

Imaging Bone and Soft Tissue Tumors with the Proliferation Marker [¹⁸F]Fluorodeoxythymidine

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Abstract **Purpose:** We have determined the ability of positron emission tomography (PET) with the thymidine analogue 3'-deoxy-3' [¹⁸F]fluorothymidine (FLT) to detect manifestation sites of bone and soft tissue tumors, to assess tumor grading, and to differentiate malignant from benign tumors. **Materials and Methods:** In this prospective bicenter trial, FLT-PET was done in 22 patients with established or suspected soft or bone tissue lesions. Routine diagnostic procedures included incisional biopsy, magnetic resonance imaging, and/or contrast-enhanced spiral computed tomography in all patients and [¹⁸F]fluorodeoxyglucose (FDG)-PET in 15 patients. Forty-five to 60 minutes after i.v. injection of 350 to 425 MBq FLT, emission and transmission scanning was done. Tracer uptake in the tumor was evaluated semiquantitatively by calculation of mean and maximum standardized uptake values (FLT-SUV) and compared with respective values of FDG. Results were correlated to histopathology and tumor grading. **Results:** FLT-PET detected all malignant bone or soft tissue tumors (17 of 17). Mean FLT-SUV in benign lesions was 0.7 (range, 0.3-1.3), and 1.3 in low-grade sarcoma (grade 1; range, 1.0-1.6), 4.1 (range, 2.2-6.0; $P = 0.002$) and 6.1 (range, 2.5-8.3; $P = 0.001$) in grade 2 and grade 3 tumors, respectively. FLT but not FDG uptake correlated significantly with tumor grading ($r = 0.71$ versus $r = 0.01$), and a cutoff value of 2.0 for FLT-SUV discriminated between low- and high-grade tumors. **Conclusion:** In this clinical study, the proliferation marker FLT was suitable for imaging malignant bone or soft tissue tumors. FLT but not FDG uptake correlated significantly with the tumor grade, suggesting FLT as superior PET tracer for noninvasive grading of sarcomas.

Tumors originating from soft tissue or bone comprise a multitude of entities with different grades of malignancy, biological behavior, and therapeutic options. The incidence is relatively low (~2-3 per 100,000 in the United States), and malignant tumors account for 0.6% of all cancers (1). Treatment strategies rely on tumor stage at initial presentation, histologic subtype, and tumor grading assessed by incisional biopsy. The tumor grade represents an important factor related to metastasis and overall survival, therefore being especially helpful in selecting patients who may benefit from adjuvant

chemotherapy (2). Magnetic resonance imaging, helical computed tomography (CT), angiography, and bone scintigraphy remain the mainstay diagnostic tests for imaging primary tumors and screening for distant metastases (3-5).

Positron emission tomography (PET) allows noninvasive assessment of cancer biology and may aid in staging tumors, detecting relapse, or differentiating scar from residual tumors. Numerous studies have evaluated potential advantages of imaging sarcoma with PET and the glucose analogue [¹⁸F]fluorodeoxyglucose (FDG; refs. 6-20, reviewed in ref. 21). FDG-PET was also suggested for noninvasive tumor grading (7, 9, 10, 12, 13, 19). The vast majority of these studies showed significantly higher uptake of FDG in high-grade compared with low-grade sarcomas. However, an overlap of tumoral FDG uptake has been reported in a considerable number of patients. Furthermore, false-positive PET-findings were reported in aggressive benign tumors and inflammatory lesions (10, 22, 23). FDG is also inadequate to differentiate low-grade malignant lesions from benign tumors. Several preliminary studies suggested early discrimination between nonresponders, and patients responding to adjuvant chemotherapy and persistent FDG uptake was shown to be a strong predictor for early relapse (24-30). Yet, the exact role of FDG-PET for diagnosis, follow-up, and response assessment of patients with bone or soft tissue sarcoma remains to be determined (31-33).

Multimodality imaging integrating metabolic and morphologic imaging (e.g., FDG-PET/CT) may overcome some of these

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Grant support: Deutsche Forschungsgemeinschaft (German Research Foundation) grant Bu-1424/1 (KFO 120, P3).

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©2008 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-07-4294

shortcomings. Another approach is the use of more specific radiopharmaceuticals. Deregulated cell cycle progression is a prerequisite of cancer and presumably more specific than altered glucose consumption. Imaging proliferation potentially increases the specificity and enables early response assessment to antineoplastic treatment. [¹¹C]thymidine represents the native pyrimidine nucleoside and was therefore introduced as radiopharmaceutical for imaging proliferation. The thymidine analogue 3'-deoxy-3' [¹⁸F]fluorothymidine (FLT) has been reported to be more stable and can accumulate both in proliferating tissues and malignant tumors (34). In malignant lymphoma, we have recently shown the superior differentiation of low-grade from more aggressive tumors (35). There is only one clinical study reporting that FLT-PET is feasible for imaging of soft tissue sarcomas of the extremities (36).

The aim of this study was to determine if PET with the proliferation marker FLT enables also detection of malignant tumors of bone structures, differentiation of benign from malignant tumors, and if FLT-PET allows noninvasive assessment of tumor grading.

Patients and Methods

Patient characteristics and routine staging. This prospective study comprised 22 patients (17 men and 5 women) with a mean age of 49.3 y (range, 18-78 y; Table 1). Patients with newly diagnosed soft tissue or bone tumors suspicious for a malignant tumor or already histologically confirmed malignant tumors who were admitted to the University Hospitals in Ulm (14 patients) or Munich (Klinikum rechts der Isar, Technical University Munich; 8 patients) for pretherapeutic staging and treatment planning were included. Patients with a history of a malignant tumor or patients who previously underwent radiotherapy or chemotherapy were excluded from this series. PET imaging was done

in 14 patients before and in 8 patients after incisional biopsy. Biopsied tumor specimens were >10 mm in all patients. The mean diameter of all biopsied lesions was 59.6 mm (SD, 41; range, 15-150 mm). All patients gave written informed consent to participate in the study, which was approved by the ethical committee of the medical faculty of both centers.

Routine staging included clinical examination, laboratory screening, and CT or magnetic resonance imaging of the primary tumor site in all patients. In 15 patients, FDG-PET was also part of the staging procedures.

Histopathology and tumor grading. Histopathologic classification was carried out by experienced pathologists (P.M. or G.W.) in all patients according to the current WHO recommendations using H&E-stained sections (37). For grading of soft tissue sarcomas, the French grading system (French Federation of Cancer Centers) was used (38). For grading of malignant tumors of bone, a similar classification system was used based on tumor differentiation, mitotic Fig. count, and presence of necrotic tissue.

