A Pilot Study to Evaluate 3′-Deoxy-3′-^{18}F-Fluorothymidine PET for Initial and Early Response Imaging in Mantle Cell Lymphoma

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Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma. Proliferation activity is considered an important prognostic marker. Immunohistochemical analysis from core biopsy or lymph node may not represent the proliferation rate. We investigated the in vivo proliferation marker 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) to characterize MCL. **Methods:** Eight untreated MCL patients were recruited prospectively. ^{18}F-FLT PET/CT was performed 45 min after injection of ^{18}F-FLT. ^{18}F-FDG PET/CT served as reference. Mean ^{18}F-FLT standardized uptake values were assessed per lesion and compared with respective ^{18}F-FDG uptake. Correlation of mean ^{18}F-FLT and ^{18}F-FDG uptake in the hottest lesion to Ki67 immunostaining was performed. Five patients underwent repetitive early ^{18}F-FLT PET. **Results:** All lymphoma lesions identified by ^{18}F-FDG PET/CT showed increased ^{18}F-FLT uptake. Semiquantitative analysis revealed a high mean ^{18}F-FLT standardized uptake value of 9.9 (range, 5.5–15.9). Mean ^{18}F-FLT uptake and Ki67 expressions showed a strong positive correlation. **Conclusion:** PET using ^{18}F-FLT as a biomarker for proliferative activity showed a high sensitivity for MCL. ^{18}F-FLT uptake shows a correlation with proliferation. Our results warrant further analysis of ^{18}F-FLT PET in MCL.

Key Words: positron emission tomography; mantle cell lymphoma; ^{18}F-FLT; ^{18}F-FDG

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Mantle cell lymphoma (MCL) is a subtype of B-cell non-Hodgkin lymphoma characterized by the translocation t(11;14)(q13;q32) resulting in nuclear overexpression of cyclin D1 in most patients. In addition to the constitutive expression of the cell cycle regulatory protein cyclin D1, various aberrations in apoptotic and DNA damage response pathways have been reported. At initial diagnosis, most patients present with an advanced disease stage (Ann Arbor stage III or IV). Despite major advances in the clinical management of MCL, including treatment with monoclonal anti-CD20–specific antibodies (rituximab), high-dose cytarabin, and consolidation myeloablative therapy followed by hematopoietic stem cell support, MCL is still considered a noncurable disease (*1–4*).

^{18}F-FDG PET is a noninvasive imaging technique that is suggested for posttreatment imaging and has been proven useful for routine staging or interim assessment of diffuse large B-cell lymphoma and Hodgkin lymphoma (*5–8*). In MCL, the sensitivity of ^{18}F-FDG PET is close to 100%, but ^{18}F-FDG PET has not yet proven to be beneficial for either response assessment or posttreatment surveillance (*9–11*). Introduction of the thymidine analog 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT), a PET tracer derived from the cytostatic drug azidovudine, allows in vivo imaging of proliferating tissues and malignant tumors (*12*). Here, we present a pilot study that evaluates initial and early interim ^{18}F-FLT PET and provide evidence for the suitability of ^{18}F-FLT as an imaging biomarker for noninvasive characterization of MCL.

MATERIALS AND METHODS

Patients and Clinical Data

This study was approved by the ethics committee of the Medical Faculty of Technische Universität München. Eight patients met the inclusion criteria (first diagnosis of MCL, indication for systemic treatment, age ≥ 18 y, and full contractual capability) and were included after signing the informed consent form. According to the initial staging, 1 patient presented with stage II disease and 7 patients with stage IV disease, as indicated by the reference methods (clinical evaluation, bone marrow biopsy, and CT scan). The MCL international prognostic index (MIPI) score was calculated as described earlier (*13*). Patient characteristics are shown in Table 1.

