrow and skeleton responded to therapy with a significant decrease ($p<0.001$). Conclusion: 18F-FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of MM. Despite its limited sensitivity, the performance of 18F-FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. On the other hand, 18F-NaF PET/CT does not seem to add significantly to 18F-FDG PET/CT in treatment response evaluation of MM patients undergoing HDT and ASCT, at least shortly after therapy.
Response to Reviewers:	

Dear Editor,

Please find below our response to the Reviewers' comments on our manuscript entitled "Treatment response evaluation with ^{18}F-FDG PET/CT and ^{18}F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation"

by C. Sachpekidis, J. Hillengass, H. Goldschmidt, B. Wagner, U. Haberkorn, K. Kopka, A. Dimitrakopoulou-Strauss

Reviewer #1:
Adequate. No remarks

Reviewer #2:
Authors have made extensive modification on the original manuscript based on our comment. But two questions still remain to be answered.

1. Authors provided the results of PFS and OS in the revised manuscript in Table 1. Data without appropriate analysis doesn't lead to convincing conclusions. Authors did not proceed to survival analysis due to lack of late follow-up data for all patients. Why don't authors proceed to progression-free survival analysis?

   Authors' response: We proceeded to progression-free survival analysis for 28 patients, since, as already mentioned, one patient was lost to follow-up. By the time of writing 12 patients demonstrated progression. We dichotomized patients in PET/CT-positive (complete response) and PET/CT-negative (non-complete response) after therapy (follow-up scan). 6/12 patients demonstrated complete ^{18}F-FDG response and 6/12 patients had non-complete ^{18}F-FDG response. The results of Kaplan-Meier analysis and a graph are now presented in the Point to point discussion (please see below Table 1, Figure 1). No statistically significant difference in PFS was observed between the ^{18}F-FDG-positive and ^{18}F-FDG-negative patients. We would prefer not to include the results of this

analysis in the manuscript, since the number of events is still small. However, if the Reviewer insists, we could present them data as supplementary data.

Due to the fact that only one patient showed complete response in ^{18}F-NaF PET/CT (^{18}F-NaF negative follow-up PET/CT), we did not perform similar dichotomization and survival analysis for this tracer.

	Median (months)	Mean (months)
follow-up ^{18}F-FDG negative	29.4	34
follow-up ^{18}F-FDG positive	39	30

Table 1. Mean and median PFS values of the 12 patients demonstrating progression, dichotomized according to the result of follow-up ^{18}F-FDG PET/CT. The PFS difference between these two groups was not statistically significant (log-rank p=0.848).

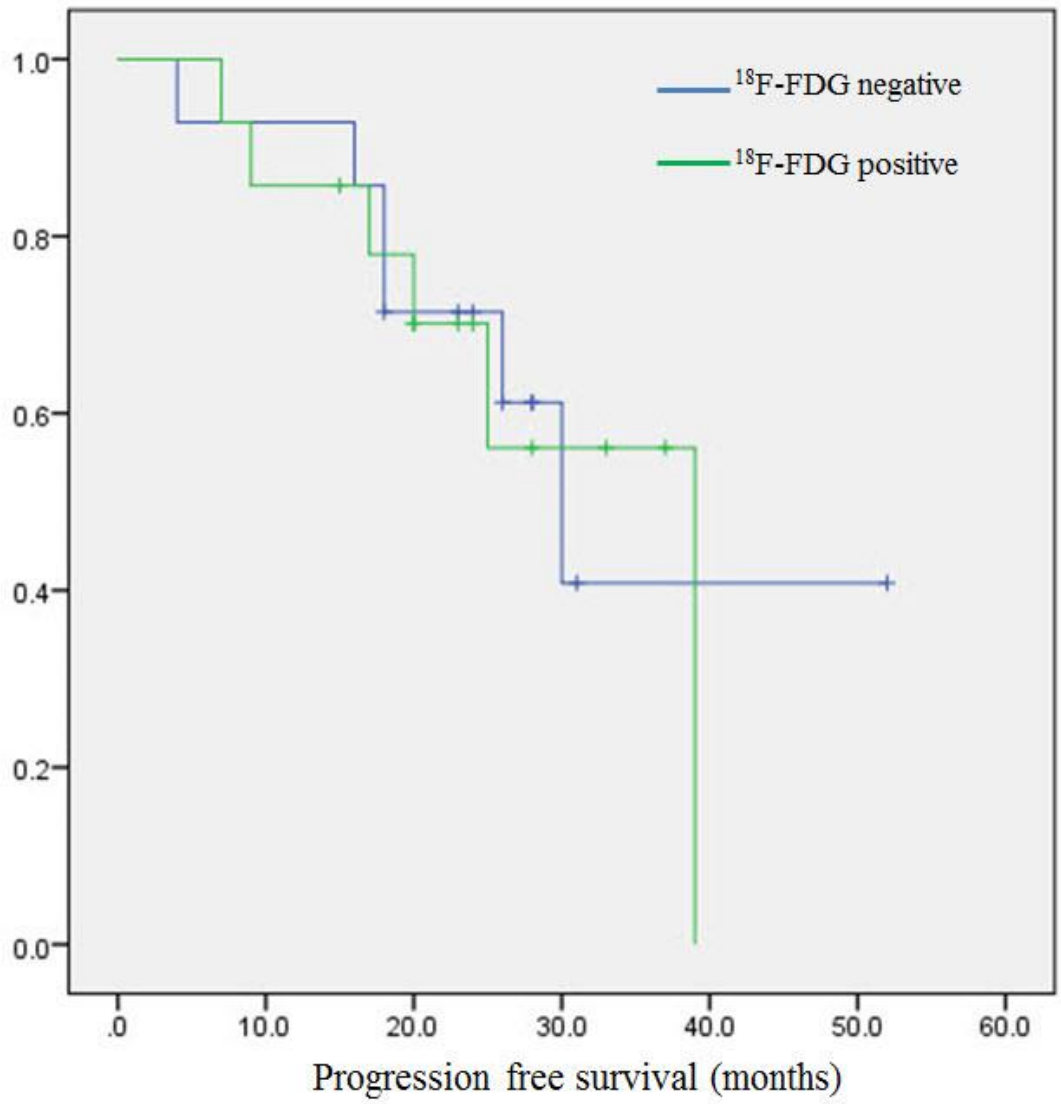


Figure 1: Kaplan-Meier plot of PFS. No statistically significant difference in PFS between patients with negative (blue curve) and positive (green curve) follow-up ^{18}F-FDG PET/CT (log-rank p = 0.848).

2. I just want to know how to calculate the dosages of 18F-FDG and 18F-NaF. Table 2 is unnecessary. The dosages can be described with interval numbers.

   Authors' response: Table 2 was removed and dosage ranges of both tracers are now provided in text (pg 5, para 2, ln 6-7). There was a maximum limit of 250 MBq for each PET exam, as defined by the federal radiation protection agency. The administered dose was not weight-dependent. We tried to apply as much

activity as possible with respect to the predefined upper limit. Nevertheless, due to technical reasons (e.g. delays in delivery of tracer), in very few cases relative low doses of tracer activity were administered (for example baseline ^{18}F-FDG PET/CT of patient 13).

Treatment response evaluation with ^{18}F-FDG PET/CT and ^{18}F-NaF PET/CT in multiple myeloma patients undergoing high-dose chemotherapy and autologous stem cell transplantation

C. Sachpekidis[1,2], J. Hillengass[3], H. Goldschmidt[3,4], B. Wagner[3], U. Haberkorn[1,5], K. Kopka[6], A. Dimitrakopoulou-Strauss[1]

[1]Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center (DKFZ), Heidelberg, Germany

[2]Department of Nuclear Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland

[3]Department of Internal Medicine V, University Hospital Heidelberg, Heidelberg, Germany

[4]National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Germany

[5]Department of Nuclear Medicine, University of Heidelberg, Heidelberg, Germany

[6]Department of Radiopharmaceutical Chemistry, German Cancer Research Center (DKFZ), Heidelberg, Germany

correspondence address:

Christos Sachpekidis, M.D.
Medical PET Group-Biological Imaging
Clinical Cooperation Unit Nuclear Medicine
German Cancer Research Center
Im Neuenheimer Feld 280
D-69210 Heidelberg
Emails: christos_saxpe@yahoo.gr

Key Words: ^{18}F-FDG; ^{18}F-NaF; PET/CT; high-dose chemotherapy; autologous stem cell transplantation; two-tissue compartment model

ABSTRACT

The aim of this study was to assess the combined use of the radiotracers ^{18}F-FDG and ^{18}F-NaF in treatment response evaluation of a group of multiple myeloma (MM) patients undergoing high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) by means of static (whole-body) and dynamic PET/CT (dPET/CT). **Patients and methods:** 34 patients with primary, previously untreated MM scheduled for treatment with HDT followed by ASCT were enrolled in the study. All patients underwent PET/CT scanning with ^{18}F-FDG and ^{18}F-NaF before and after therapy. Treatment response by means of PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria. The evaluation of dPET/CT studies was based on qualitative evaluation, semi-quantitative (SUV) calculation, and quantitative analysis based on 2-tissue compartment modelling and a non-compartmental approach leading to the extraction of fractal dimension (FD). **Results:** An analysis was possible in 29 patients: 3 with clinical complete response (CR) and 26 with non-CR (13 patients near complete response-nCR, 4 patients very good partial response-VGPR, 9 patients partial response-PR). After treatment, ^{18}F-FDG PET/CT was negative in 14/29 patients and positive in 15/29 patients, showing a sensitivity of 57.5% and a specificity of 100%. According to the EORTC 1999 criteria, ^{18}F-FDG PET/CT-based treatment response revealed CR in 14 patients (^{18}F-FDG PET/CT CR), PR in 11 patients (^{18}F-FDG PET/CT PR) and progressive disease in 4 patients (^{18}F-FDG PET/CT PD). In terms of ^{18}F-NaF PET/CT, 4/29 pts (13.8%) had a negative baseline scan, thus failed to depict MM. Regarding the patients, for which a direct lesion-to-lesion comparison was feasible, ^{18}F-NaF PET/CT depicted 56 of the 129 ^{18}F-FDG positive lesions (43%). Follow-up ^{18}F-NaF PET/CT showed persistence of 81.5% of the baseline ^{18}F-NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned to