Synthesis of [¹⁸F]FLT. [¹⁸F]FLT was produced using the method of Machulla et al. (39) with minor modifications. [¹⁸F]fluoride was produced via the ¹⁸O(p,n)¹⁸F nuclear reaction by bombardment of isotopically enriched [¹⁸O]H₂O with a 16.5-MeV proton beam at a PETtrace cyclotron. The detailed synthesis protocol is described elsewhere (35). The radiochemical purity was >99% confirmed via radioanalytical high performance liquid chromatography (Phenomenex Luna C18 column; 250 cm × 4.6 mm; 10% ethanol in water; flow rate, 0.5 mL/min). The specific activity was ~120 GBq/μmol. The final product was sterile and pyrogen free.

PET. In both centers, FLT-PET imaging was done using a high-resolution full-ring scanner (ECAT HR+; Siemens/CTI), which produces 47 contiguous slices per bed position with a slice thickness of 3.4 mm. Axial field of view is 15.5 cm per bed position. At least five bed positions were measured in each patient covering a total field of view of 77.5 cm. The emission scan included the base of the skull, neck, thorax, abdomen, pelvis, and proximal femora in all patients. Additionally, in case of a peripheral tumor, affected limbs (legs or arms) were imaged. Patients fasted for at least 6 h before PET imaging. Static emission scans

Table 1. Patient characteristics, histologic subtype of bone or soft tissue tumors, grading, and mean or maximum FLT or FDG-uptake in the primary tumor

No	Sex/Age	Histopathology	Tissue type	Tumor size (mm)	Tumor grading	FLT-SUV mean	FDG-SUV mean	FLT-SUV maximum	FDG-SUV maximum
1	W/30	Fibromyxoid sarcoma	Bone	80 × 60 × 130	1	N/a	N/a	N/a	NA
2	M/19	Ewing's sarcoma	Bone	150 × 125 × 173	3	2.54	N/a	3.15	NA
3	M/29	Liposarcoma (pleomorphic)	Soft	47 × 20 × 68	2	2.89	10.8	4.45	18.2
4	M/71	Osteosarcoma	Bone	27 × 29 × 29	2	2.22	3.7	2.93	6.7
5	W/35	Leiomyosarcoma of bone	Bone	54 × 84 × 80	2	4.14	N/a	4.86	NA
6	W/53	Myxoid chondrosarcoma	Bone	102 × 89 × 90	2	3.28	21.9	4.08	31.2
7	M/74	Merkel-cell tumor (malignant)	Soft	25 × 28 × 25	1	1.55	N/a	1.84	NA
8	M/55	Histiocytoma (malignant)	Bone	17 × 28 × 30	2	N/a	N/a	N/a	NA
9	W/49	Schwannoma (malignant)	Soft	35 × 33 × 38	3	4.80	N/a	8.70	NA
10	M/63	Histiocytoma (malignant)	Bone	20 × 20	2	6.08	7.4	6.77	12.1
11	M/47	Mesenchymal spindle cell tumor	Soft	34 × 37 × 30	3	7.0	18.2	9.81	29.6
12	M/68	Spindle cell sarcoma	Soft	56 × 64 × 56	3	7.61	4.3	8.34	5.6
13	M/78	Myxofibroid sarcoma	Soft	78 × 64 × 98	2	6.12	9.1	7.42	11.8
14	M/67	Malignant histiocytosis	Soft	25 × 12 × 23	3	7.27	6.4	12.73	7.8
15	M/77	MPNST	Soft	15 × 11 × 23	3	4.21	6.8	5.25	10.1
16	M/78	Epithelioid sarcoma + MPNST	Soft	38 × 46 × 52	3	8.34	6.2	9.05	8.5
17	F/34	Leiomyosarcoma	Soft	80 × 115 × 65	1	1.0	5.1	1.41	6.7
18	M/26	Schwannoma	Soft	26 × 35 × 19	Benign	0.77	1.3	1.14	1.8
19	M/42	Enchondroma	Bone	9 × 13 × 110	Benign	1.25	N/a	1.98	NA
20	M/38	Necrosis of the femoral head	Bone	15 × 20 × 18	Benign	1.77	2.9	2.43	4.7
21	M/33	Hibernoma	Soft	32 × 33 × 40	Benign	0.27	3.0	0.31	3.3
22	M/18	Osteochondroma	Bone	15 × 11 × 15	Benign	0.53	1.5	0.62	1.8

Abbreviations: MPNST, malignant peripheral nerve sheath tumor; NA, not available.

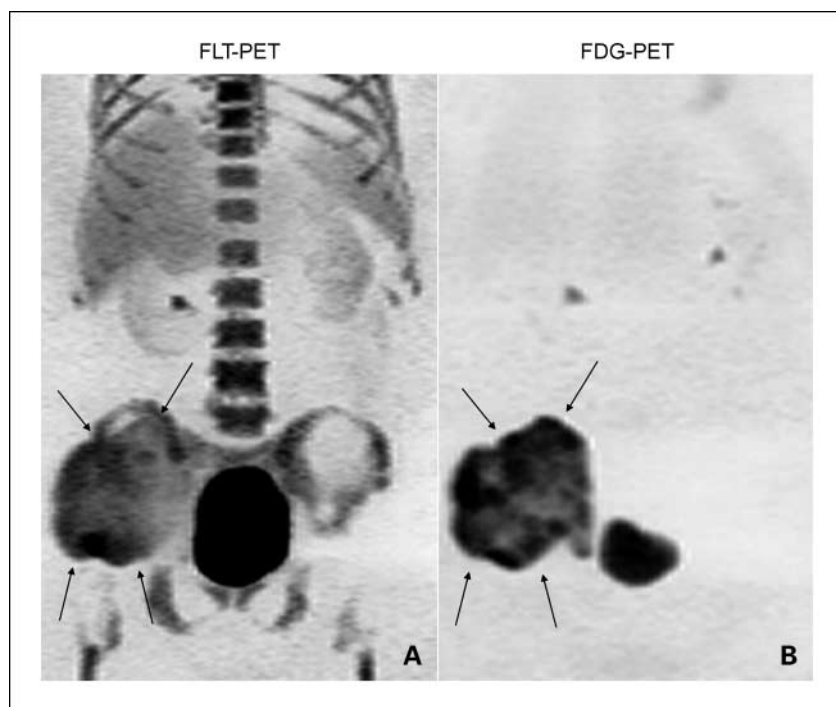


Fig. 1. *A*, biodistribution of FLT in a patient with Ewing's sarcoma (grade 3) 60 min after i.v. injection of the radiotracer (maximum intensity projection of FLT-PET; patient no. 2). Besides intense uptake of FLT in bone marrow (vertebral column, ribs, and pelvis), intense uptake of FLT in the primary tumor is visible (arrows); weak uptake in renal calices and intense uptake in the bladder. *B*, FDG-PET (maximum intensity projection) of the same patient as shown in *A*, done 1 day before FLT-PET, indicating intense FDG uptake in the malignant primary. Only weak uptake in renal calices and bladder due to renal excretion of the tracer.

