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^{18}F-FLT PET/CT in the evaluation of pheochromocytomas and paragangliomas: A pilot study

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Abstract

Context—^{18}F-FDG PET/CT has been proved to be a highly sensitive method for pheochromocytomas/paragangliomas (PHEOs/PGLs) associated with succinate dehydrogenase (SDH) mutations. This finding has been attributed to altered tumor cell metabolism resulting from these mutations and does not provide additional prognostic information to genotype. Therefore, identification of new biomarkers for aggressiveness is needed. A high Ki-67 index was proposed to be an additional prognostic factor.

Objectives—This pilot study aimed to evaluate 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) PET/CT, a PET proliferation tracer, as a potential imaging agent in a series of 12 PHEO/PGL patients with different genetic backgrounds, to compare ^{18}F-FLT uptake with ^{18}F-FDG PET/CT, and to evaluate classical factors of aggressiveness.

Patients and Methods—Twelve patients (7 metastatic and 5 non-metastatic) were prospectively evaluated with ^{18}F-FDG and ^{18}F-FLT and followed for at least 2 years after the initial imaging work-up.

Outcome measures—Uptake was assessed at a lesion level, visually and quantitatively by maximum standard uptake values (SUVmax) for both tracers. ^{18}F-FLT uptake was compared to

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risk factors known to be linked with a poor prognosis in PGLs (*SDHB*-mutated status, lesion size, dopaminergic phenotype) and with ^{18}F-FDG uptake.

Results—In 12 patients, 77 lesions were assessed. All lesions had low ^{18}F-FLT uptake (median SUVmax, 2.25; range, 0.7–4.5). There was no apparent superiority of ^{18}F-FLT uptake in progressive lesions and most of the lesions showed a mismatch, with high ^{18}F-FDG uptake (median SUVmax, 10.8; range, 1.1–79.0) contrasting with low ^{18}F-FLT uptake.

Conclusions—This study suggests that PHEOs/PGLs—even those that progress—do not exhibit intense ^{18}F-FLT uptake. It provides the first *in vivo* demonstration that proliferation may not be a major determinant of ^{18}F-FDG uptake in these tumors. These findings provide new insight into the biological behavior of PGL and suggest that antiproliferative agents may be suboptimal for treatment of these tumors.

Key Terms

pheochromocytoma and paraganglioma; ^{18}F-fluorothymidine; ^{18}F-fluorodeoxyglucose; positron emission tomography; proliferation; glycolysis

Introduction

Pheochromocytomas (PHEOs) and extraadrenal paragangliomas (PGLs) are neural crest cell-derived tumors associated with either the sympathetic (thoraco-abdominal PGLs) or parasympathetic (mainly head and neck PGLs) nervous systems.

Approximately 40% of PHEOs and PGLs carry a germline mutation in one of at least 18 genes (1). These mutations are associated with transcriptome changes that are currently subdivided into 2 main clusters. Cluster 1 is enriched with genes (i.e., succinate dehydrogenase complex, subunits A-D (SDHx)) that are associated with the hypoxic response (mainly HIF-2α) and cluster 2 contains tumors mutated for genes that activate kinase signaling and protein translation (i.e., Ret Proto-Oncogene-MEN2) (2).

Overall, the malignancy risk for PHEOs/PGLs has been estimated to be 10%, with an increased risk in sympathetic PGLs belonging to the cluster 1 subgroup. Interestingly, these tumors also exhibit increased 2-deoxy-2-[18F]-fluoro-D-glucose (^{18}F-FDG) uptake on PET/CT compared to cluster 2 tumors (3). ^{18}F-FDG PET/CT has been proven to provide prognostic information in several endocrine cancers such as thyroid carcinomas of follicular origin (4), medullary thyroid carcinoma (5), and gastroenteropancreatic neuroendocrine tumors (GEP-NETs) (6). In these tumors, acquisition of the ^{18}F-FDG metabolic pattern is progressive during the dedifferentiation process. In contrast, the ^{18}F-FDG PET/CT phenotype was found to be mainly dependent on the presence of a pseudohypoxic phenotype (activation of the HIF-signaling pathway despite normal oxygen supply) in PHEOs/PGLs, a finding that is in large part dependent on the genetic background (cluster 1 genes) (2, 3, 7, 8). Therefore, the ^{18}F-FDG PET/CT phenotype does not provide prognostic information in PHEOs/PGLs, and SDHx-tumors may have an indolent course even with highly elevated uptake values of FDG.