^{18}F-FDG negative. Treatment response according to ^{18}F-NaF PET/CT revealed CR in 1 patient (^{18}F-NaF PET/CT CR), PR in 5 patients (^{18}F-NaF PET/CT PR), SD in 12 patients (^{18}F-NaF PET/CT SD), and PD in 7 patients (^{18}F-NaF PET/CT PD). Dynamic ^{18}F-FDG and ^{18}F-NaF PET/CT studies showed that $SUV_{average}$, SUV_{max}, as well as the kinetic parameters K_1, influx and FD from reference bone marrow and skeleton responded to therapy with a significant decrease ($p<0.001$). **Conclusion:** ^{18}F-FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of MM. Despite its limited sensitivity, the performance of ^{18}F-FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. On the other hand, ^{18}F-NaF PET/CT does not seem to add significantly to ^{18}F-FDG PET/CT in treatment response evaluation of MM patients undergoing HDT and ASCT, at least shortly after therapy.

INTRODUCTION

High-dose chemotherapy (HDT) with melphalan followed by autologous stem cell transplantation (ASCT) is the standard of care for multiple myeloma (MM) patients aged 65 years or younger[1,2,3,4,5]. In the last years the incorporation of novel agents (thalidomide, lenalidomide, bortezomib) into induction regiments and maintenance therapy of MM has improved the quality of treatment response, which in turn has led to extended progression free survival (PFS) and overall survival (OS) rates[4,6,7]. This previously unreported, prolonged survival of MM patients renders accurate assessment of response to therapy a necessity. Treatment response evaluation in MM is based on well-defined laboratory parameters and in case of a complete serological response the assessment of plasma cell percentage in bone marrow usually acquired from the iliac crest [8,9].

^{18}F-FDG PET/CT is a sensitive functional imaging modality. The updated International Myeloma Working Group (IMWG) criteria consider patients with focal skeletal lesions and increased uptake with underlying osteolytic destruction in one of the new imaging modalities as indicative of active myeloma[10,11]. Although its routine application in the follow-up of MM is not yet recommended, ^{18}F-FDG PET/CT appears to be useful in the monitoring of MM and has been proposed to strengthen the evaluation of the quality of treatment response[12,13,14,15,16].

^{18}F-NaF is a PET tracer used for skeletal imaging, which accumulates in both osteoblastic and osteolytic lesions, reflecting regional blood flow and bone remodeling[17,18,19,20]. ^{18}F-NaF PET/CT is evolving as an important imaging method for the assessment of malignant bone diseases[21,22,23]. Despite being suggested as a potential valuable tool in the assessment of MM[24,25,26,27,28], three recently published prospective studies have yielded rather discouraging results, regarding the

performance of ^{18}F-NaF PET/CT in evaluation of myeloma bone disease[29,30,31]. Nevertheless, the data regarding application of ^{18}F-NaF PET/CT in MM are still considered to be limited.

The aim of this prospective study was to assess the combined use of the radiotracers ^{18}F-FDG and ^{18}F-NaF in treatment response evaluation of a group of MM patients undergoing HDT followed by ASCT by means of static (whole-body) and dynamic PET/CT (dPET/CT).

MATERIALS AND METHODS

Patients

The evaluation included initially 34 patients confirmed to suffer from MM based on the criteria established by the IMWG, at the time point of patient recruitment, and scheduled for treatment with HDT followed by ASCT[32]. All patients had primary disease and had never received chemotherapy. Their mean age was 59.1 years (range 38-73 years). Table 1 presents analytically the characteristics of the patients investigated. Patients with a negative baseline ^{18}F-FDG PET/CT were excluded from the statistical analysis in order to avoid bias in the interpretation of the results (n=5 patients). Patient data on PFS and OS up to July 2016 (time of writing) are also presented. The analysis was conducted in accordance to the declaration of Helsinki with approval of the ethical committee of the University of Heidelberg and the federal agency of radiation protection.

PET/CT data acquisition

All patients underwent PET/CT scanning with ^{18}F-FDG and ^{18}F-NaF before and after therapy with HDT and ASCT. The mean time between baseline and follow-up study was 95 days (range 47-228 days) (Table 1). The double tracer study in each patient was completed in two consecutive days. For reasons of radiation protection the patients were intravenously administered with a maximum dosage of 250 MBq ^{18}F-FDG (range 85-246 MBq) on the first day and respectively a maximum dosage of 250 MBq ^{18}F-NaF (range 167-247 MBq) on the second day. Data acquisition consisted of two parts for each tracer: the dynamic part (dPET/CT studies of the lower lumbar spine and the pelvic skeleton) and the static part (whole body PET/CT). Details regarding data acquisition are described in a previous publication of our group[29].

PET/CT data analysis

Data analysis was based on: visual (qualitative) analysis, semi-quantitative evaluation based on SUV calculations, and quantitative analysis of the ^{18}F-FDG and ^{18}F-NaF PET/CT scans, performed before (baseline PET/CT) and after (follow-up PET/CT) treatment.

Qualitative analysis was based on visual assessment of the PET/CT scans, according to criteria applied in previous studies from our group[29, 33] . Briefly, bone marrow/skeletal foci presenting with significantly enhanced ^{18}F-FDG uptake, for which another benign aetiology was excluded, were considered indicative for myeloma. Afterwards, the results of ^{18}F-NaF PET/CT were correlated to those of ^{18}F-FDG PET/CT, which served as reference. The basic concept regarding ^{18}F-NaF PET/CT evaluation was that only lesions that correlated with respective lesions on ^{18}F-FDG PET/CT were considered as MM-indicative[29].

Semi-quantitative evaluation was based on volumes of interest (VOIs) and on subsequent calculation of SUVs. VOIs were drawn with an isocontour mode (pseudo-snake) and were placed over sites of MM involvement as well as over reference tissue[34]. Bone marrow (in the case of ^{18}F-FDG) and skeleton (in the case of ^{18}F-NaF) of the 5th lumber vertebra and os ilium if without focal tracer enhancement served as reference tissue.

Quantitative evaluation of the dynamic ^{18}F-FDG and ^{18}F-NaF PET/CT data, derived from reference tissue of the pelvis, was performed using a dedicated software and based on a two-tissue compartment model, with methods already reported in literature and performed previously from our group[29,35,36,37,38,39,40,41]. The application of a two-tissue compartment model leads to the extraction of the kinetic parameters K_1, k_2, k_3

and k_4 as well as influx (Ki) that describe specific molecular processes for each tracer. In case of ^{18}F-FDG, K_1 reflects the carrier-mediated transport of ^{18}F-FDG from plasma to tissue while k_2 reflects the transport of the radiopharmaceutical back from tissue to plasma, and k_3 represents the phosphorylation rate while k_4 the dephosphorylation rate of the glucose analogue. Influx (K_i) is derived from the equation = $(K_1 \times k_3)/(k_2 + k_3)$. In case of ^{18}F-NaF, rate constants K_1 and k_2 describe the fluoride ions exchange with hydroxyl groups of hydroxyapatite crystal of the bone and the reverse, while k_3 and k_4 represent the formation of fluoroapatite and the opposite[25]. Influx (K_i) is related to Ca^{2+} influx and bone apposition rate and, presumably, represents bone remodelling rate[42].

In addition to performing compartment analysis, a non-compartment model based on the fractal dimension (FD) for the time-activity data was also applied. FD is a parameter of heterogeneity based on the box counting procedure of chaos theory and was calculated for the time activity data in each individual voxel of a VOI. The values of FD vary from 0 to 2 showing the more deterministic or chaotic distribution of the tracer activity via time in a VOI[43].

Treatment response evaluation by laboratory and imaging

Treatment response evaluation was performed according to the clinical gold standard, based on the European Bone Marrow Transplantation Criteria, introduced by Bladé et al[8] and modified by the IMWG uniform response criteria for multiple myeloma[9]. These criteria served as reference standard in our study.

Treatment response by means of ^{18}F-FDG PET/CT was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria leading to four groups of therapy response (complete response, ^{18}F-FDG PET/CT CR; partial response, ^{18}F-FDG PET/CT PR; stable disease, ^{18}F-FDG PET/CT SD; progres-

sive disease, ^{18}F-FDG PET/CT PD) [44]. Due to lack of defined treatment monitoring criteria based on ^{18}F-NaF PET/CT, we also applied the EORTC 1999 criteria for this tracer.

Moreover, quantitative data derived from dynamic PET/CT studies from the pelvis were also applied in treatment response evaluation. In particular, the kinetic parameters retrieved from application of two-tissue compartment modelling as well as FD in reference bone marrow or skeleton were compared before and after therapy.

Statistical analysis

Data were statistically evaluated using the STATA/SE 12.1 (StataCorp) software on an Intel Core (2 · 3.06 GHz, 4 GB RAM) running with Mac OS X 10.8.4 (Apple Inc., Cupertino, CA, USA). The statistical evaluation was performed using the descriptive statistics and Wilcoxon rank-sum test. Moreover, we calculated the sensitivity and specificity of ^{18}F-FDG PET/CT for determination of remission status based on the clinical gold standard[8,9]. The results were considered significant for p less than 0.001 ($p<0.001$).