were started 45 to 60 min after injection of 350 to 425 MBq [^{18}F]FLT (mean, 385 MBq). The acquisition time was 4 or 5 min per bed position, respectively. Three or 4 min transmission scans with a germanium-68/gallium-68 ring source were done for attenuation correction. Emission data were corrected for random coincidences and dead time, and reconstructed by filtered backprojection (Hanning filter with cutoff frequency 0.4 cycles per bin). The matrix size was 128×128 pixels with a pixel size of 4.0×4.0 mm. The image pixel counts were calibrated to activity concentrations (Becquerel/milliliter) and decay corrected using the time of tracer injection as reference.

At the center of Munich, FDG-PET was done in eight patients using a hybrid PET/CT scanner (Siemens Biograph 16); at the PET center of Ulm, three patients had additional FDG-PET using a Siemens ECAT HR+ scanner (as mentioned above) and four patients with the use of a PET/CT scanner (GE Discovery LS). Patients fasted for least 6 h before the PET scan, and blood glucose levels were measured before administration of FDG. All measured values were <150 mg/dl. The CT acquisition protocol included a low-dose CT in all 15 patients (26 mAs; 120 kV; 0.5s per rotation; 5-mm slice thickness) from the top of the skull to mid thigh for attenuation correction followed by the PET scan. In case of a peripheral tumor, imaging of the affected extremities (legs or arms) was done additionally. Sixty minutes after i.v. injection of 370 to 550 MBq of FDG, PET scans were acquired in two-dimensional mode with an acquisition time of 3 or 4.5 min per bed position. Forty or 37 contiguous slices were acquired per bed position; the matrix size was 128×128 with a slice thickness of 3.4 mm or 5 mm, respectively. Images were reconstructed by an attenuation-weighted ordered-subsets expectation maximization algorithm (4 iterations; 8 subsets) followed by a postreconstruction smoothing Gaussian filter (5-mm full-width at half maximum). Emission data were corrected for randoms, dead time, scatter, and attenuation, and the same reconstruction algorithm was applied as for the conventional FLT- or FDG-PET data.

Visual interpretation of FLT-PET. All images were evaluated by two experienced nuclear medicine physicians without knowledge of the results of histopathologic classification or standard imaging procedures. Focally increased FLT uptake in soft tissues or bone with usually mild or homogenous activity were interpreted as manifestation sites of a malignant tumor. If both readers produced differing results, respective scans were reanalyzed and a consensus was made.

Quantification of FLT or FDG uptake. In each tumor, the transaxial section with the highest metabolic activity was selected. Circular regions of interest with a diameter of 1.5 cm were placed in the area with the highest tumor activity as described earlier (40). In small tumors, side-by-side viewing of pretherapeutic helical CT or magnetic resonance imaging was used to assure correct placement of the regions of interest. Mean and maximum standardized uptake values (mean or maximum FLT-SUV) were calculated from each regions of interest using the formula: $\text{SUV} = \text{measured activity concentration (Bq/g)} \times \text{body weight (g)} / \text{injected activity (Bq)}$. Regions of interests were also placed in reference segments of the following organs: lungs, liver, bone marrow, spleen, bone, intestines, and brain. Normal tissue biodistribution was similar to that described in an earlier study in patients with malignant lymphoma (data not shown; ref. 35). In patients 1 and 8, transmission scanning was not practicable due to technical reasons and, consecutively, SUVs could not be calculated. Respective uptake of FDG in primary bone or soft tissue tumors was semiquantitatively assessed as described for FLT.

Data analysis. Statistical analyses were done using PRISM software version 5.0 (GraphPad). After completion of the study, results of histopathology, tumor grading, routine staging procedures, and findings at FLT-PET were compared. Data are presented as mean, median, range, and SD. Differences were considered significant at a P value of <0.05 . Linear regression analysis was done to test for a potential correlation of FLT or FDG uptake and tumor grading using Origin software 7.0 (OriginLab). Receiver operating characteristic analysis was done to evaluate performance characteristics of FLT- or FDG-PET for differentiating benign from malignant tumors.

Results

Histopathology and tumor grading. Twelve of 22 patients were included due to a newly diagnosed soft tissue lesion; 10 patients presented with lesions of bone structures (Table 1). Overall, histopathology revealed malignant tumors in 17 patients and benign lesions in 5 patients (Table 1). Soft tissue masses comprised 10 malignant tumors including spindle cell sarcoma ($n = 2$), liposarcoma, leiomyosarcoma, myxofibroid

and epitheloid sarcoma, schwannoma, histiocytosis, a peripheral nerve sheath tumor and a Merkel tumor, as well as 2 benign tumors (schwannoma and hibernoma).

Seven malignant bone tumors comprised fibromyxoid sarcoma ($n = 2$), Ewing's sarcoma, osteosarcoma, leiomyosarcoma, and histiocytoma ($n = 2$). Two patients had benign tumors of bone (enchondroma and osteochondroma), whereas one had necrosis of the right femoral head. Histopathologic grading according to the French (soft tissue sarcoma) or WHO (sarcoma of bone) classification system revealed 3 low-grade tumors and 14 high-grade tumors (7 patients with grade 2 and 7 patients with grade 3 tumors).

Imaging malignant soft and bone tissue tumors with FLT-PET. FLT-PET produced images of high contrast of both proliferating bone marrow and high-grade bone or soft tissue tumors (Figs. 1 and 2). In all patients, histologically proven malignant tumors presented with focal FLT uptake higher than surrounding background activity (17 of 17; sensitivity, 100%). Mean FLT uptake in all malignant tumors was 4.7 (range, 1.0-8.3) and significantly higher compared with the four benign tumors (mean FLT uptake, 0.7; range, 0.3-1.8; $P < 0.0001$). There was a similar FLT uptake in soft tissue tumors (mean FLT-SUV, 4.7; range, 1.0-7.6) compared with tumors of bone (mean FLT-SUV, 4.5; range, 2.5-8.3).