Classical indicators of poor prognosis include: *SDHB* mutation, tumor size > 5 cm, tumor location (extra-adrenal), age < 30 years at first presentation, and metastatic disease (9-11). Recently, some new studies have proposed to predict metastatic potential and/or tumor aggressiveness using characteristics such as a dopaminergic phenotype (i.e. detection of dopamine or its metabolite methoxytyramine) (12, 13), the presence of tumor necrosis, high Ki-67 index and/or mitotic count (14), overexpression of HIF-α and its target genes in tumors (15, 16), or extremely high mRNA copy numbers of a variant of carboxypeptidase E in tumors (17). Identification of *in vivo* biomarkers of aggressiveness would be of particular interest in the assessment of these tumors.

3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) has been proposed as a PET proliferation tracer even though it is not incorporated into DNA due to phosphorylation by cytosolic thymidine kinase-1 (TK1). The assumption is that the concentration of FLT nucleotides in cells is proportional to TK1 activity and, therefore, to cellular proliferation. The role of ^{18}F-FLT in oncology is still debated but several studies have shown promising results for tumor grading and in the evaluation of treatment response (18).

The aims of the present study were to evaluate ^{18}F-FLT PET/CT in a series of 12 PHEO/PGL patients with varying genetic backgrounds, compare ^{18}F-FLT uptake with a metabolic pattern on ^{18}F-FDG PET/CT, and evaluate classical factors of aggressiveness.

Materials and Methods

Patients

Twelve non-consecutive adult patients (10 men and 2 women; median age, 43 years; range, 27–70 years) with PGLs (as defined by the reference standard—see below) were prospectively included between January and July 2012 (and followed up over the course of at least two years). All patients were studied at the National Institutes of Health (NIH). The protocol (NCT00004847) was approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH. All patients provided written informed consent. The inclusion criteria were at least one PGL (as defined by the reference standard—see below) at the time of the study. Exclusion criteria included: age below 18 years, pregnancy, or recent (< 2 months) systemic treatment.

Reference Standard to define PGL

PGL lesions were confirmed histologically when surgery was performed on patients with nonmetastatic disease (patients # 1, 2, 5, 8 and 9). When surgery was not indicated due to the presence of metastases (patients # 3, 4, 6, 7, 10, 11, 12), lesions were characterized as PGL-related (either primary or metastatic) based on the positivity (characteristic appearance) on conventional imaging—either contrast-enhanced computed tomography (ceCT) or contrast-enhanced magnetic resonance imaging (ceMRI)—that corresponded with positivity on either ^{18}F-FDOPA or ^{18}F-FDA PET/CT (3, 19, 20); these findings were further supported by positive PGL-specific biochemistry and positive genetic testing results.

The following data were collected: metastatic status (defined by presence of tumor cells at non-neural crest-derived sites), *genetic testing*, adrenal versus extra-adrenal location, tumor

size (patient carrying at least one tumor diameter > 5 cm, which is the optimal cutoff from Eisenhofer's analysis (13)), dopaminergic phenotype (i.e., presence of dopamine and/or its metabolite methoxytyramine in urine and/or plasma (13)), and age at diagnosis. Patients were followed (clinically, biochemically, and with imaging) for at least two years after imaging.

Imaging protocol for ^{18}F-FDG and ^{18}F-FLT PET/CT scans

All patients underwent ^{18}F-FDG and ^{18}F-FLT PET/CT scanning. ^{18}F-FLT was provided by Cardinal Health. A fixed ^{18}F-FLT dose of 195 MBq was injected. For ^{18}F-FDG PET scanning, patients fasted for at least 4 hours before intravenous injection of 5 MBq/kg ^{18}F-FDG (median total injected dose, 390 MBq; range, 340–590 MBq) and blood glucose levels were measured just before injection to ensure a value below 200 mg/dL. For both radiopharmaceuticals, PET/CT acquisitions were performed on a Biograph-128 mCT PET/CT scanner (Siemens Medical Solutions). For both studies, PET data acquisition started at 60 minutes (min) after injection. Emission images were obtained in 3-dimensional mode, with 3-min/bed-position (BP) and 5-min/BP rates for ^{18}F-FDG and ^{18}F-FLT, respectively. The acquisitions were done from the thighs to the head. Co-registered CT studies for attenuation correction and anatomic co-registration were performed with the following imaging parameters: 120 kV, 115 mA and a 1.5 mm section thickness. Co-registered images were displayed on a workstation (DeltaViewer - MedImage), with 3D fused navigation along the axial, coronal, and sagittal planes and maximum intensity projection (MIP) rendering.