RESULTS

Treatment response evaluation based on the clinical gold standard

Patient population characteristics, as well as the results of treatment response evaluation are reported in Table 1. All patients showed at least partial clinical response after completion of HDT and ASCT. 5 MM patients had a negative baseline ^{18}F-FDG PET/CT scan and were, therefore, excluded from the statistical analysis. These 5 patients were also MM-negative on ^{18}F-NaF PET/CT. Regarding the remaining 29 patients, 3 of them demonstrated complete response (CR) and 26 demonstrated non-CR. In particular, 13 patients showed near complete response (nCR), 4 patients very good partial response (VGPR), and 9 patients showed partial response (PR) according to the clinical evaluation criteria.

^{18}F-FDG PET/CT evaluations

Baseline ^{18}F-FDG PET/CT demonstrated 129 MM-indicative focal lesions in 22 patients. The comparison between ^{18}F-FDG PET and the underlying low-dose CT findings in these 22 patients revealed 86 circumscribed osteolytic lesions in CT that correlated with the ^{18}F-FDG avid PET lesions (66.7%). In 5 patients the number of lesions was too large to be exactly calculated (more than 20 lesions). 2 patients demonstrated an intense diffuse pattern of bone marrow uptake without focal lesions. No baseline EMD was detected. After treatment, ^{18}F-FDG PET/CT became negative in 14 patients, while it remained positive in 15 patients. In correlation with the clinical gold standard, ^{18}F-FDG PET/CT after therapy was true positive in 15/26 patients with non-CR, and false negative in 11/26 patients with non-CR, resulting in a sensitivity of 57.7%. On the other hand, ^{18}F-FDG PET/CT was true negative in 3/3 patients with CR, resulting in a specificity of 100%. ^{18}F-FDG PET/CT demonstrated no false positive results in the skeleton. Two patients demonstrated on follow-up ^{18}F-FDG

PET/CT pelvic lymphadenopathy and liver lesions. However, after correlation with clinical data, these findings were attributed to inflammatory/post-therapeutic changes and fungus infection respectively. Treatment response evaluation according to the EORTC 1999 criteria revealed 14 patients with CR (^{18}F-FDG PET/CT CR), 11 patients with PR (^{18}F-FDG PET/CT PR), and 4 patients with PD due to development of new bone marrow lesions (^{18}F-FDG PET/CT PD) (Table 2) (Figures 1, 3).

^{18}F-NaF PET/CT evaluations

Regarding ^{18}F-NaF PET/CT evaluations, 4/29 (13.8%) patients failed to depict any MM lesions on the baseline PET/CT. In the remaining patients 108 lesions were demonstrated on baseline ^{18}F-NaF PET/CT. Follow-up ^{18}F-NaF PET/CT showed that 88 of the 108 (81.5%) baseline MM-indicative lesions were still ^{18}F-NaF positive after treatment. In terms of treatment response, 1 patient showed ^{18}F-NaF PET/CT-CR, 5 patients ^{18}F-NaF PET/CT-PR, 12 patients ^{18}F-NaF PET/CT-SD, and 7 patients ^{18}F-NaF PET/CT-PD (Table 2) (Figures 2, 4).

Comparison between ^{18}F-FDG PET/CT and ^{18}F-NaF PET/CT findings

In 5 patients with innumerable ^{18}F-FDG positive lesions, ^{18}F-NaF PET/CT revealed a more limited disease extent on baseline scan. The 2 patients, who demonstrated an intense diffuse pattern of bone marrow uptake on ^{18}F-FDG PET/CT, were ^{18}F-NaF negative. Regarding the 22 patients, for who a direct lesion-to-lesion comparison was feasible, ^{18}F-NaF PET/CT depicted 56 of the 129 ^{18}F-FDG positive lesions (43%). 57 of the 88 lesions (64.7%) that were still positive on follow-up ^{18}F-NaF PET/CT had already turned to ^{18}F-FDG negative, thus were falsely classified as MM-positive by the follow-up ^{18}F-NaF PET/CT, according to the criteria applied in the study.

Survival data

The data on PFS and OS up to July 2016 (time of writing) are presented in Table 1. The follow-up time ranged from 15 to 52 months. Only one patient had died. 12 patients demonstrated progression, while 18 patients were progression-free. One patient was lost to follow-up. Due to lack of late follow-up data for all patients, we did not proceed to survival analysis in order to compare the survival rates between the different groups.

Kinetic analysis data

The results of dPET/CT evaluations from reference tissue before and after therapy are presented in Tables 3, 4. In terms of both ^{18}F-FDG dPET/CT and ^{18}F-NaF dPET/CT, the patients responded to therapy with a statistically significant decrease of the semi-quantitative parameters $SUV_{average}$ and SUV_{max}, as well as of the quantitative parameters K_1, influx (K_i) and FD ($p<0.001$ respectively). The changes in the respective TACs for both tracers are presented in Figures 5, 6.

DISCUSSION

Assessment of treatment response in MM is based on certain, well-defined laboratory parameters[8,9]. Nevertheless, novel imaging modalities such as PET/CT are nowadays considered valuable tools in improving the definition of response to therapy especially in case of complete response where the percentage of plasma cells in the bone marrow is assessed only from a single location at the iliac crest. Several studies have highlighted the potency of ^{18}F-FDG PET/CT in accurate response evaluation to therapy in MM[16, 45, 46, 47] while the role of ^{18}F-NaF PET/CT in the evaluation of myeloma lesions is still being investigated. In the present prospective study we assessed the combined use of ^{18}F-FDG PET/CT and ^{18}F-NaF PET/CT in treatment response evaluation of MM patients undergoing HDT with melphalan followed by ASCT.

In patient-based analysis ^{18}F-FDG PET/CT showed a sensitivity of 57.7%, a result similar to results previously reported by Derlin et al. who found sensitivities of 54.6% and 50.0% for correct determination of remission status after stem cell transplantation using also the same clinical criteria as gold standard[14,47]. A possible explanation for this relatively limited sensitivity is that MM cells have a rather low proliferation rate and some lesions might be too small to be depicted, given that 6 of the false negative patients (66.7%) were clinically characterized as nCR, a disease stage with very low tumour mass[14, 48]. The phenomenon of achievement of clinical CR with persistence of ^{18}F-FDG PET/CT positivity after therapy in MM has been studied by Zamagni et al and Bartel et al. These groups have highlighted the fact that MM patients with conventionally defined CR but with persistence of ^{18}F-FDG PET/CT positive lesions have a higher risk of progression than ^{18}F-FDG PET/CT negative patients[12,13]. Moreover, the Zamagni

group has proven that the achievement of conventional CR and ^{18}F-FDG PET/CT negativity ensured a significantly prolonged progression free survival (PFS) and an extended overall survival (OS) compared to the achievement of conventional CR but with persistence of ^{18}F-FDG avidity after therapy[16]. The specificity of ^{18}F-FDG PET/CT was 100% with 3/3 patients clinically characterized as CR being ^{18}F-FDG negative. ^{18}F-FDG PET/CT is considered a relatively specific imaging modality regarding MM response assessment, due to its ability to differentiate between active disease and fibrotic lesions[15,46]. As expected, a direct comparison of the treatment response assessed by the clinical gold standard and ^{18}F-FDG PET/CT was not feasible.

The performance of ^{18}F-NaF PET/CT was rather limited. In patient-based analysis, baseline ^{18}F-NaF PET/CT was negative in 13.8% of the ^{18}F-FDG PET/CT positive MM patients. Moreover, baseline ^{18}F-NaF PET/CT depicted only 43% of the ^{18}F-FDG positive lesions, a result in accordance with a previous study of our group involving 67 MM patients, in which ^{18}F-NaF PET/CT detected only 39% of the MM lesions demonstrated on ^{18}F-FDG PET/CT[29]. Regarding follow-up studies, ^{18}F-NaF PET/CT showed persistence of the majority (81.5%) of the baseline ^{18}F-NaF positive MM lesions after treatment, despite the fact that 64.7% of them had turned ^{18}F-FDG negative as a response to HDT and ASCT. The reason for this discordance between ^{18}F-FDG and ^{18}F-NaF PET/CT findings lies on the different molecular mechanisms of the two tracers. ^{18}F-FDG represents a direct parameter of tumour metabolism. A decline in the tumour ^{18}F-FDG uptake is expected to be seen with a loss of viable cancer cells, which is in the case in patients with partial or complete response[49]. On the other hand, ^{18}F-NaF uptake mechanism corresponds to osteoblastic activity. The

accumulation of ^{18}F-NaF in osteolytic lesions, as in case of MM, takes place in the accompanying, even minimal, reactive osteoblastic changes[19].

To date there is little information available about the role of ^{18}F-NaF PET in treatment monitoring of systemic cancer therapy. Hillner et al. have recently assessed the impact of ^{18}F-NaF PET results in a set of 2,217 oncological patients receiving systemic therapy. Their results showed a high impact of the modality in patients with progressive osseous metastatic disease, with a 40% change in treatment plan after ^{18}F-NaF PET[21]. The authors stressed, however, the non-tumor specific nature of the tracer as an indicator of reactive bone formation in response to various insults, and the limitation of being subject to the flare phenomenon associated with systemic therapy.