The average maximum FLT uptake in malignant tumors was 6.4 (range, 1.8-12.7). Maximum FLT uptake in soft tissue tumors was 7.2 (range, 1.8-12.7) and significantly higher

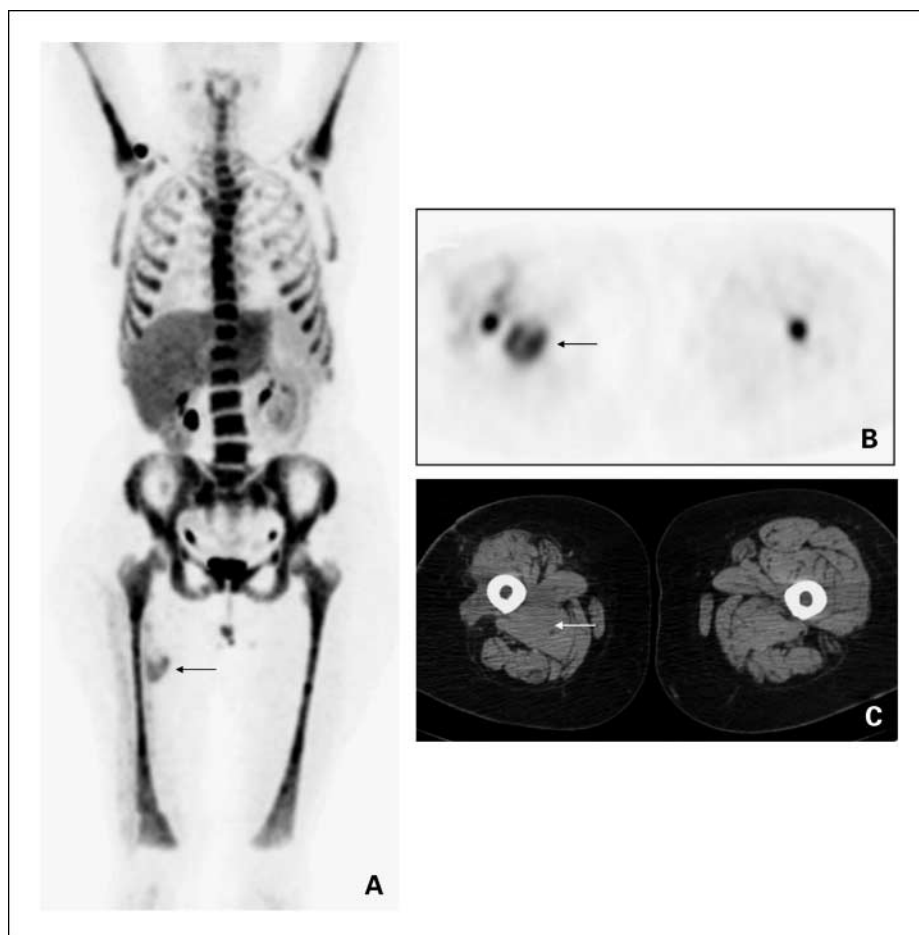
compared with malignant bone tumors (4.7; range, 3.2-6.8; $P < 0.05$).

Three patients had multiple lung metastases from soft tissue sarcoma or sarcoma of bone with a minimum size of >20 mm. In all patients, focal pulmonary FLT uptake was observed corresponding to lung metastases present at conventional imaging (helical CT or FDG-PET; Fig. 3).

Imaging benign tumors with FLT-PET. Three of 4 benign tumors did not show increased FLT uptake compared with background activity, whereas one presented with mild focal tracer uptake (specificity, 75%). Mean FLT-SUV in benign lesions was 0.7 (range, 0.3-1.3). In the three soft tissue tumors including schwannoma, enchondroma, and hibernoma, uptake was quite low with a mean FLT-SUV of <0.8 in all lesions. In the one patient with enchondroma of the distal femur, increased FLT uptake was observed compared with the contralateral bone and was, therefore, misinterpreted as malignant. Consecutively, SUV calculation revealed slightly increased tracer uptake in this lesion (mean FLT-SUV, 1.3). The lesion representing necrosis of the femoral head was correctly interpreted as benign because of a lower uptake compared with the contralateral side. However, this lesion had somewhat increased FLT uptake (mean FLT-SUV, 1.8), although clearly lower than neighboring normal bone marrow activity.

Assessment of tumor grading. In low-grade sarcoma (grade 1), mean FLT-SUV was 1.3 (range, 1.0-1.6) and significantly lower compared with grade 2 (4.1; range, 2.2-6.0; $P = 0.002$)

Fig. 2. A, biodistribution of FLT in a patient with soft tissue sarcoma (grade 2) 60 min after i.v. injection of the radiotracer (maximum intensity projection of FLT-PET; patient no. 3). Medium intense uptake of FLT in the primary tumor compared with intense uptake in adjacent bone marrow (vertebral column, ribs, pelvis, and femora). B, transaxial section of the primary tumor. C, corresponding transaxial section of helical CT.