^{18}F-FLT and ^{18}F-FDG PET/CT scanning were performed within 1 month of each other in all but one case, with ^{18}F-FLT performed first in 2 patients (#2 and 6) and ^{18}F-FDG performed first in the 10 other patients. For patient #10, the ^{18}F-FLT scan was performed 4 months after the ^{18}F-FDG scan.

^{18}F-FDG and ^{18}F-FLT PET image analysis

On ^{18}F-FLT and ^{18}F-FDG PET/CT images, uptake was assessed for each lesion (determined on the basis of CT) visually (intensity of uptake in comparison to the surrounding background and to reference organs) and quantitatively. Uptake values were classified according to their location: soft-tissue for lesions located in head and neck area, mediastinum, lung, liver, extrahepatic abdomen and pelvis, and bone. In the case of multiple lesions, a cut-off of 5 lesions per area was fixed.

For quantitative assessment, body weight maximum standardized uptake values (SUVmax) were calculated using the following formula: SUV equals decay-corrected tracer tissue concentration (in Bq/g) injected dose (in Bq) normalized by the patient's body weight (in g). In both ^{18}F-FLT and ^{18}F-FDG PET, volumes of interest (VOI) were determined manually over the lesions using co-registered CT. Some small lesions that could not be separated easily from surrounding physiologic uptake (such as in the cases of small liver and bone lesions), were considered ‘non-measurable. The largest diameter of each lesion was measured.

Physiological ^{18}F-FLT uptake was also quantified by SUVmax in the following normal organs: muscle (right para-vertebral muscle at the level of L4), for use as an intra-patient non-proliferative negative control, and bone marrow (in the L4 vertebral body) and lymphatic tissue (including non-specific inflammatory lymph nodes, tonsils, and thymic remnants) for use as intra-patient positive controls; indeed hematopoietic cells in bone marrow and (to a lesser extent) lymphatic tissue were highly ^{18}F-FLT positive, as expected for active proliferative tissues (21, 22). Liver uptake was also measured.

Histopathologic analyses and immunohistochemical staining

Histopathologic analyses and immunohistochemical staining were performed in 5 patients with nonmetastatic disease in whom tumor tissue were collected prospectively after ^{18}F-FLT PET scan. Cell and tissue morphology and immunohistochemical staining for (chromogranin and synaptophysin) confirmed the diagnosis of PGL and Ki-67 levels were assessed. No patient with metastatic disease underwent surgery for ethical reasons: PGL diagnosis could non-invasively be confirmed using imaging, biochemistry, and medical history, and PGL biopsy can lead to severe adverse events related to catecholamine release.

Genetic testing

Genetic testing included gene sequencing of SDHB, SDHD, RET, and VHL as well as assessment of large gene rearrangements of SDHB and SDHD. When all these were negative, patients were qualified ‘apparently sporadic’. Patients were not tested for mutations in the recently described genes SDHA, SDHAF2, and TMEM127.

Statistical analysis

Descriptive quantitative data were expressed as either a median or given a range. The correlation between ^{18}F-FLT SUV and ^{18}F-FDG SUV was assessed using the Spearman rank correlation coefficient. A P-value <0.05 was considered statistically significant.

Results

Patients

Twelve patients with PHEO/PGL were studied. The genetic statuses represented were as follows: 5 SDHB, 2 SDHD, 4 apparently sporadic, and 1 MEN2. 7 were carrying metastatic lesions and 5 had only primary lesions (1 adrenal, 4 extra-adrenal). 4 patients were at the initial stages of PGL disease, 2 were evaluated for recurrence, and 6 for disease progression. 3 patients had previously received ^{131}I-MIBG radiation therapy (1 had his last dose 4 months prior), 2 CVD chemotherapy (1 received his last dose 2 months prior), and 2 external beam radiation therapy (EBRT) over the rachis. 2 patients had a dopaminergic phenotype. One patient had an adrenal PGL and 11 patients had extra-adrenal PGLs. 7 patients had a history of a voluminous (larger than 5 cm) primary PHEO/PGL lesion. Patient characteristics are detailed in Table 1.

Three patients (patients #10-12) had progressive disease (all associated with the *SDHB* mutated genotype, 2/3 with dopaminergic phenotype). Other patients had stable or slowly progressive disease.

^{18}F-FLT uptake in PGL lesions

Seventy-seven lesions were assessed. Of those, 14 lesions were in patients with only primary lesions and 63 lesions were in patients carrying metastatic lesions. Metastatic sites included: soft tissue (lymph nodes) (19), bones (16), liver (10), and lungs (18). Of the 77 lesions, 64 were measurable using SUVmax. Thirteen small lesions were non-measurable due to surrounding tissue activity: 2 primary PGLs, 6 bone lesions, and 5 liver lesions.