The experience from ^{99m}Tc-MDP bone scintigraphy (BS), the analogue of ^{18}F-NaF PET for conventional nuclear medicine, is much larger. Although the response to treatment is evident through a decrease in ^{99m}Tc-MDP uptake in BS, several studies have shown that an increased activity of the bone-seeking tracer in the area of a tumour lesion may persist for several months after therapy, partly in terms of the healing, osteoblastic, reactive process[,50,51,52,53,54]. Garcia et al. evaluated the combined use of ^{99m}Tc-MDP BS and ^{18}F-FDG PET in treatment response assessment of bone metastases in 25 patients suffering from breast and lung cancer. According to their results, 5 patients with improvement on ^{18}F-FDG PET scans demonstrated PD and/or SD on ^{99m}Tc-MDP BS. Clinical follow-up, serial tumor markers and radiological findings confirmed the ^{18}F-FDG PET findings, leading to the conclusion that some of the BS results should be interpreted as representing a persistent bone reaction, not active metastatic disease[55]. This previous experience with bone matrix radiotracers was the reason that the sensitivity and specificity of ^{18}F-NaF PET/CT were not assessed with regard to the clinical gold standard.

Apart from the conventional evaluation of whole-body PET/CT scans, we performed quantitative assessment of the dynamic PET/CT data derived from reference bone marrow (^{18}F-FDG), and reference skeleton (^{18}F-NaF) of the pelvis after application of two-tissue compartment modelling. The quantitative aspect is a major advantage of PET, which is neglected when using whole-body protocols and visual/qualitative evaluation as the only diagnostic tool. Only limited data exist on quantitative assessment of tracer kinetics in MM. Our group has recently shown that the ^{18}F-FDG kinetic parameters K_1, influx (K_i), as well as SUV from reference bone marrow of the os ilium, correlated significantly with bone marrow malignant plasma cell infiltration rate[40]. The herein presented results revealed that in the case of ^{18}F-FDG, tracer uptake (reflected by $SUV_{average}$ and SUV_{max}), its transport capacity (K_1), and its influx rate (K_i) responded to HDT and ASCT with a significant decrease. These findings are in agreement with previous findings from Dimitrakopoulou-Strauss et al., who studied a group of MM patients undergoing anthracycline-based chemotherapy with dynamic ^{18}F-FDG PET/CT prior to the onset of therapy and after the first cycle. The authors found a significant decrease ($p<0.000$) of SUV, FD, V_B, and influx (K_i) for ^{18}F-FDG as derived from reference bone marrow of the os ilium, in response to treatment[56]. The herein presented results provide more evidence in the direction of establishment of ^{18}F-FDG PET/CT as a tool for treatment response evaluation in MM; we proved that in a group of patients that clinically responded to therapy with at least PR, certain parameters involved in ^{18}F-FDG metabolism also responded with a significant decrease of their values. Considering that the particular ^{18}F-FDG parameters correlate with the bone marrow malignant plasma cell infiltration rate, an indicator of myeloma burden and one of the myeloma defining events[10], our data stress the capacity of ^{18}F-

FDG dynamic PET/CT to demonstrate bone marrow changes in response to treatment in a molecular level.

Interestingly, ^{18}F-NaF-associated kinetic parameters demonstrated similar changes as ^{18}F-FDG in response to therapy. In particular, ^{18}F-NaF uptake ($SUV_{average}$ and SUV_{max}), the rate of fluoride ions exchange with hydroxyl groups of hydroxyapatite crystal of the bone (K_1), Ca^{2+} influx, bone apposition rate and, presumably, bone remodelling rate (K_i) decreased significantly after HDT and ASCT. Myelomatous bone disease after ASCT is little understood[57]. The fact that bone marrow transplantation may affect the skeleton has been demonstrated by Gandhi et al. in an heterogeneous group of oncological patients, one of which suffered from MM. The authors showed that 3 months after ASCT there was a significant decline of bone mineral density in the femoral neck and a non-significant trend towards reduction in the lumbar spine[58]. Terpos et al. have shown that bone formation markers do not normalise until the eighth month post-ASCT, providing an indication that bone formation may delay in normalising[59]. Further, in one of the few published treatment monitoring studies by means of dynamic ^{18}F-NaF PET, Installé et al. have demonstrated in 14 patients with Paget's disease receiving bisphosphonates therapy that SUV_{max}, K_1, and influx constant K_i decreased significantly as response to treatment[60]. To the best of our knowledge, these are the first data regarding bone turnover changes in MM patients receiving HDT and ASCT, evaluated by means of dynamic PET/CT.

In addition to two-tissue compartment modelling for tracer kinetics assessment, we also applied a non-compartmental approach based on the box counting procedure of the chaos theory for the analysis of dPET data, resulting in another index representative of tissue heterogeneity, fractal dimension (FD)[43]. Fractal geometry has

found use in pathology for assessment of irregularities[61]. Our group has shown that FD of ^{18}F-FDG correlates significantly with the degree of bone marrow malignant plasma cell infiltration rate[40]. In the present study we found that FD for both tracers decreased significantly, reflecting a decline of the heterogeneity of the concentration of both tracers over time in response to treatment, a result in accordance with the changes of compartment-derived kinetic parameters.

This study has some limitations. Firstly, the number of patients enrolled was relatively small. Therefore, further studies with a larger study population are warranted to generalize the herein presented results. Secondly, most of the PET/CT positive findings were not histopathologically confirmed. However, this is usually not possible in the clinical setting. Another limitation is the confinement of the dynamic PET/CT studies only in the anatomic area of the pelvis, since whole-body dynamic studies cannot be performed. We used a two-bed position protocol for the dynamic PET acquisition, which allows the study of a relatively large field of view of 44 cm. Nevertheless, new PET/CT scanners allow dynamic studies over several bed positions by using a continuous bed movement, thus, facilitating the use of dynamic protocols and reducing the whole acquisition time. Finally, the lack of late follow-up data for all patients prevented us from proceeding to survival analysis between the different patient groups, which will be the topic of a future publication of our group.

CONCLUSION

In the present study ^{18}F-FDG PET/CT demonstrated a sensitivity of 57.7% and a specificity of 100% in treatment response evaluation of 29 MM patients undergoing HDT and ASCT, using the clinical response criteria as reference standard. Despite its limited sensitivity, the performance of ^{18}F-FDG PET/CT was satisfactory, given that 6/9 false negative patients in follow-up scans (66.7%) were clinically characterized as nCR, a disease stage with very low tumor mass. In contrary, ^{18}F-NaF PET/CT did not aid significantly in treatment response evaluation of MM patients, at least in an early phase. Dynamic PET/CT studies demonstrated a decrease of SUVs and specific kinetic parameters in reference tissue for both ^{18}F-FDG and ^{18}F-NaF as response to treatment, reflecting changes in a molecular level before any morphological changes take place.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Furthermore, the study was approved by the Ethical Committee I of the University of Heidelberg and the Federal Radiation Protection Agency.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Acknowledgment:

The study was part of a special research area project (SFB TRR 79) funded by the German Research Foundation.

FIGURE LEGENDS

Figure 1: A 70-years old stage III MM patient scheduled for HDT and ASCT, undergoing ^{18}F-FDG PET/CT before and after therapy. Maximum intensity projection (MIP) ^{18}F-FDG PET/CT before therapy (left) revealed a mixed pattern of ^{18}F-FDG uptake with intense, diffuse uptake in the axial skeleton and multiple, focal bone marrow lesions. Follow-up ^{18}F-FDG PET/CT MIP three months after ASCT (right) demonstrated a complete remission of both diffuse bone marrow uptake as well as focal myeloma-indicative lesions (^{18}F-FDG PET/CT-CR). ^{18}F-FDG uptake in cervical, abdominal and inguinal lymph nodes in the follow-up scan was attributed to inflammatory reaction after therapy, thus considered benign. Response according to clinical criteria was CR and according to the ^{18}F-FDG PET EORTC criteria also CR.

Figure 2: Whole body ^{18}F-NaF PET/CT MIP before and after therapy of the same patient as in figure 1. Baseline ^{18}F-NaF PET/CT (left) demonstrated several ^{18}F-NaF positive skeletal lesions, which partly corresponded to respective lesions on ^{18}F-FDG PET/CT (Fig. 1) and were considered myeloma-indicative, as well as several degenerative changes mostly in the spine. Follow-up ^{18}F-NaF PET/CT MIP after therapy (right) showed remission of some of the MM-indicative lesions but at the same time persistence of several of them (^{18}F-NaF PET/CT-PR). Response according to clinical criteria was CR and according to the ^{18}F-NaF PET criteria applied in our study PR.

Figure 3: A 68-years old stage III MM patient scheduled for HDT and ASCT undergoing ^{18}F-FDG PET/CT before and after therapy. Transaxial ^{18}F-FDG PET/CT in the cervical level before therapy (upper row) revealed an ^{18}F-FDG

avid, MM-indicative lesion in the transverse process of the 4th cervical vertebrae. The patient underwent a follow-up ^{18}F-FDG PET/CT 49 days after ASCT (lower row), which demonstrated complete metabolic remission of the MM lesion. According to the EORTC 1999 criteria, the patient was characterized as ^{18}F-FDG PET/CT-CR. According to clinical criteria, the patient`s response was nCR.

Figure 4: Transaxial ^{18}F-NaF PET/CT before and after therapy of the same patient as in figure 3. Baseline ^{18}F-NaF PET/CT (upper row) revealed also the ^{18}F-FDG avid, myeloma-indicative lesion in the transverse process of the 4th cervical vertebrae as ^{18}F-NaF positive. In contrary to ^{18}F-FDG PET/CT (Fig.3), the lesion demonstrated a persistence of the ^{18}F-NaF accumulation in the follow-up ^{18}F-NaF PET/CT (lower row) after HDT and ASCT (^{18}F-NaF PET/CT-SD).