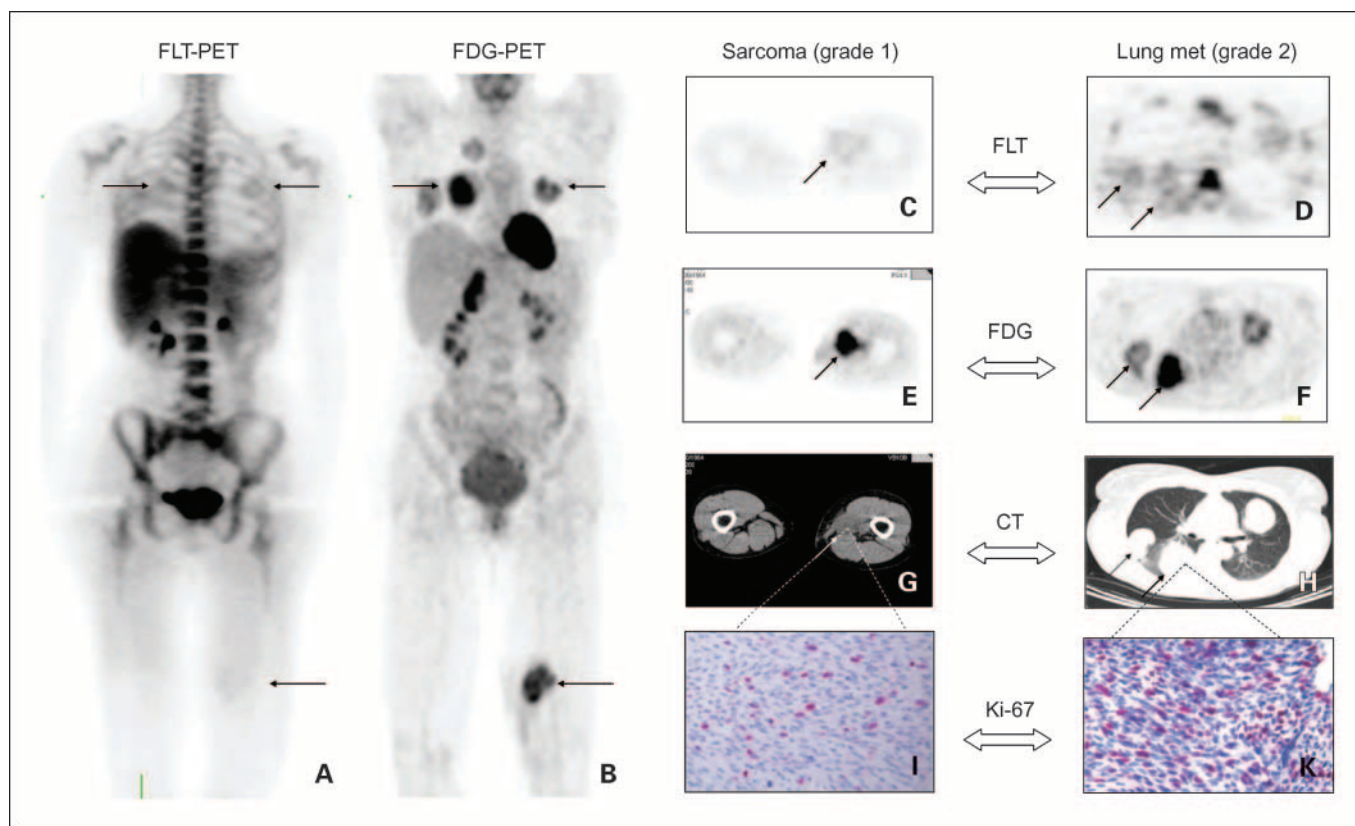


Fig. 3. *A*, FLT-PET (maximum intensity projection) of patient 17 with low-grade soft tissue sarcoma of the left distal thigh (arrows). Faint FLT-uptake in the malignant primary (mean FLT-SUV, 1.0) and mild uptake in pulmonary metastases (mean FLT-SUV, 2.2). *B*, FDG-PET (maximum intensity projection) of the same patient, intense tracer uptake in the malignant primary and in pulmonary metastases. *C* and *D*, transaxial sections of FLT-PET showing faint or mild tracer uptake in soft tissue sarcoma and pulmonary metastases. *E* and *F*, intense uptake of FDG in corresponding sections. *G* and *H*, corresponding sections of helical CT showing the primary tumor and large pulmonary metastases in both lungs (arrows). *I*, anti-Ki-67 immunostaining (MIB-1) of tissue derived from soft tissue sarcoma (*C*, *E*, and *G*) indicating low proliferation fraction (<5% brown-labeled nuclei). *K*, Ki-67 immunohistochemistry of a pulmonary metastasis (grade 2) indicates higher proliferative activity (15% proliferating cells).

and grade 3 tumors (6.1; range, 2.5-8.3; $P = 0.001$). Maximum FLT values also differed significantly between low-grade tumors (1.6; range, 1.4-1.8) and grade 2 (5.3; range, 2.9-7.4; $P = 0.002$) or grade 3 tumors (7.9; range, 3.2-12.7; $P = 0.001$).

Mean and maximum FLT uptake correlated significantly with tumor grading ($P < 0.001$) and a cutoff value of 2.0 for mean SUV (respective value of maximum SUV, 2.3) discriminated between low- and high-grade tumors. Using receiver operating characteristic analysis, mean FLT-SUV distinguished between benign and malignant tumors with an area under the curve of 0.96 (maximum FLT-SUV, 0.94).

There was an overlap of FLT-uptake in benign tumors and low-grade sarcomas presumably related to physiologically increased tracer uptake in proliferating bone marrow in the one patient with enchondroma. Omitting this bone marrow lesion from the analysis, a cutoff value of 0.8 for mean FLT-SUV (maximum FLT-SUV, 1.2) differentiated between all benign and malignant tumors.

Comparison of FLT- and FDG-PET. Fifteen of 22 patients (11 of 17 patients with malignant bone or soft tissue tumors) underwent PET imaging with both tracers enabling a direct comparison. In malignant tumors, mean average FDG-SUV was 9.5 (range, 3.7-21.9) and average maximum FDG-SUV was 14.8 (range, 6.7-31.2). Both mean and maximum FDG-SUV were significantly higher compared with benign lesions (average

mean SUV, 2.2; range, 1.3-3.0; average maximum SUV, 2.9; range, 1.6-4.7; $P < 0.0001$), resulting in the same sensitivity for detection of malignant tumors (100%). There was focal FDG uptake in 3 of 4 benign lesions; however, a threshold of 3.4 for mean SUV differentiated between benign and malignant tumors (area under the curve, 1.0; $P < 0.05$; respective values for maximum FDG-SUV: cutoff value, 5.2; area under the curve, 1.0; $P < 0.05$). Compared with FLT, linear regression analysis indicated no significant correlation of mean or maximum FDG-SUV and tumor grading (mean FDG-SUV: $r = 0.012$; $P = 0.97$; maximum FDG-SUV: $r = 0.016$; $P = 0.96$; Fig. 4).

Discussion

Recently, the thymidine analogue FLT was suggested for noninvasive assessment of proliferation and tumor grading (34, 35). This is the first clinical study indicating that FLT-PET is suitable for imaging sarcomas of bone structures. Due to low background in peripheral bones and soft tissues, malignancy associated FLT-uptake was easily detectable also in bone sarcomas (sensitivity, 100%). Routine staging procedures detected sarcoma-related pulmonary metastases in three patients, and focal pulmonary uptake of FLT was present in all of them. However, lesions were >20 mm in all patients, and the high sensitivity may not hold up in a larger study comprising also smaller tumors.

An important finding of our study is a significantly higher FLT-uptake in high-grade (grade 2-3) compared with low-grade tumors ($P < 0.0001$). No overlap was observed and a cutoff value of 2.0 for mean FLT-SUV differentiated between low- and high-grade tumors. Linear regression analysis also indicated significant correlation of FLT uptake and tumor grading ($P < 0.001$). This observation is consistent with a recent study reporting a sensitivity of 100% and significant correlation of maximum FLT-SUV to proliferation fraction and tumor grading in a series of 19 patients with soft tissue sarcoma (36).

Interestingly, in a patient with leiomyosarcoma, faint FLT-uptake of the primary tumor (mean SUV, 1.0) corresponded to low-grade histology (grade 1), whereas in pulmonary metastases, FLT uptake was markedly higher (FLT-SUV, 2.2). Rebiopsy of the pulmonary lesion indicated a more aggressive histology (grade 2). Tumor grade was therefore correctly assigned in both primary tumor and metastases, whereas FDG-PET showed intense uptake in both lesions (Fig. 3). These findings suggest a potential role of FLT-PET for noninvasive tumor grading and warrant further analysis in a larger series.