All lesions had rather low ^{18}F-FLT uptake, with an average SUVmax of 2.25 [0.7– 4.5]. In contrast, ^{18}F-FLT uptake in normal bone marrow was high in all patients, with an average SUVmax of 16.6 [12.9–22.1]. (In patients #7, 11, and 12, this was non-measurable due to disseminated metastatic bone disease). In 4 patients (#2, 3, 5 and 9). FLT uptake was noted in 13 lymph nodes/lymphatic tissue-related sites (including tonsils and thymic remnants) thought unrelated to presence of PHEO/PGL (based on negativity of ^{18}F-FDOPA and ^{18}F-FDA studies). These lesions had an average SUVmax of 6.0 [3.9–10.9].

We observed high ^{18}F-FLT uptake in normal liver (average SUVmax 9.8 [6.8–16.0]) in all patients, reflecting hepatic glucuronidation of FLT.

Quantification of background ^{18}F-FLT uptake in muscle, the nonproliferative control, showed an average SUVmax of 1.7 [1.2–2.3]. Figure 1 displays the ^{18}F-FLT SUVmax in lesions and reference organs.

^{18}F-FLT in PGL compared with clinical criteria of poor prognosis (per-patient analysis)

No significant differences in tumor FLT SUVmax values were observed between patients segregated by presence or absence of poor prognostic factors, including SDHB vs. non-SDHB (2.9 [0.9-3.9] vs 1.9 [0.7-4.5]), age ≤30 vs >30 year at diagnosis (2.35 [0.9-3.9] vs 2.05 [0.7-4.5]), metastatic vs nonmetastatic disease (2.05 [0.7-4.5] vs 2.45 [1.4-3.1]), presence or absence of large (>5 cm) lesion (3.25 [1.3-4.5] vs 2.45 [1.4-3.1], and elevated vs normal dopamine secretion (median 2.9 [range : 0.9-3.9] vs 2.0 [0.7-4.5]). There was also no significant difference in uptake of lesions in patients who had progressive vs. stable disease during follow-up (2.4 [0.9-3.9] versus 2.0 [0.7-4.5]). Figure 2 displays the average ^{18}F-FLT SUVmax in lesions according to these prognostic factors and follow-up.

Comparison of ^{18}F-FLT uptake in PGL with ^{18}F-FDG uptake

On visual analysis, the majority of lesions (66/77) showed a discrepancy between ^{18}F-FLT and ^{18}F-FDG uptake, with high ^{18}F-FDG uptake contrasting with low or absent ^{18}F-FLT uptake. Figure 3 shows an example of an *SDHB* patient with a primary abdominal PGL, and Figure 4, an example of an *SDHB* patient with widespread metastatic disease.

Overall, the average ^{18}F-FDG SUVmax of the 77 lesions was 10.8 [1.1-79.0], compared to an average SUVmax of 2.25 [0.7–4.5] for ^{18}F-FLT. Correlation analysis demonstrated a weak positive correlation between ^{18}F-FLT and ^{18}F-FDG uptake expressed by SUVmax

values (Spearman's rho 0.62, P-value <0.05 – Figure 5 shows the scatter plot). Similarly, correlation analysis conducted on subgroups (*SDHB*-related tumors, non-*SDHB*-related tumors) demonstrated a weak positive correlation between the ^{18}F-FLT and ^{18}F-FDG SUVmax values (Spearman's rho 0.40, P-value 0.11 for SDHB and Spearman's rho 0.59, P-value <0.05 for non-SDHB).

Ki-67 immunostaining

In vitro proliferative activity as assessed by Ki-67 index was low (less than 5%) in all surgically resected lesions (patients # 1, 2, 5, 8 and 9). Average ^{18}F-FLT and ^{18}F-FDG SUVmax were 2.8 and 6.5 in patient # 1, 2.7 and 14.5 in patient # 2, 3.1 and 10.4 in patient # 5, 2.9 and 53.2 in patient # 8 and 3.1 and 46.9 in patient # 9.

Discussion

This study shows that in our 12 patients, PHEOs/PGLs did not exhibit intense ^{18}F-FLT uptake, even those that progressed rapidly and/or exhibited high ^{18}F-FDG uptake. It also provides the first *in vivo* demonstration that proliferation is probably not a major determinant of ^{18}F-FDG uptake in these lesions.