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TABLE 1
Patient Characteristics (n = 8)

Patient no.	Age (y)	Sex	Stage (Ann Arbor)	Histology (*14*)	Treatment	Ki67 (%)	MIPI score	MIPI-Ki67score	SUVmax		SUVmax ^{18}F-FLT	
									^{18}F-FDG	^{18}F-FLT	Mean ± SD	Range
1	87	Male	IVAE	Classic	R	35	6.6 (high)	7.3 (high)	NA	NA	NA	
2	71	Male	IVAE	Classic	R-Benda	10	6.1 (intermediate)	6.3 (intermediate)	4.0	6.2	3.9 ± 2.1	2.0–6.2
3	80	Female	IIA	Classic	R-Benda	5	7.1 (high)	7.2 (high)	10.7	8.6	8.2 ± 0.7	7.7–8.6
4	65	Male	IVBE	Classic	MCL-2	10	6.0 (intermediate)	6.2 (intermediate)	5.6	7.7	5.8 ± 1.2	4.5–7.7
5	75	Female	IVB	Blastoid	R-Benda	85	6.2 (intermediate)	8.0 (high)	11.7	19.6	17.6 ± 2.6	14.5–19.6
6	74	Female	IVBE	Classic	R-CHOP	40	7.1 (high)	7.9 (high)	9.6	15.1	11.4 ± 2.2	9.3–15.1
7	47	Male	IVA	Blastoid	MCL-2	80	5.4 (low)	7.1 (high)	11.5	14.5	12.2 ± 2.9	8.2–14.5
8	67	Male	IVA	Classic	R-CHOP	1	6.3 (intermediate)	6.4 (intermediate)	8.5	9.5	7.6 ± 1.3	6.3–9.5

R = rituximab; NA = not applicable in patient with no uptake; R-Benda = rituximab, bendamustin; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; MCL-2 (analog) = rituximab-maxiCHOP, alternating with rituximab high-dose cytarabin (*3*).

Histology and Immunohistochemistry

Lymphomas were classified according to the updated World Health Organization classification system (*14*). Slides of 5- to 6-μm sections cut from formalin-fixed paraffin-embedded tissues were deparaffinized and stained with hematoxylin and eosin (Dako), dehydrated, and then covered with a coverslip. For immunohistochemistry, 2-μm sections were deparaffinized. Antigen retrieval was performed by pressure cooking in citrate buffer (pH 6) for 7 min. All 8 patients were cyclin D1–positive as assessed by immunohistochemistry (clone sp4; DCS Innovative Diagnostik-Systeme). Proliferation was analyzed using the proliferation marker Ki67 (monoclonal antibody clone MIB-1; Dako). Two independent hematopathologists analyzed high-power fields (×40) of each primary MCL.

Imaging and Data Analysis

Baseline ^{18}F-FLT PET and ^{18}F-FDG PET/CT examinations were performed within 1 wk before therapy, together with routine staging modalities (clinical examination, CT, bone marrow biopsy). ^{18}F-FLT PET was repeated in 5 patients at an average of 6.2 d (median, 6.0; range, 5.0–7.0 d) after the start of the first course of immunochemotherapy. ^{18}F-FLT was synthesized as previously described (*15*). PET was performed 45 min after injection of approximately 300 MBq of ^{18}F-FLT (range, 270–340 MBq) as previously described (*16*). All PET scans were evaluated by 2 observers unaware of the clinical data and the results of other imaging studies. Circular regions of interest (diameter, 1.5 cm) were placed in the area with the highest tumor activity, as previously published (*17*). Mean standardized uptake values (SUVs) were calculated from each region of interest using the formula SUV = measured activity concentration (Bq/g) × body weight (g)/injected activity (Bq).

Statistical Analysis

Statistical analyses were performed using PASW Statistics software (version 18.0; SPSS, Inc.). Because of the small sample size and the rather explorative nature of the study, no formal tests were conducted. The arithmetic mean, median, and range were reported for description of quantitative data. Correlation coefficients according to Pearson (r) or, if appropriate, according to Spearman (ρ), were calculated with 95% confidence intervals.