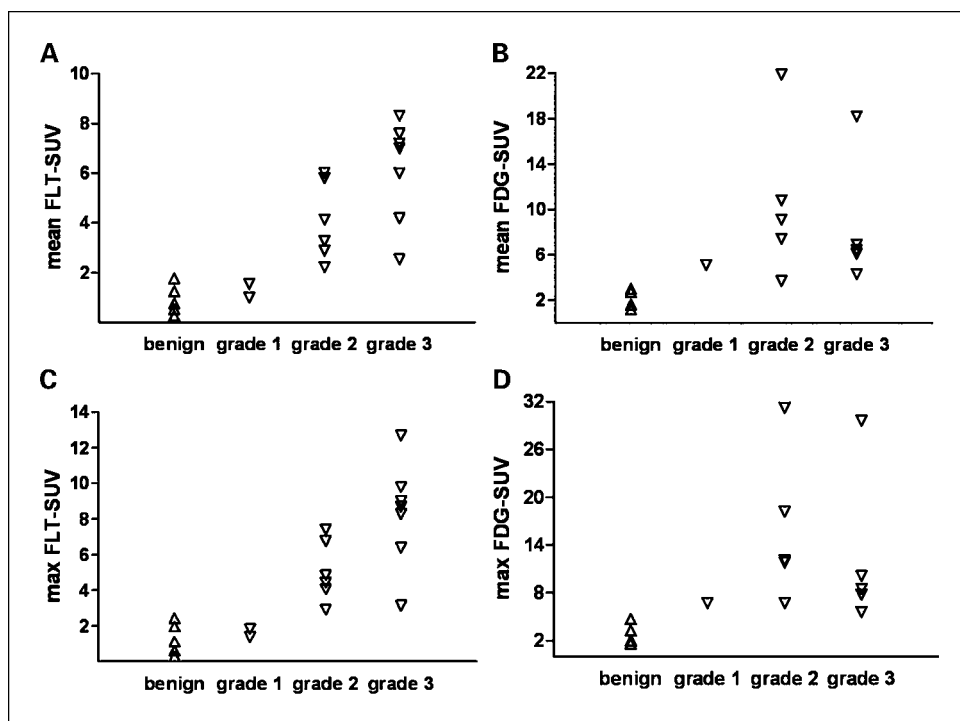
PET using the glucose analogue FDG is now an established imaging modality for detection and staging of cancer (41). In bone and soft tissue sarcomas, FDG-PET was reported to be highly sensitive and especially relevant for estimation of individual prognosis. However, accuracy regarding tumor grading and differentiation from benign and malignant tumors can be reduced by nonspecific uptake in inflammatory cells and aggressive benign tumors (10, 22, 23). In the present study, a similar overlap of benign tumors and low-grade sarcomas has been observed. FLT uptake was in the range of background activity in the majority of these lesions. Increased tracer uptake was observed in benign bone lesions containing normal bone marrow such as the proximal or distal femur. This is presumably related to increased proliferative activity of normal

bone marrow (35). Omitting lesions containing normal bone marrow from the analysis, FLT-SUV was < 1.0 in benign tumors and a threshold of 0.8 for FLT-SUV differentiated benign from malignant tumors. This finding further supports the hypothesis that imaging proliferation may be more specific compared with FDG-PET (42, 43).

In the majority of the patient collective, a direct comparison of both FLT- and FDG-PET was possible. Whereas a threshold of 3.4 for FDG-SUV also reliably differentiated benign from malignant tumors, linear regression analysis indicated no significant correlation of the FDG uptake to the tumor grade (Fig. 4). Several authors reported significantly higher uptake of FDG in high-grade compared with low-grade sarcomas, whereas benign and grade 1 sarcomas could not be reliably differentiated (7, 9, 10, 12, 13, 19). A study comprising a mixed population of 89 bone and soft tissue sarcomas also showed a significant correlation of FDG uptake and tumor grading (44). However, an overlap of FDG uptake in low- and high-grade tumors was obvious in a considerable number of patients. The comparison of FLT- to FDG-PET in the current study is limited by the number of patients. Furthermore, FDG uptake for grade 1 and 2 tumors was higher compared with previous studies. Therefore, a clinical study including a higher patient number is warranted to further show the superiority of FLT over FDG.

The rationale to use FLT as a surrogate marker for cellular proliferation is based on its substrate specificity for the cell cycle-regulated protein TK1. Barthel et al. (45) recently reported that *in vivo* uptake of FLT is closely related to TK1 activity and cellular concentration of ATP. However, FLT acts as chain terminator and is therefore not incorporated into DNA and, hence, not a direct measure of proliferation (46). Recent publications indicate that the metabolism of FLT is complex, especially in a posttherapy situation (47–52). Kenny and coworkers (48) have found a heterogeneous pattern of FLT

Fig. 4. A, scattergram of mean and maximum FLT-SUV values (C) in biopsied lesions obtained from benign (five patients), grade 1 (two patients), grade 2 (six patients), and grade 3 tumors (seven patients). Significantly higher FLT uptake in high grade (grades 2 and 3) compared with low grade (grade 1) and benign bone or soft tissue tumors ($P < 0.0001$). Linear regression analysis indicated a significant correlation between FLT uptake in biopsied lesions (mean or maximum FLT-SUV) and tumor grading ($P < 0.001$). B and D, scattergram of corresponding mean (B) and maximum FDG-SUV values (D) obtained from four benign tumors, one in grade 1, five in grade 2, and five in grade 3 tumors, indicating no significant correlation of FDG-SUV and tumor grading (mean FDG-SUV, $P = 0.97$; maximum FDG-SUV, $P = 0.96$).



uptake in primary tumors and metastases from breast cancer. Heterogeneity of tumors potentially explains the relatively high variability of FLT uptake also observed in our study (Figs. 1 and 2).

In daily clinical practice, discrimination between benign and malignant tumors is based on histologic analysis of biopsied lesions including mitotic Fig. counting and immunostaining of e.g., proliferating cells using the Ki-67 specific antibody MIB-1. However, the prognostic value of proliferation fraction as assessed by immunohistochemistry remains a matter of debate. A biopsy is required in all cases but is suggested to be representative for all lesions only in patients with high-grade sarcoma. In patients with low-grade sarcoma, the heterogeneity of tumor proliferation can frequently not be identified except when biopsies from several tumor manifestation sites are done. Therefore, transformation to a more aggressive histology may be underestimated (Fig. 3). In patients with low-grade sarcoma, whole body FLT-PET may indicate progression in areas with increased FLT-uptake and guide biopsy for further verification.