These findings differ from highly proliferative cancers (i.e., lymphoma, lung cancer) that exhibit high ^{8}F-FLT tumor uptake values (18). However, the low ^{18}F-FLT uptake found in PGL is concordant with findings observed in well-differentiated GEP tumors. In one study, none of the tumors (primary lesions or metastases) were positive on ^{18}F-FLT PET/CT whereas ^{18}F-FDG PET/CT was positive in 7/10 cases (23). ^{18}F-FLT PET/CT was also found to be less sensitive than ^{18}F-FDG PET/CT in the diagnosis of residual lymph node and distant metastases from differentiated thyroid carcinoma (25). A case of primary papillary thyroid carcinomas detected by ^{18}F-FDG PET/CT were also found to have low ^{18}F-FLT uptake, a finding that was attributed to a low proliferation activity (24).

To our best knowledge, ^{18}F-FLT PET/CT has not been evaluated in other endocrine malignancies. Our results are consistent with the low proportion of PHEOs/PGLs with high Ki-67 indices (26-28) and provide *in vivo* evidence of low proliferation rates of PHEOs/PGL metastases. This is also consistent with preclinical models showing that PHEOs/PGL tumor cells were mostly at the resting state of the cell cycle (the so-called G0/G1 transition) (29). ^{18}F-FLT SUVmax was also not associated with presence of certain criteria for poor prognosis.

Our study also provides the first *in vivo* demonstration that proliferation is not a major determinant of ^{18}F-FDG uptake in these tumors, including *SDHx*-related tumors which often exhibit highly elevated uptake values. Several studies have found that ^{18}F-FDG tumor uptake is higher in patients with *SDHx* germline mutations compared to other tumor types (*RET, NF1*), regardless of the tumor location (3, 7). This finding has been attributed to activation of the HIF-α pathway despite normal or even high oxygen supply (also called the pseudohypoxic phenotype). However, it is notable that there are some discrepancies with *in vitro* studies that failed to identify an overexpression of glycolytic enzymes or HIF-α proteins in *SDHx*-related tumors in comparison to other subtypes (30). The present study

provides preliminary *in vivo* evidence that increased ^{18}F-FDG uptake in these patients is unrelated to proliferation and the role of glucose uptake by PHEOs/PGLs is currently largely unexplained. Based on our recent metabolomic study, it has been proposed that glucose might be directly or indirectly involved in the metabolism of myoinositol/ascorbate, glutamine/glutamate, methionine/taurine and catecholamines (31).

We acknowledge several limitations to our study: 1) absence of pathological confirmation of all lesions. Nevertheless, this is not a serious issue since PHEOs/PGLs concentrate specific radiotracers; 2) PET/CT acquisition was performed at a single study point (starting at about 60 min). However, pharmacokinetic studies showed that tracer washout is negligible and that a SUV at 60 min is correlated with the net influx constant Ki (32); 3) very small number of patients with widely heterogeneous characteristics. Some patients had also been treated in the past with therapeutic approaches that may have modified ^{18}F-FLT uptake (table 1). However, all of these patients were refractory to these therapies.

Our findings also provide new insight into the biological behavior of PGL and suggests that antiproliferative agents may not be very effective in treatment of these tumors.

Conclusion

In this limited pilot study, ^{18}F-FLT uptake was relatively low in PGL tumors, even progressive ones. This signifies a slow proliferative rate despite high ^{18}F-FDG uptake present in the same lesions. This suggests that antiproliferative drugs may not be effective in the treatment of these patients.

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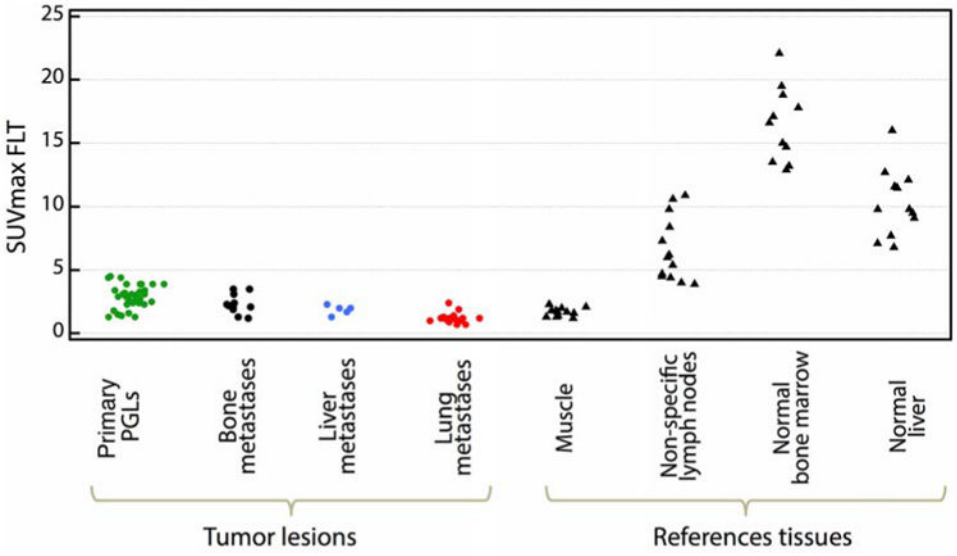