RESULTS

^{18}F-FLT Uptake Values in MCL and Correlation with ^{18}F-FDG Uptake Parameters

In 7 of 8 patients ^{18}F-FLT PET (Fig. 1, representative images) and ^{18}F-FDG PET scans showed increased uptake, whereas, after removal of a single lymph node, 1 patient showed no residual lymphoma tissue on PET. Initial mean uptake of ^{18}F-FLT in lymphoma manifestations (mean ^{18}F-FLT SUV_{mean}) was 9.9 (median, 8.2; range, 5.5–15.9). Corresponding maximum ^{18}F-FLT uptake values ranged from 6.2 to 19.6, resulting in a mean of maximum SUV (SUV_{max}) of 11.6 (median, 9.5). Corresponding uptake values for the baseline ^{18}F-FDG PET scan were 7.4 for mean SUV_{mean} (median, 7.6; range, 3.0–10.3) and 8.8 for mean SUV_{max} (median, 9.6; range, 4.0–11.7). Mean SUV_{mean} and mean SUV_{max} were higher for ^{18}F-FLT than for ^{18}F-FDG, reaching statistical significance only for SUV_{mean} (P = 0.043

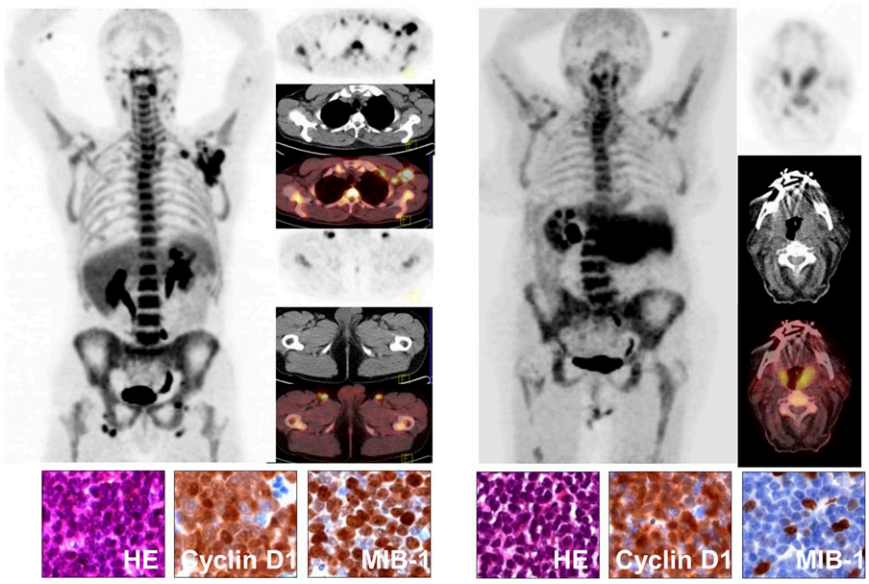

FIGURE 1. (Left) ^{18}F-FLT PET images of patient 7 with blastoid MCL. Representative hematoxylin and eosin staining, cyclin D1, and Ki67 (MIB-1) immunohistochemistry (80% positivity). (Right) ^{18}F-FLT PET images of patient 4 with classic MCL. Representative hematoxylin and eosin staining, cyclin D1, and Ki67 (MIB-1) immunohistochemistry (10% positivity). HE = hematoxylin and eosin.

and 0.051, respectively). Correlation of ^{18}F-FLT and ^{18}F-FDG uptake values was revealed to be strongly positive (Spearman ρ, +0.76; 95% confidence interval [CI], +0.01 to +0.96; Fig. 2A). Up to 5 lesions in every patient were measured, revealing a heterogeneous uptake pattern (Table 1). However, intrapatient variability of uptake values appeared to be less than the interpatient variability.

Correlation of ^{18}F-FLT Uptake Parameters to Ki67 Expression and MIPI Score

Ki67 immunohistochemistry was performed in all PET-positive patients (n = 7). Ki67-positive lymphoma cells ranged between 1% and 85% (mean, 33%; median, 10%). Correlation analysis between initial ^{18}F-FLT uptake and Ki67 proliferation index showed a strong correlation, namely higher Ki67 proliferation in patients with higher initial ^{18}F-FLT uptake values (Pearson r, +0.91; 95% CI, +0.50 to +0.99). The MIPI ranged from 5.4 to 7.1 (mean, 6.4; median, 6.3), and the MIPI including Ki67 (MIPI-Ki67) ranged from 6.2 to 8.0 (mean, 7.1; median, 7.2). Mean initial ^{18}F-FLT uptake values and MIPI-Ki67 showed a strong positive correlation (Pearson r, +0.84; 95% CI, +0.25 to +0.98; Fig. 2B). There was no considerable correlation between ^{18}F-FLT uptake and MIPI (Spearman ρ, +0.14; 95% CI, −0.69 to +0.81).