Several limitations have to be taken into account when transferring our results to the clinic. Our findings apply to a particular patient collective that does not cover all subtypes of bone or soft tissue tumors. Furthermore, SUV values were used to semiquantitatively assess the amount of tracer uptake in tumor manifestations. Uptake in small lesions may be underestimated due to partial volume effects. In the present series, lesions for which SUV calculation has been done had a size larger than 20 mm in all patients. However, partial volume

effects may influence the diagnostic accuracy of FLT-PET in a larger series. Moreover, calculation of SUVs can vary substantially between institutions and cannot be assigned to other centers. As previously reported for the standard radiotracer FDG, false-positive findings may also occur at FLT-PET because an increased proliferation rate is not specific for malignant tumors. Due to physiologically high FLT uptake in bone marrow, differentiation of benign from malignant tumors may be problematic and detection of tumor manifestations in central bone structures may be prevented.

In conclusion, the thymidine analogue FLT is suitable for imaging malignant soft and bone tissue tumors. Molecular imaging of proliferation with FLT-PET seems also superior for noninvasive assessment of tumor grading compared with the standard radiotracer FDG. These promising results warrant analysis in a larger series comprising a greater variety of tumor entities.

Disclosure of Potential Conflicts of Interest

M. Juweid has a major relationship with Synarc, Inc., San Francisco, CA.

Acknowledgments

We thank our colleagues Petra Watzlowik, Karin Kantke, Michael Herz, and Andreas Vogt for their excellent contributions; and Christine Praus and our technical staff members Brigitte Dzewas, Coletta Kruschke, Peter Birkmaier, and Juliane Hirling for the support.

References

- Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin* 1999;49:8–31.
- Coindre JM, Terrier P, Guillou L, et al. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. *Cancer* 2001;91:1914–26.
- Panicek DM, Gatsonis C, Rosenthal DI, et al. CT and MR imaging in the local staging of primary malignant musculoskeletal neoplasms: Report of the Radiology Diagnostic Oncology Group. *Radiology* 1997;202:237–46.
- Madewell JE, Ragsdale BD, Sweet DE. Radiologic and pathologic analysis of solitary bone lesions. Part I: internal margins. *Radiol Clin North Am* 1981;19:715–48.
- Pettersson H, Springfield D, Enneking WF. Radiologic management of musculoskeletal tumors. Philadelphia: Springer Publishers; 1999.
- Kern KA, Brunetti A, Norton JA, et al. Metabolic imaging of human extremity musculoskeletal tumors by PET. *J Nucl Med* 1988;29:181–6.
- Adler LP, Blair HF, Markley JT, et al. Noninvasive grading of musculoskeletal tumors using PET. *J Nucl Med* 1991;32:1508–12.
- Kole AC, Plaat BE, Hoekstra HJ, Vaalburg W, Molenaar WM. FDG and L-[1-¹¹C]-tyrosine imaging of soft-tissue tumors before and after therapy. *J Nucl Med* 1999;40:381–6.
- Eary JF, Conrad EU, Bruckner JD, et al. Quantitative [F-18]fluorodeoxyglucose positron emission tomography in pretreatment and grading of sarcoma. *Clin Cancer Res* 1998;4:1215–20.
- Aoki J, Watanabe H, Shinozaki T, et al. FDG PET of primary benign and malignant bone tumors: standardized uptake value in 52 lesions. *Radiology* 2001;219:774–7.
- Lucas JD, O'Doherty MJ, Cronin BF, et al. Prospective evaluation of soft tissue masses and sarcomas using fluorodeoxyglucose positron emission tomography. *Br J Surg* 1999;86:550–6.
- Schulte M, Brecht-Krauss D, Heymer B, et al. Fluorodeoxyglucose positron emission tomography of soft tissue tumours: is a non-invasive determination of biological activity possible? *Eur J Nucl Med* 1999;26:599–605.
- Schulte M, Brecht-Kraus D, Heymer B, et al. Grading of tumors and tumorlike lesions of bone: evaluation by FDG-PET. *J Nucl Med* 2000;41:1695–701.
- Franzius C, Daldrup-Link HE, Sciuk J, et al. FDG-PET for detection of pulmonary metastases from malignant primary bone tumors: comparison with spiral CT. *Ann Oncol* 2001;12:479–86.
- Tateishi U, Yamaguchi U, Seki K, et al. Glut-1 expression and enhanced glucose metabolism are associated with tumour grade in bone and soft tissue sarcomas: a prospective evaluation by [18F]fluorodeoxyglucose positron emission tomography. *Eur J Nucl Med Mol Imaging* 2006;33:683–91.
- Brenner W, Conrad EU, Eary JF. FDG PET imaging for grading and prediction of outcome in chondrosarcoma patients. *Eur J Nucl Med Mol Imaging* 2004;31:189–95.
- Hawkins DS, Schuetz SM, Butrynski JE, et al. [18F]Fluorodeoxyglucose positron emission tomography predicts outcome for Ewing sarcoma family of tumors. *J Clin Oncol* 2005;23:8828–34.
- Schwarzbach MH, Hinz U, Dimitrakopoulou-Strauss A, et al. Prognostic significance of preoperative [18-F] fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging in patients with resectable soft tissue sarcomas. *Ann Surg* 2005;241:286–94.
- Ioannidis JP, Lau J. 18F-FDG PET for the diagnosis and grading of soft-tissue sarcoma: a meta-analysis. *J Nucl Med* 2003;44:717–24.
- Eary JF, O'Sullivan F, Powitan Y, et al. Sarcoma tumor FDG uptake measured by PET and patient outcome: a retrospective analysis. *Eur J Nucl Med Mol Imaging* 2002;29:1149–54.
- Bastiaannet E, Groen H, Jager PL, et al. The value of FDG-PET in the detection, grading and response to therapy of soft tissue and bone sarcomas; a systematic review and meta-analysis. *Cancer Treat Rev* 2004;30:83–101.
- Kubota R, Kubota K, Yamada S, et al. Microautoradiographic study for the differentiation of intratumoral macrophages, granulation tissues and cancer cells by the dynamics of fluorine-18-fluorodeoxyglucose uptake. *J Nucl Med* 1994;35:104–12.
- Shreve PD, Anzai Y, Wahl RL. Pitfalls in oncologic diagnosis with FDG PET imaging: physiologic and benign variants. *Radiographics* 1999;19:61–77.
- Schuetz SM, Rubin BP, Vernon C, et al. Use of positron emission tomography in localized extremity soft tissue sarcoma treated with neoadjuvant chemotherapy. *Cancer* 2005;103:339–48.
- van Ginkel RJ, Hoekstra HJ, Pruim J, et al. FDG-PET to evaluate response to hyperthermic isolated limb perfusion for locally advanced soft-tissue sarcoma. *J Nucl Med* 1996;37:984–90.
- Stroobants S, Goeminne J, Seegers M, et al. 18FDG positron emission tomography for the early prediction of response in advanced soft tissue sarcoma treated with imatinib mesylate (Glivec). *Eur J Cancer* 2003;39:2012–20.
- Schulte M, Brecht-Krauss D, Werner M, et al. Evaluation of neoadjuvant therapy response of osteogenic sarcoma using FDG PET. *J Nucl Med* 1999;40:1637–43.
- Franzius C, Sciuk J, Brinkschmidt C, Jurgens H, Schober O. Evaluation of chemotherapy response in primary bone tumors with F-18 FDG positron emission tomography compared with histologically assessed tumor necrosis. *Clin Nucl Med* 2000;25:874–81.
- Jones DN, McCowage GB, Sostman HD, et al.