Figure 1. FLT SUVmax in lesions and reference organs

PGLs: includes all PGLs (primary tumos only) from patients with metastatic and non metastatic.disease. The median number of primary PGLs assessed per patient was 3 (range : 1-9).

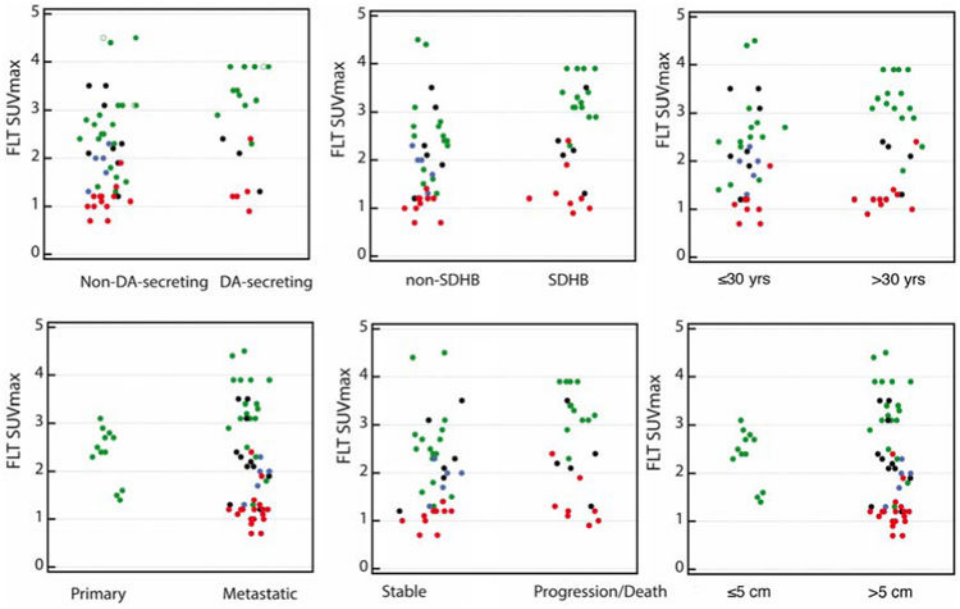

Figure 2. Lesion FLT SUVmax compared with criteria of poor prognosis

The color-code of the dots is the same as in figure 1.

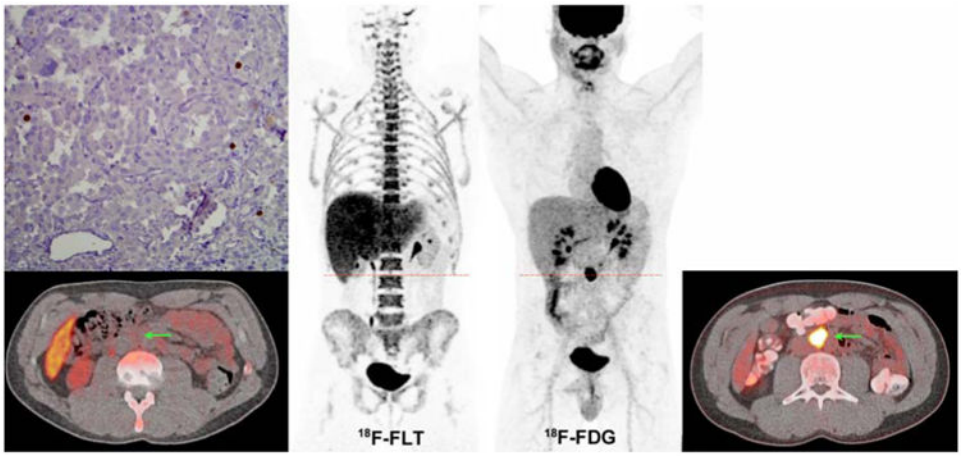

Figure 3. Single retroperitoneal primary PGL in an *SDHB* patient (#3)

^{18}F-FDG (MIP image and axial CT-fused image at the level of the PGL lesion) shows particularly high uptake (^{18}F-FDG SUVmax of 59.8). This lesion was corresponded to a 2.6-cm abdominal extra-adrenal mass (arrows). ^{18}F-FLT (MIP image and axial CT-fused image at the level of the PGL lesion) shows low uptake (^{18}F-FLT SUVmax of 2.5). Low Ki-67 staining.