Early Response Assessment by ^{18}F-FLT Uptake Parameters

Five patients participated in the early response assessment phase of this study. One week after the start of treatment, ^{18}F-FLT uptake showed a mean SUV_{mean} decrease of 45% (range, −15% to 96%). The corresponding mean SUV_{max} decrease was 44% (range, −15% to 94%). We observed a heterogeneous change in ^{18}F-FLT uptake, with an SUV decrease greater than 80% in 2 patients (SUV_{mean}, 84% and 96%; SUV_{max}, 84% and 94%), whereas in 2 patients ^{18}F-FLT uptake decreased by only 20% and 38% for SUV_{mean} (25% and 35% for SUV_{max}, respectively, Fig. 3), and in 1 patient the uptake even increased (15% each for SUV_{mean} and SUV_{max}, respectively).

Clinical Response Assessment

One patient received rituximab monotherapy. All other patients received combined immunochemotherapy (Table 1). All patients responded to antibody therapy or immunochemotherapy (with 6 patients achieving complete response as assessed by conventional CT staging and 2 patients with a partial response, 1 after cycle 6 and 1 after cycle 3, with therapy ongoing). Because of the low number or patients, no correlation between ^{18}F-FLT or MIPI and response was assessed.

DISCUSSION

All evaluable patients presented with intense uptake of the radionucleoside ^{18}F-FLT, and all of the MCL lesions identified by conventional imaging modalities (spiral CT, ^{18}F-FDG PET/CT) were visible by ^{18}F-FLT PET. The good visibility of aggressive lymphoma by ^{18}F-FLT PET is in agreement with several previously published trials in other subentities (*17,18*). Several studies have analyzed the ^{18}F-FDG PET avidity of MCL (*9,10*). These reports indicate that the sensitivity in detecting bone marrow involvement is rather low (*9*), and our results indicate that this is even more the case for ^{18}F-FLT PET.

A combined clinical and biologic score (MIPI) has recently been established that allows a reliable estimation of the individual clinical course. In addition, cell proliferation assessment (Ki67) is a strong prognostic marker (*13*). These results confirmed a previous transcriptome analysis that identified a predictive 20-gene proliferation signature (*19*). Even in this small pilot study, we were able to detect a strong positive correlation between proliferation, as assessed by Ki67 staining or MIPI-Ki67, and ^{18}F-FLT