- Monitoring of neoadjuvant therapy response of soft-tissue and musculoskeletal sarcoma using fluorine-18-FDG PET. *J Nucl Med* 1996;37:1438–44.
30. Hawkins DS, Rajendran JG, Conrad EU, Bruckner JD, Eary JF. Evaluation of chemotherapy response in pediatric bone sarcomas by [¹⁸F]-fluorodeoxy-D-glucose positron emission tomography. *Cancer* 2002;94:3277–84.
 31. Reske SN, Kotzerke J. FDG-PET for clinical use. Results of the 3rd German Interdisciplinary Consensus Conference, Onko-PET III, 21 July and 19 September 2000. *Eur J Nucl Med* 2001;28:1707–23.
 32. Gambhir SS, Czernin J, Schwimmer J, Silverman DH, Coleman RE, Phelps ME. A tabulated summary of the FDG PET literature. *J Nucl Med* 2001;42:1–93S.
 33. Gupta N, Gill H, Graeber G, et al. Dynamic positron emission tomography with F18 fluorodeoxyglucose imaging in differentiation of benign from malignant lung/mediastinal lesions. *Chest* 1998;114:1105–11.
 34. Shields AF, Grierson JR, Dohmen BM, et al. Imaging proliferation *in vivo* with [¹⁸F]FLT and positron emission tomography. *Nat Med* 1998;4:1334–6.
 35. Buck AK, Bommer M, Stilgenbauer S, et al. Molecular imaging of proliferation in malignant lymphoma. *Cancer Res* 2006;66:11055–61.
 36. Cobben DC, Elsinga PH, Suurmeijer AJ, et al. Detection and grading of soft tissue sarcomas of the extremities with 18F-3'-fluoro-3'-deoxy-L-thymidine. *Clin Cancer Res* 2004;10:1685–90.
 37. Fletcher CDM, Unni K, Mertens F. WHO classification of tumors. Pathology and genetics of tumour of soft tissue and bone. Lyon: IARC press; 2002.
 38. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. *J Clin Oncol* 1997;15:350–62.
 39. Machulla HJ, Blocher A, Kuntzsch M, et al. Simplified labeling approach for synthesizing 3'-deoxy-3'-[¹⁸F]fluorothymidine ([¹⁸F]FLT). *J Radioanal Nucl Chem* 2000;24:843–6.
 40. Herrmann K, Wieder HA, Buck AK, et al. Early response assessment using 3'-deoxy-3'-[¹⁸F]fluorothymidine-positron emission tomography in high-grade non-Hodgkin's lymphoma. *Clin Cancer Res* 2007;13:3552–8.
 41. von Schulthess GK, Steinert HC, Hany TF. Integrated PET/CT: current applications and future directions. *Radiology* 2006;238:405–22.
 42. van Waarde A, Cobben DC, Suurmeijer AJ, et al. Selectivity of [¹⁸F]FLT and [¹⁸F]FDG for differentiating tumor from inflammation in a rodent model. *J Nucl Med* 2004;45:695–700.
 43. Buck AK, Halter G, Schirmeister H, et al. Imaging proliferation in lung tumors with PET: [¹⁸F]FLT versus [¹⁸F]FDG. *J Nucl Med* 2003;44:1426–31.
 44. Folpe AL, Lyles RH, Sprouse JT, Conrad EU III, Eary JF. (F-18) fluorodeoxyglucose positron emission tomography as a predictor of pathologic grade and other prognostic variables in bone and soft tissue sarcoma. *Clin Cancer Res* 2000;6:1279–87.
 45. Barthel H, Perumal M, Latigo J, et al. The uptake of 3'-deoxy-3'-[¹⁸F]fluorothymidine into L5178Y tumours *in vivo* is dependent on thymidine kinase 1 protein levels. *Eur J Nucl Med Mol Imaging* 2005;32:257–63.
 46. Shields AF, Briston DA, Chandupatla S, et al. A simplified analysis of [¹⁸F]3'-deoxy-3'-fluorothymidine metabolism and retention. *Eur J Nucl Med Mol Imaging* 2005;259:1269–75.
 47. Perumal M, Pillai RG, Barthel H, et al. Redistribution of nucleoside transporters to the cell membrane provides a novel approach for imaging thymidylate synthase inhibition by positron emission tomography. *Cancer Res* 2006;66:8558–64.
 48. Kenny LM, Vigushin DM, Al-Nahhas A, et al. Quantification of cellular proliferation in tumor and normal tissues of patients with breast cancer by [¹⁸F]fluorothymidine-positron emission tomography imaging: evaluation of analytical methods. *Cancer Res* 2005;65:10104–12.
 49. Muzi M, Mankoff DA, Grierson JR, Wells JM, Vesselle H, Krohn KA. Kinetic modeling of 3'-deoxy-3'-fluorothymidine in somatic tumors: mathematical studies. *J Nucl Med* 2005;46:371–80.
 50. Dittmann H, Dohmen BM, Kehlbach R, et al. Early changes in [¹⁸F]FLT uptake after chemotherapy: an experimental study. *Eur J Nucl Med Mol Imaging* 2002;29:1462–9.
 51. Barthel H, Cleij MC, Collingridge DR, et al. 3'-deoxy-3'-[¹⁸F]fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy *in vivo* with positron emission tomography. *Cancer Res* 2003;63:3791–8.
 52. Leyton J, Latigo JR, Perumal M, Dhaliwal H, He Q, Aboagye EO. Early detection of tumor response to chemotherapy by 3'-deoxy-3'-[¹⁸F]fluorothymidine positron emission tomography: the effect of cisplatin on a fibrosarcoma tumor model *in vivo*. *Cancer Res* 2005;65:4202–10.