Figure 5: Time activity curves (TACs) depicting ^{18}F-FDG concentration during the 60 minutes of dynamic PET acquisition in reference bone marrow before (upper row) and after therapy with HDT and ASCT (lower row). The curves are derived from bone marrow of the os ilium that served as reference (blue curve with green dots) and from the common iliac artery (curve with gold dots). Small decrease in the radiotracer concentration in reference tissue VOIs after therapy. The corresponding kinetic parameters responded to therapy also with a decrease.

Figure 6: Time activity curves (TACs) depicting ^{18}F-NaF concentration during the 60 minutes of dynamic PET acquisition in reference skeleton before (upper row) and after therapy with HDT and ASCT (lower row). The curves

are derived from osseous tissue of the os ilium that served as reference (blue curve with green dots) and from the common iliac artery (curve with gold dots). Decrease in the radiotracer concentration in reference tissue VOIs after therapy. The corresponding kinetic parameters responded to therapy also with a decrease.

Table 1 Characteristics, treatment response and survival rates of the patients investigated in the study.

Patient number	Stadium	Age	Gender	Time between ASCT-follow-up PET (days)	Clinical response	^{18}F-FDG PET/CT response	^{18}F-NaF PET/CT response	Progression after therapy	PFS (months)	OS (months)
1	1	66	F	78	PR	PR	Negative baseline scan	Yes	39	44
2	3	65	M	228	CR	CR	PR	Yes	16	49
3	3	57	M	117	nCR	PR	SD	Yes	17	32
4	3	70	M	97	CR	CR	PR	Yes	30	43
5	3	73	M	132	nCR	PR	SD	No	37	37
6	1	53	M	64	PR	CR	SD	No	31	31
7	3	46	F	81	nCR	CR	SD	Yes	26	35
8	3	53	F	83	VGPR	PR	SD	Lost to follow-up		
9	3	69	F	52	PR	CR	CR	Yes	4	41
10	3	49	M	120	nCR	CR	Negative baseline scan	No	28	28
11	3	43	M	93	nCR	PD	PD	No	28	28
12	3	60	M	67	nCR	PR	PD	Yes	9	18 (dead)
13	3	68	M	75	nCR	PR	PR	Yes	25	25
14	3	69	M	101	nCR	CR	SD	No	28	28
15	3	68	F	49	nCR	CR	SD	No	24	24
16	3	53	M	70	PR	CR	SD	No	26	26
17	3	62	F	145	nCR	PD	PD	Yes	20	27
18	3	70	M	51	VGPR	PR	PD	No	33	33
19	1	60	M	115	PR	PR	SD	No	24	24
20	3	59	F	47	VGPR	PR	SD	No	23	23
21	3	60	M	86	CR	CR	SD	No	52	52
22	1	63	F	106	nCR	CR	Negative baseline scan	No	23	23
23	3	59	F	120	PR	CR	SD	Yes	18	24
24	3	48	M	102	nCR	CR	PR	No	18	18
25	3	61	M	89	PR	PR	Negative baseline scan	No	20	20
26	3	45	M	67	PR	PD	PD	No	20	20
27	3	71	M	125	PR	CR	PR	Yes	18	19
28	3	59	F	128	nCR	PR	PD	Yes	7	19
29	3	62	F	55	VGPR	PD	PD	No	15	15
30	1	62	M	105	VGPR	Negative baseline scan (excluded)	Negative baseline scan			
31	3	69	F	136	VGPR	Negative baseline scan (excluded)	Negative baseline scan			
32	3	38	M	48	VGPR	Negative baseline scan (excluded)	Negative baseline scan			

33	3	46	M	98	nCR	Negative baseline scan (excluded)	Negative baseline scan			
34	3	54	M	86	nCR	Negative baseline scan (excluded)	Negative baseline scan			

M, male; F, female; CR, complete response; nCR, near complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease.
All patients were alive at the time of writing, with the exception of patient no 12.

Table 2 Treatment response of the 29 MM patients according to clinical criteria, ^{18}F-FDG PET/CT criteria and ^{18}F-NaF PET/CT criteria.

Clinical response	**^{18}F-FDG PET/CT response**	**^{18}F-NaF PET/CT response**
CR= 3 patients nCR= 13 patients VGPR= 4 patients PR= 9 patients	CR= 14 patients PR= 11 patients PD= 4 patients	CR= 1 patient PR= 5 patients SD= 12 patients PD= 7 patients

CR, complete response; nCR, near complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease

Table 3 Descriptive statistics of mean and median values prior and after HDT and ASCT for the ^{18}F-FDG semi-quantitative and quantitative parameters in reference bone marrow. The values of parameters K_1, k_2, k_3, k_4 and influx are 1/min. SUVs and FD have no unit.

Parameter	**Mean prior**	**Median prior**	**Mean after**	**Median after**
$SUV_{average}$*	2.2	1.9	1.6	1.3
SUV_{max}*	3.9	3.3	2.5	2.0
K_1*	0.214	0.186	0.158	0.144
k_2*	0.710	0.693	0.570	0.571
k_3	0.054	0.050	0.050	0.045
k_4	0.025	0.020	0.028	0.020
influx*	0.014	0.013	0.011	0.009
FD*	1.146	1.138	1.086	1.065

*significant probabilities ($p<0.001$)

Table 4 Descriptive statistics of mean and median values prior and after HDT and ASCT for the ^{18}F-NaF semi-quantitative and quantitative parameters in reference skeleton. The values of parameters K_1, k_2, k_3, k_4 and influx are 1/min. SUVs and FD have no unit.

Parameter	**Mean prior**	**Median prior**	**Mean after**	**Median after**
$SUV_{average}$*	8.7	8.4	6.9	6.3
SUV_{max}*	14.6	13.8	10.5	10.0
K_1*	0.200	0.177	0.143	0.116
k_2	0.413	0.421	0.329	0.272
k_3	0.279	0.249	0.250	0.229
k_4	0.015	0.013	0.013	0.012
influx*	0.076	0.070	0.059	0.054
FD*	1.382	1.390	1.340	1.342

*significant probabilities ($p<0.001$)

REFERENCES

[1]Attal M, Harousseau JL, Stoppa AM et al. A prospective, randomised trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergrouppe Francais du Myelome. *N Engl J Med*. 1996;335: 91-97.

[2]Harousseau JL. Autologous transplantation for multiple myeloma. *Ann Oncol*. 2008; 19 Suppl 7: vii 128-133.

[3]Bladé J, Rosiñol L, Cibeira MT, Rovira M, Carreras E. Hematopoietic stem cell transplantation for multiple myeloma beyond 2010. *Blood*. 2010; 115:3655-3663.

[4]Palumbo A., Anderson K. Multiple myeloma. *N Engl J Med*. 2011;364:1046-1060.

[5]Engelhardt M, Terpos E, Kleber M et al. European Myeloma Network. European Myeloma Network recommendations on the evaluation and treatment of newly diagnosed patients with multiple myeloma. *Haematologica*. 2014;99:232-242.

[6]Cavo M, Tacchetti P, Patriarca F et al. GIMEMA Italian Myeloma Network. Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study. *Lancet*. 2010; 376:2075-2085.

[7]Cavo M, Rajkumar SV, Palumbo A et al. International Myeloma Working Group. International Myeloma Working Group consensus approach to the treatment of multiple myeloma patients who are candidates for autologous stem cell transplantation. *Blood*. 2011;117:6063-6073.

[8]Bladé J, Samson D, Reece D et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. *Br J Haematol*. 1998;102:1115-1123.

[9]Durie BG, Harousseau JL, Miguel JS et al. International Myeloma Working Group. International uniform response criteria for multiple myeloma. *Leukemia*. 2006; 20: 1467-1473.

[10]Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15:e538-548.

[11] Zamagni E, Nanni C, Gay F, et al.18F-FDG PET/CT focal, but not osteolytic,

lesions predict the progression of smoldering myeloma to active disease. *Leukemia*. 2016;30:417-422.

[12]Bartel TB, Haessler J, Brown TL et al. F18-fluorodeoxyglucose positron emission tomography in the context of other imaging techniques and prognostic factors in multiple myeloma. *Blood*. 2009; 114:2068-2076.

[13]Zamagni E, Patriarca F, Nanni C et al. Prognostic relevance of 18F-FDG PET/CT in newly diagnosed multiple myeloma patients treated with up-front autologous transplantation. *Blood*. 2011; 118:5989-5995.

[14]Derlin T, Weber C, Habermann CR et al. 18F-FDG PET/CT for detection and localization of residual or recurrent disease in patients with multiple myeloma after stem cell transplantation. *Eur J Nucl Med Mol Imaging*. 2012; 39:493-500.

[15]Caers J, Withofs N, Hillengass J et al. The role of positron emission tomography-computed tomography and magnetic resonance imaging in diagnosis and follow up of multiple myeloma. *Haematologica*; 2014; 99:629-637.

[16]Zamagni E, Nanni C, Mancuso K et al. PET/CT Improves the Definition of Complete Response and Allows to Detect Otherwise Unidentifiable Skeletal Progression in Multiple myeloma. *Clin Cancer Res*. 2015; 21: 4384-4390.

[17]Blau M, Ganatra R, Bender MA. 18 F-Fluoride for bone imaging. *Semin Nucl Med*. 1972; 2:31-37.

[18]Hawkins RA, Choi Y, Huang SC et al. Evaluation of the skeletal kinetics of fluorine-18-fluoride ion with PET. *J Nucl Med. 1992*; 33:633-642.

[19]Even-Sapir E, Mishani E, Flusser G et al. 18F-Fluoride positron emission tomography and positron emission tomography/computed tomography. *Semin Nucl Med*. 2007; 37:462-469.

[20]Grant FD, Fahey FH, Packard AB et al. Skeletal PET with 18 F-fluoride: applying new technology to an old tracer. *J Nucl Med*. 2008; 49:68-78.