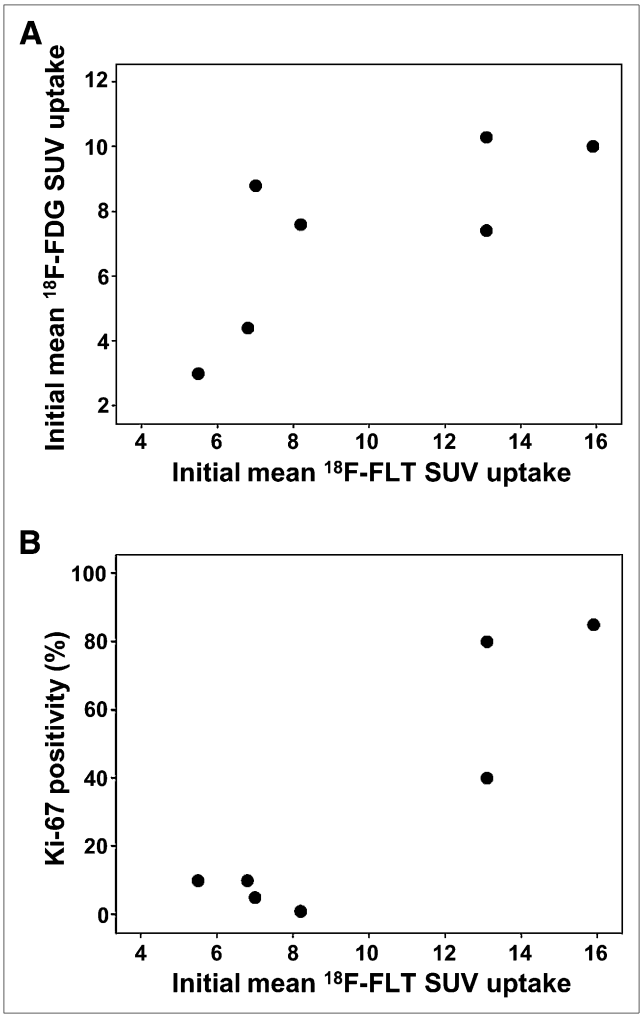

FIGURE 2. Correlation between initial mean ^{18}F-FLT SUV_{mean} and initial mean ^{18}F-FDG-SUV_{mean} uptake (A) and initial mean ^{18}F-FLT SUV_{mean} uptake and Ki67 positivity (%) (B).

uptake. This correlation indicates that ^{18}F-FLT PET is a proper sensitive tool to estimate the proliferative activity of MCL, which has been shown earlier for ^{18}F-FDG PET when comparing blastoid versus classic MCL (*9*).

Four of 5 patients who entered the early response assessment part of the study showed a substantial decrease in ^{18}F-FLT uptake. Because of the low number of patients and the short follow-up period, descriptive results have been presented regarding individual changes of the ^{18}F-FLT SUV early after start of therapy. Because all patients in this study responded to treatment, we cannot at this point come to a conclusion on the predictive value of ^{18}F-FLT PET for response assessment. Negativity for minimal residual disease (MRD) assessed by polymerase chain reaction after 3–4 cycles of immunochemotherapy has recently been shown to be a highly predictive marker for progression-free survival (*20*). A positive correlation between early ^{18}F-FLT PET responses and MRD negativity at midterm may allow the establishment of the clinical value of this imaging technique in MCL and may in the long term lead to therapeutic changes based on imaging results.

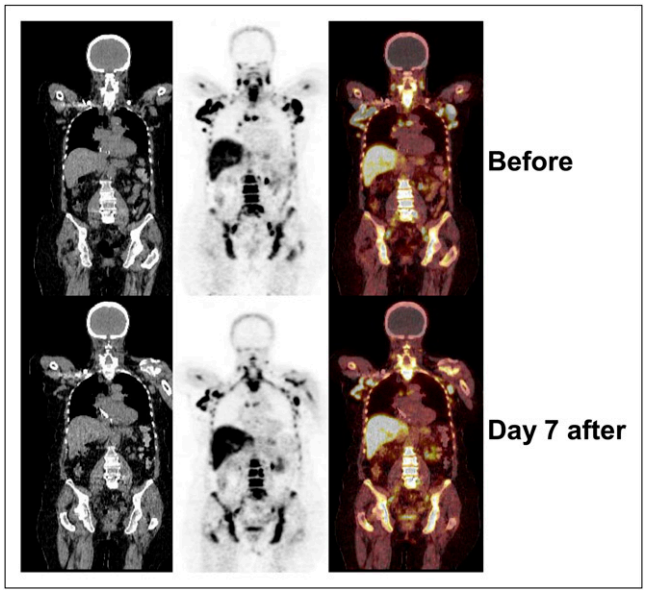

FIGURE 3. Early response assessment by ^{18}F-FLT PET. Shown are images of patient 5 before initiation of immunochemotherapy and at day 7 after initiation of chemotherapy. SUV_{mean} and SUV_{max} decreased by 38% and 35%, respectively.

CONCLUSION

Our data demonstrate that ^{18}F-FLT PET is a promising and sensitive tool for the detection of MCL lesions. Even in our limited-patient-number study, we detected a strong positive correlation (lower 95% confidence limit, modest positive correlation) of ^{18}F-FLT uptake and proliferation assessed by Ki67 staining. Our data justify further evaluation in larger cohorts, especially with regard to response and the predictive MRD levels to integrate initial or interim ^{18}F-FLT PET as a predictive tool in the clinical management of MCL patients.

DISCLOSURE STATEMENT

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

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