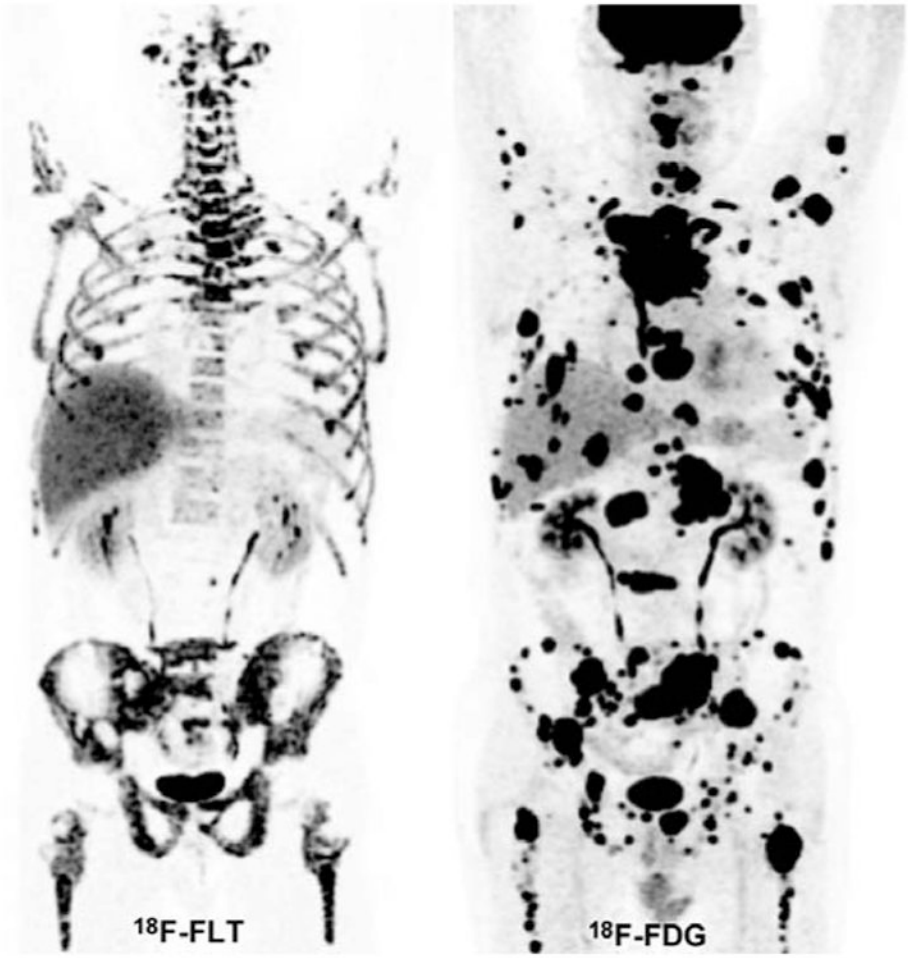

Figure 4. Disseminated metastatic disease (soft-tissue, bone, liver, and lung lesions) in an *SDHB* patient

^{18}F-FDG (MIP image) shows high tumor burden with very high ^{18}F-FDG uptake in the vast majority of the lesion. ^{18}F-FLT (MIP image) shows very low ^{18}F-FLT uptake. Bone lesions (notably in both femoral heads and bilateral iliac bones) appear to have relatively low FLT uptake compared to the surrounding background uptake in marrow.