[21]Hillner BE, Siegel BA, Hanna L, Duan F, Quinn B, Shields AF. 18F-fluoride PET used for treatment monitoring of systemic cancer therapy: results from the National Oncologic PET Registry. *J Nucl Med*. 2015; 56:222-228.

[22]Beheshti M, Mottaghy FM, Payche F et al. (18)F-NaF PET/CT: EANM procedure guidelines for bone imaging. *Eur J Nucl Med Mol Imaging*. 2015; 42:1767-1777.

[23]Lecouvet FE, Talbot JN, Messiou C, Bourguet P, Liu Y, de Souza NM; EORTC Imaging Group. Monitoring the response of bone metastases to treatment with

Magnetic Resonance Imaging and nuclear medicine techniques: a review and position statement by the European Organisation for Research and Treatment of Cancer imaging group. *Eur J Cancer.* 2014; 50:2519-2531.

[24]Tan E, Weiss BM, Mena E, Korde N, Choyke PL, Landgren O. Current and future imaging modalities for multiple myeloma and its precursor states. *Leuk Lymphoma*. 2011; 52:1630-1640.

[25]Kurdziel KA, Shih JH, Apolo AB et al. The kinetics and reproducibility of 18F-sodium fluoride for oncology using current PET camera technology. *J Nucl Med*. 2012;53:1175-1184.

[26]Nishiyama Y, Tateishi U, Shizukuishi K et al. Role of 18F-fluoride PET/CT in the assessment of multiple myeloma: initial experience. *Ann Nucl Med*. 2013; 27:78-83.

[27]Xu F, Liu F, Pastakia B.Different lesions revealed by 18F-FDG PET/CT and 18F-NaF PET/CT in patients with multiple myeloma. *Clin Nucl Med*. 2014;39:e407-409.

[28]Oral A, Yazici B, Ömür Ö, Comert M, Saydam G.18F-FDG and 18F-NaF PET/CT Findings of a Multiple Myeloma Patient With Thyroid Cartilage Involvement. *Clin Nucl Med*. 2015;40:873-876.

[29]Sachpekidis C, Goldschmidt H, Hose D et al. PET/CT studies of multiple myeloma using (18) F-FDG and (18) F-NaF: comparison of distribution patterns and tracers' pharmacokinetics. *Eur J Nucl Med Mol Imaging*. 2014; 41:1343-1353.

[30]Ak I, Onner H, Akay OM. Is there any complimentary role of F-18 NaF PET/CT in detecting of osseous involvement of multiple myeloma? A comparative study of F-18 FDG PET/CT and F-18 FDG NaF PET/CT. *Ann Hematol*. 2015; 94:1567-1575.

[31]Bhutani M, Turkbey B, Tan E et al. Bone marrow abnormalities and early bone lesions in multiple myeloma and its precursor disease: A prospective study using functional and morphologic imaging. *Leuk Lymphoma*. 2015 Dec 21:1-23. (Epub ahead of print).

[32]International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol*. 2003; 121:749-757.

[33]Sachpekidis C, Hillengass J, Goldschmidt H et al. Comparison of (18)F-FDG PET/CT and PET/MRI in patients with multiple myeloma. Am J Nucl Med Mol Imaging. 2015;5:469-478.

[34]http://www.pmod.com/files/download/v31/doc/pbas/4729.htm

[35]Sokoloff L, Smith CB. Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In: Greitz T, Ingvar DH, Widén L, editors. The metabolism of the human brain studied with positron emission tomography. New York: Raven Press; 1983; p. 123-148.

[36]Burger C, Buck A. Requirements and implementations of a flexible kinetic modeling tool. *J Nucl Med*. 1997; 38:1818-1823.

[37]Mikolajczyk K, Szabatin M, Rudnicki P, Grodzki M, Burger C. A Java environment for medical image data analysis: initial application for brain PET quantitation. *Med Inform.*1998; 23:207–214.

[38]Miyazawa H, Osmont A, Petit-Taboué MC, Tillet I, Travère JM, Young AR et al. Determination of 18F-fluoro-2-deoxy-D-glucose rate constants in the anesthetized baboon brain with dynamic positron tomography. *J Neurosci Methods*. 1993; 50:263-272.

[39]Cheng C, Alt V, Dimitrakopoulou-Strauss A, Pan L, Thormann U, Schnettler R et al. Evaluation of new bone formation in normal and osteoporotic rats with a 3-mm femur defect: functional assessment with dynamic PET-CT (dPET-CT) using a 2-deoxy-2 [(18)F] fluoro-D-glucose (^{18}F-FDG) and ^{18}F-fluoride. *Mol Imaging Biol*. 2013;15:336-344.

[40]Sachpekidis C, Mai EK, Goldschmidt H, et al. (18)F-FDG dynamic PET/CT in patients with multiple myeloma: patterns of tracer uptake and correlation with bone marrow plasma cell infiltration rate. *Clin Nucl Med*. 2015;40:e300-307.

[41]Ohtake T, Kosaka N, Watanabe T, Yokoyama I, Moritan T, Masuo M et al. Noninvasive method to obtain input function for measuring tissue glucose utilization of thoracic and abdominal organs. *J Nucl Med*. 1991; 32:1432-1438.

[42]Czernin J, Satyamurthy N, Schiepers C. Molecular mechanisms of bone 18F-NaF deposition. *J Nucl Med*. 2010; 51:1826-1829.

[43]Dimitrakopoulou-Strauss A, Strauss LG, Mikolajczyk K, et al. (2003) On the fractal nature of positron emission tomography (PET) studies. *World J Nucl Med*. 4:306-313.

[44]Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, et al. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. *Eur J Cancer.*1999; 35:1773-1782.

[45]Nanni C, Zamagni E, Celli M, et al. The value of 18F-FDG PET/CT after autologous stem cell transplantation (ASCT) in patients affected by multiple myeloma (MM): experience with 77 patients. *Clin Nucl Med.* 2013;38:e74-79.

[46]Zamagni E, Cavo M. The role of imaging techniques in the management of multiple myeloma. *Br J Haematol.* 2012;159:499-513

[47]Derlin T, Peldschus K, Münster S, et al. Comparative diagnostic performance of ^{18}F-FDG PET/CT versus whole-body MRI for determination of remission status in multiple myeloma after stem cell transplantation. *Eur Radiol.* 2013;23:570-578.

[48]Vij R, Fowler KJ, Shokeen M. New Approaches to Molecular Imaging of Multiple Myeloma. *J Nucl Med.* 2016;57:1-4.

[49]Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. *J Nucl Med.* 2009;50 Suppl 1:122S-150S.

[50]Janicek MJ, Hayes DF, Kaplan WD. Healing flare in skeletal metastases from breast cancer. *Radiology.* 1994;192:201-204.

[51]Vogel CL, Schoenfelder J, Shemano I, Hayes DF, Gams RA. Worsening bone scan in the evaluation of antitumor response during hormonal therapy of breast cancer. *J Clin Oncol.* 1995; 13:1123-1128.

[52]Galasko CS. Diagnosis of skeletal metastases and assessment of response to treatment. *Clin Orthop Relat Res.* 1995;(312):64-75.

[53]Cook GJ, Fogelman I. The role of nuclear medicine in monitoring treatment in skeletal malignancy. *Semin Nucl Med.* 2001;31:206-211.

[54]Vassiliou V, Andreopoulos D, Frangos S, Tselis N, Giannopoulou E, Lutz S. Bone metastases: assessment of therapeutic response through radiological and nuclear medicine imaging modalities. *Clin Oncol (R Coll Radiol).* 2011;23:632-645.

[55]García JR, Simó M, Soler M, Pérez G, López S, Lomeña F. Relative roles of bone scintigraphy and positron emission tomography in assessing the treatment response of bone metastases. *Eur J Nucl Med Mol Imaging.* 2005;32:1243-1244.

[56]Dimitrakopoulou-Strauss A, Hoffmann M, Bergner R, Uppenkamp M, Haberkorn U, Strauss LG. Prediction of progression-free survival in patients with multiple myeloma following anthracycline-based chemotherapy based on dynamic FDG-PET. *Clin* Nucl *Med.* 2009;34:576-584.

[57]Laroche M, Lemaire O, Bourin P, et al. Dual-energy X-ray absorptiometry and

biochemical markers of bone turnover after autologous stem cell transplantation in myeloma. *Eur J Haematol*. 2012;88:388-395.

[58]Gandhi MK, Lekamwasam S, Inman I, et al. Significant and persistent loss of bone mineral density in the femoral neck after haematopoietic stem cell transplantation: long-term follow-up of a prospective study. *Br J Haematol.* 2003;121:462-468.