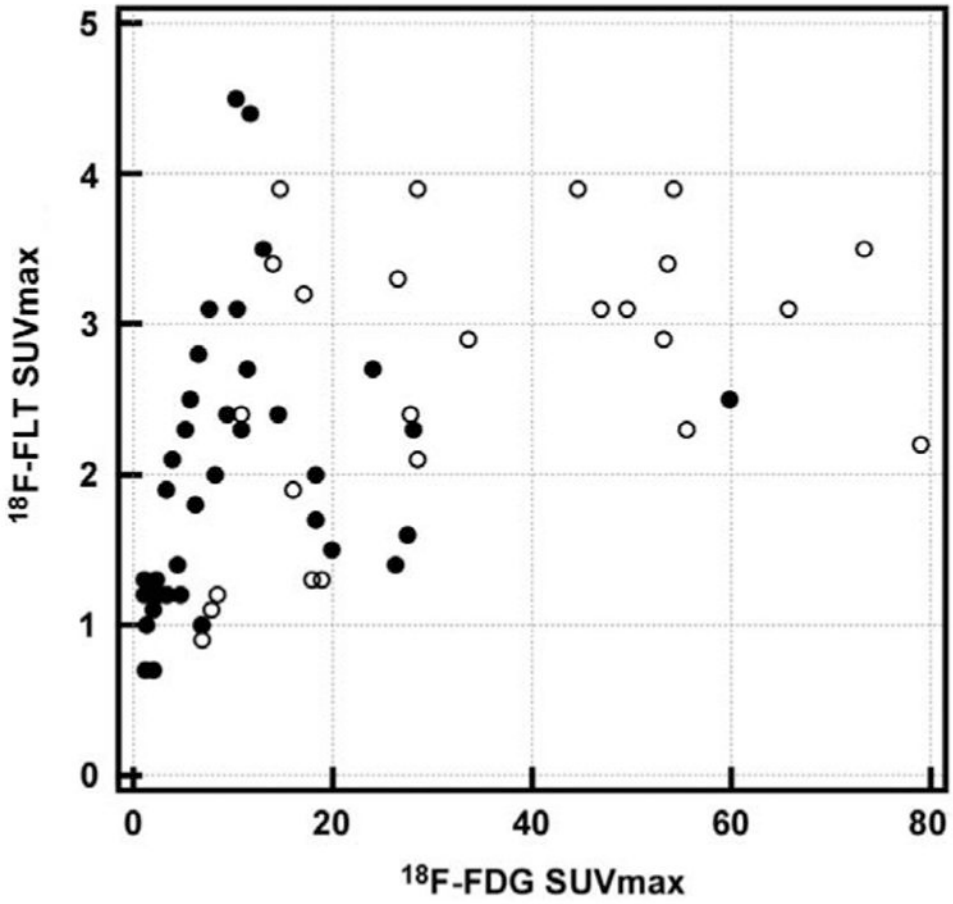

Figure 5. Scatter-plot displaying tumor ^{18}F-FLT SUVmax values in relation to their ^{18}F-FDG SUVmax

Black rounds correspond to lesions from non-*SDHB* patients. White rounds correspond to lesions from non-*SDHB* patients. ^{18}F-FLT SUVmax values in these lesions are low (average 2.25 [0.7-4.5]) whereas their corresponding ^{18}F-FDG SUVmax values are high (average 10.8 [1.1-79.0]).

Table 1

Patient clinical characteristics

Patient	Age (yrs), sex	Germline mutation	Age at intial diagnosis	Status at the time of FLT & FDG PET	Size of the largest lesion at Time of FLT/FDG	Dopamine secretion	Reason for evaluation at the time of FLT & FDG PET	Previous systemic treatments (delay between end of last treatment and PET scan)	2 year follow-up (progression or death from PGL)
#1	43, M	sporadic	43	primary	3.8 cm	Negative	Suspected primary	NONE	no
#2	43, M	SDHD	43	primary	4.8 cm	Negative	Suspected primary	NONE	no
#3	43, M	SDHD	41	primary	4.7 cm	Negative	Restaging	NONE	no
#4	48, F	MEN2	35	metastatic	12 cm	Negative	Restaging	131I-MIBG (>2 years)	no
#5	47, M	sporadic	30	metastatic	unknown	Negative	Suspected recurrence	NONE	no
#6	70, M	sporadic	67	metastatic	24 cm	Negative	Suspected recurrence	NONE	no
#7	50, F	sporadic	43	metastatic	8 cm	Negative	Restaging	131I-MIBG (8 months), sandostatin therapy (on)	no
#8	30, M	SDHB	30	primary	2.5 cm	Negative	Initial staging	NONE	no
#9	27, M	SDHB	27	primary	3 cm	Negative	Initial staging	NONE	no
#10	35, M	SDHB	19	metastatic	8 cm	Positive	Restaging	NONE	yes
#11	35, M	SDHB	25	metastatic	7 cm	Positive	Restaging	CVD (>2 years), 131I-MIBG (4 months)	Yes
#12	47, M	SDHB	41	metastatic	3 cm	Negative	Restaging	CVD (2 months)	yes

CVD: cyclophosphamide, vincristine and dacarbazine