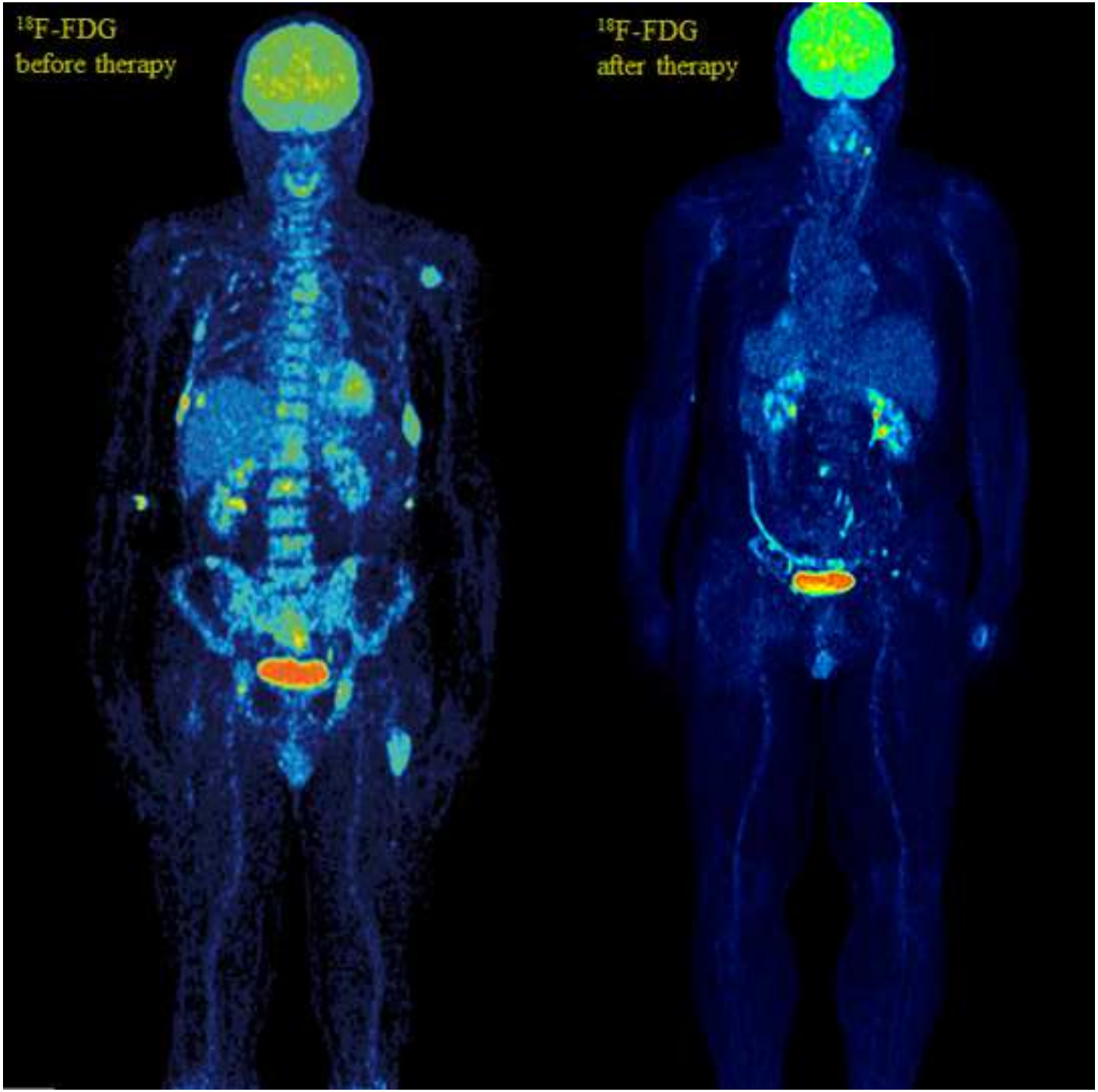
[59]Terpos E, Politou M, Szydlo R, et al. Autologous stem cell transplantation normalizes abnormal bone remodeling and sRANKL/osteoprotegerin ratio in patients with multiple myeloma. *Leukemia.* 2004;18:1420-1426.

[60]Installé J, Nzeusseu A, Bol A, Depresseux G, Devogelaer JP, Lonneux M. (18)F-fluoride PET for monitoring therapeutic response in Paget's disease of bone. *J Nucl Med.* 2005;46:1650-1658.

[61]Dey P. Basic principles and applications of fractal geometry in pathology: a review. *Anal Quant Cytol Histol*. 2005;27:284-290.

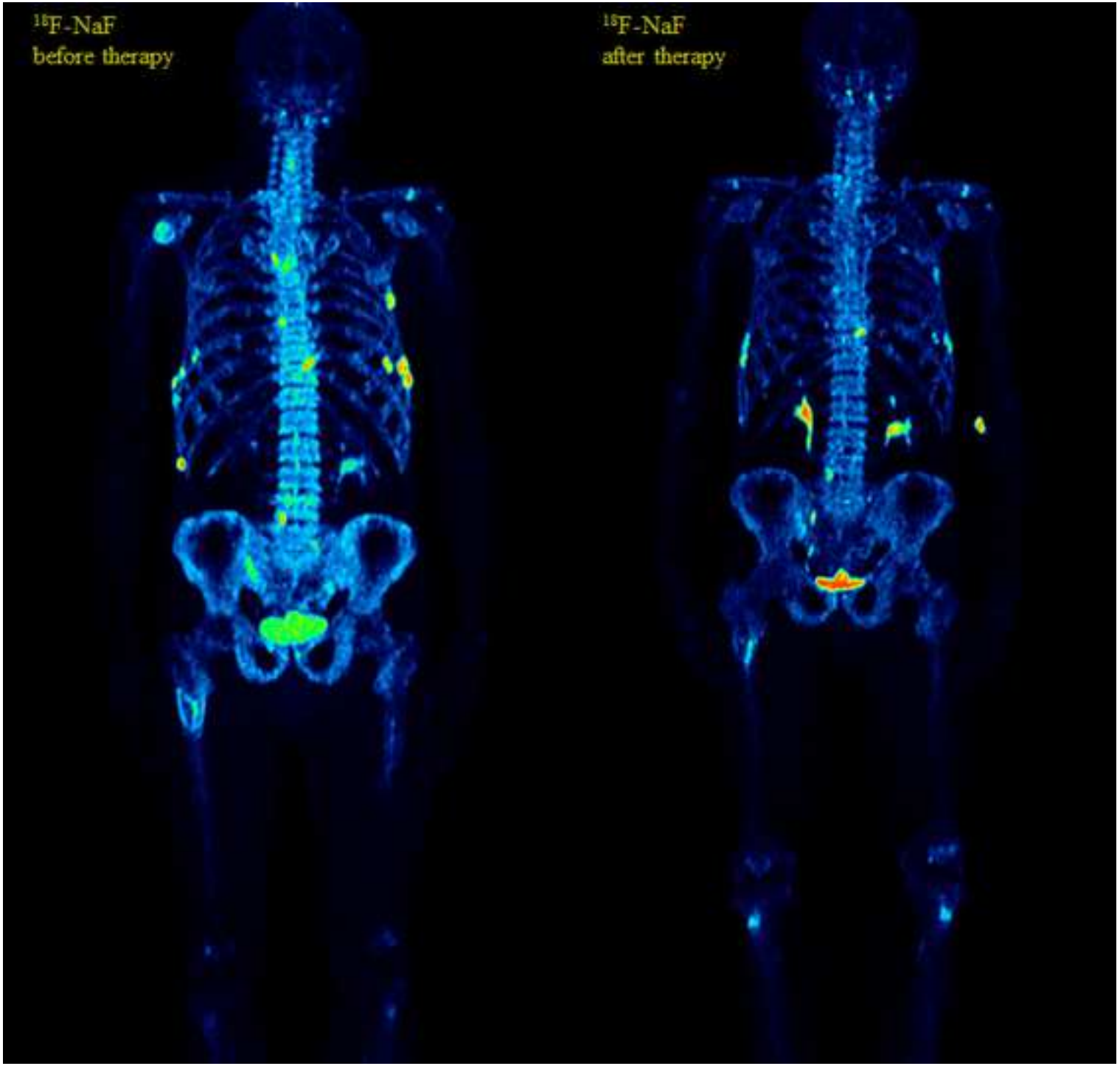
Figure

Click here to download Figure Figure 1.jpg



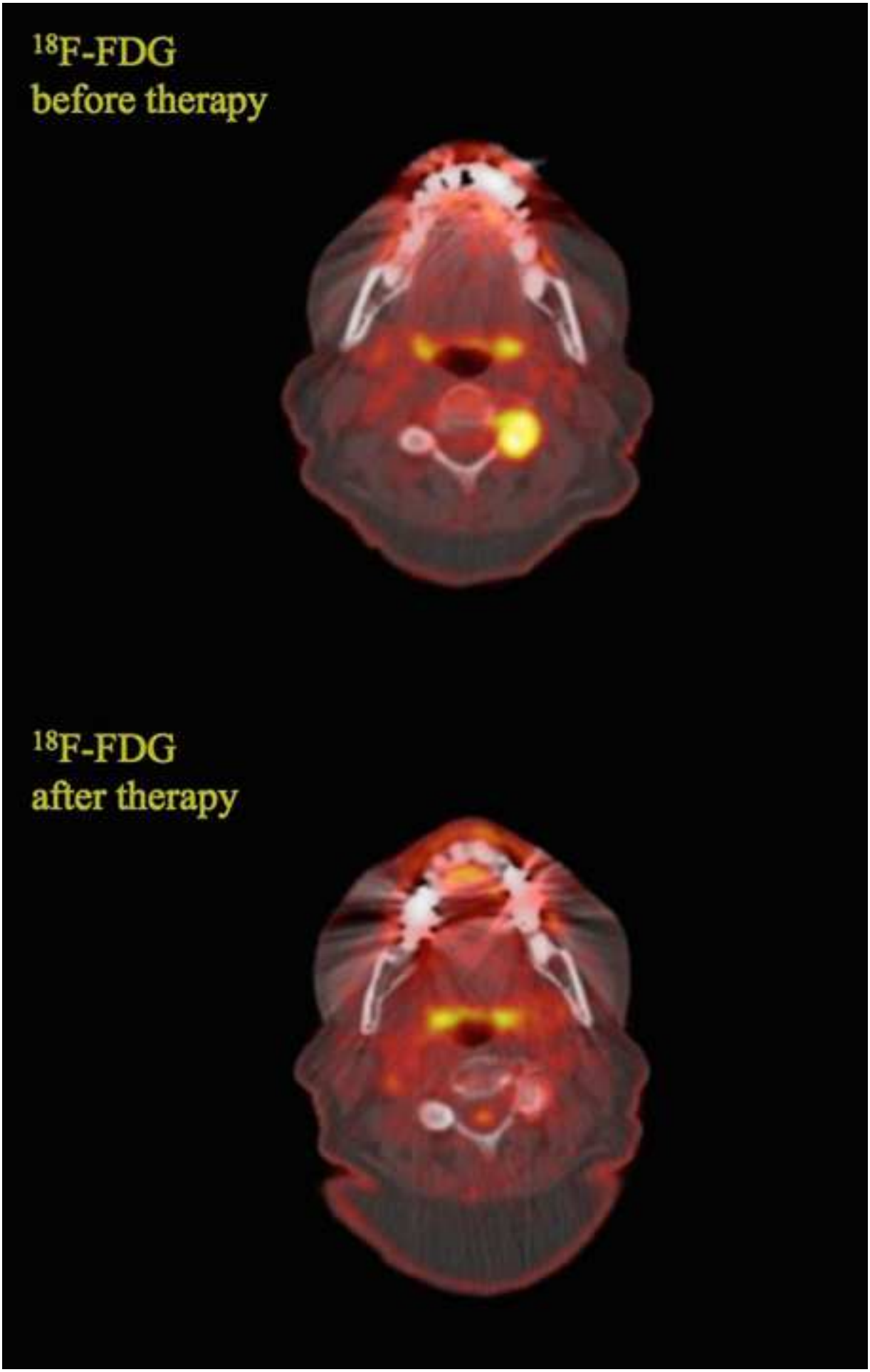
Figure

Click here to download Figure Figure 2.jpg



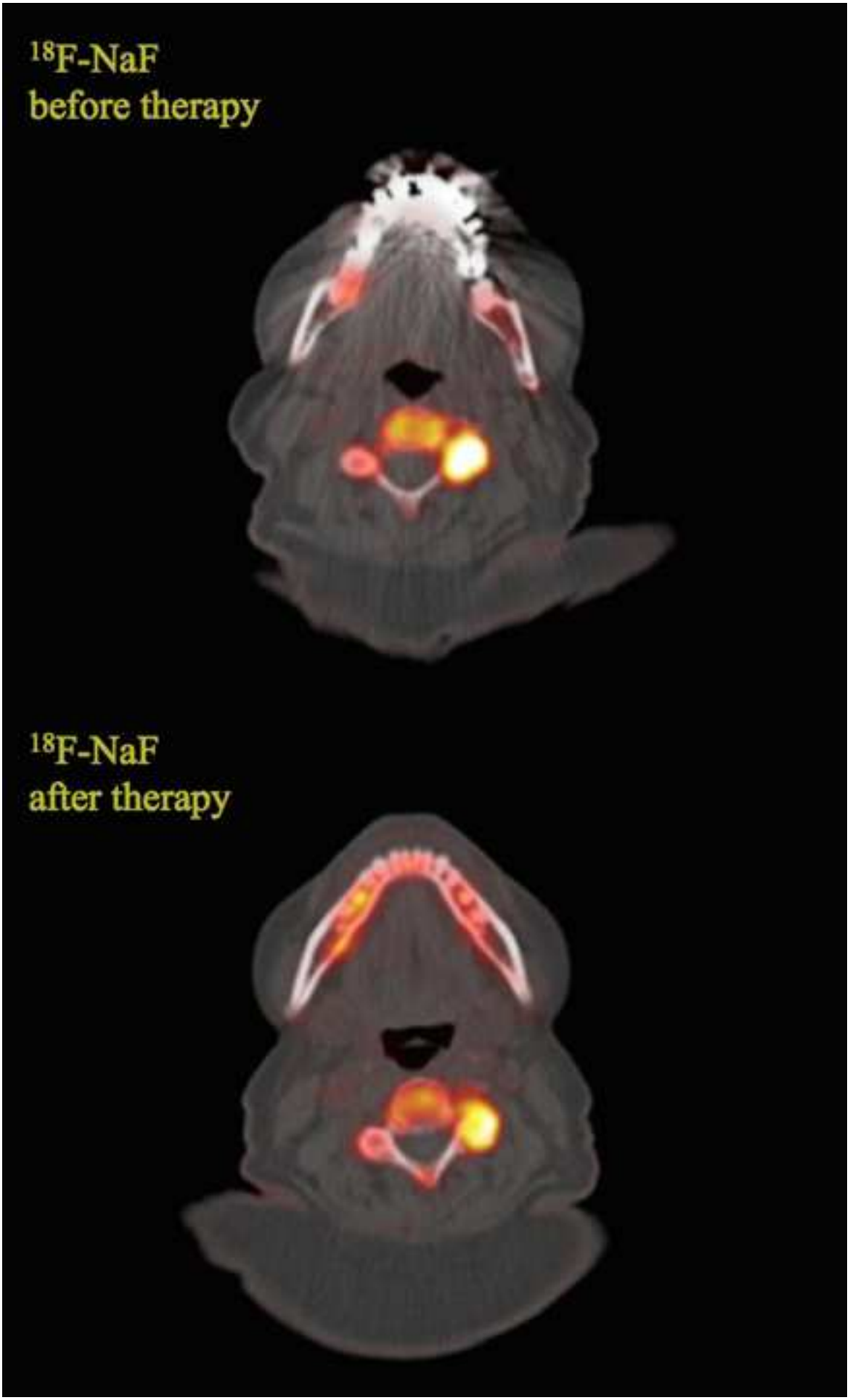
Figure

Click here to download Figure Figure 3 revised.jpg



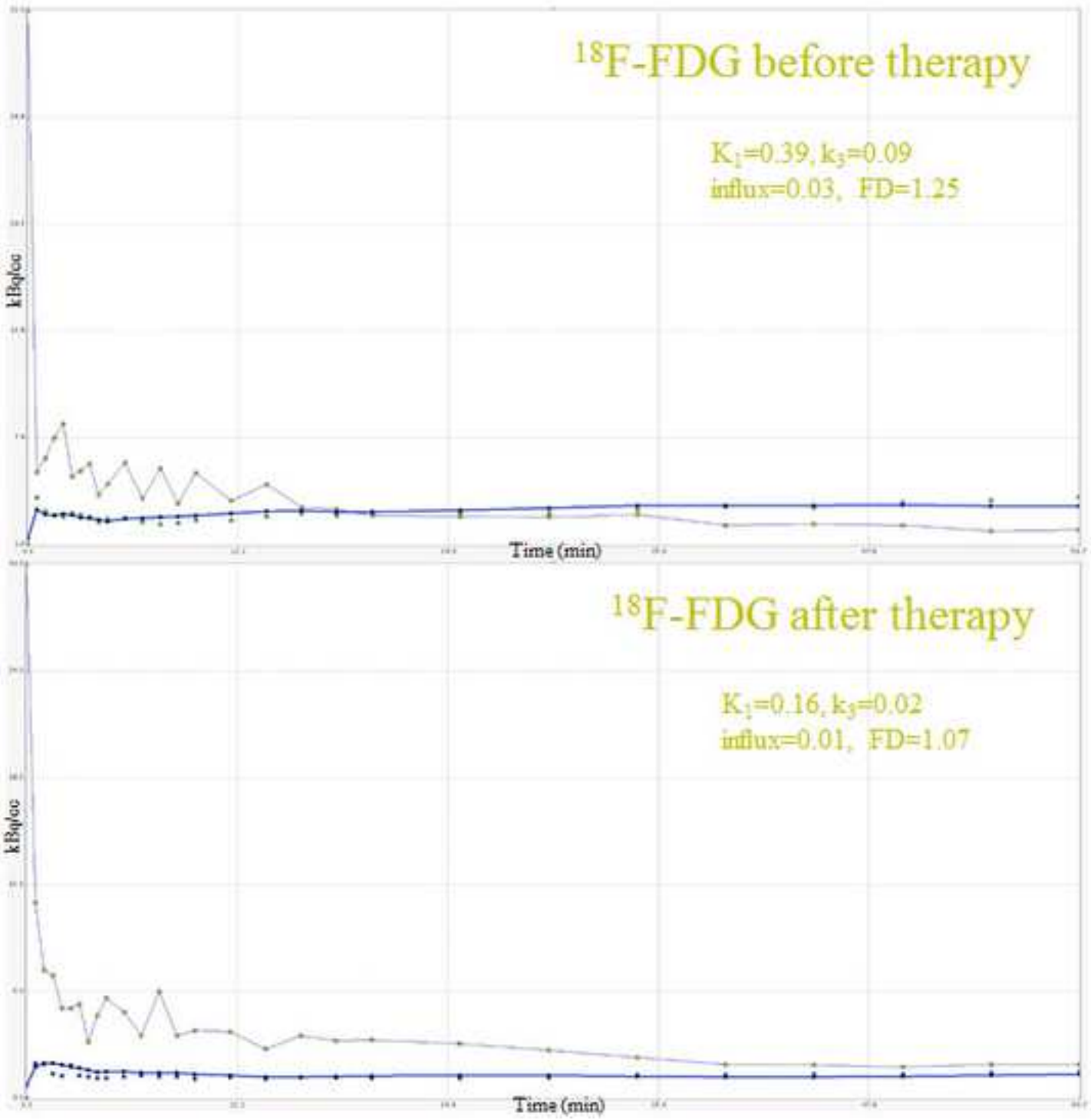
Figure

Click here to download Figure Figure 4 revised.jpg



Figure

Click here to download Figure Figure 5.jpg



Figure

Click here to download Figure Figure 6.jpg