

# Monitoring Tumor Response to Therapy with $^{18}\text{F}$ -FLT PET

**I**maging tumor cell proliferation is of high interest for both research and clinical practice in oncology. High proliferation rates are characteristic of most malignant tumors, whereas in benign tumors the fraction of proliferating cells is comparatively small. Therefore, imaging of proliferation may provide a sensitive and specific tool for differentiation of benign and malignant tumors. Furthermore, proliferation imaging may be used for detection of metastatic disease in patients with known cancer. Because many benign lesions that mimic metastases

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on morphologic imaging demonstrate low proliferation rates or absence of proliferation, imaging of proliferation has the potential to significantly improve the accuracy of cancer staging.

Finally, radiotherapy and chemotherapy rapidly decrease proliferation rates in responding tumors. This effect usually precedes a reduction in tumor size. Imaging of cellular proliferation may therefore provide an earlier readout for therapeutic effects than do size measurements by CT or MRI. Many novel targeted agents, such as protein kinase inhibitors, have a predominantly cytostatic effect and do not cause rapid tumor shrinkage. The beneficial effects of these therapies

may therefore be underestimated by conventional response criteria such as the Response Evaluation Criteria in Solid Tumors, which are based on a significant reduction of tumor size within a few weeks of therapy. Proliferation imaging is therefore of enormous interest for the further clinical development of these novel therapeutic agents.

## IMAGING OF TUMOR CELL PROLIFERATION

Because of these applications for detection and staging of cancer and for monitoring of therapy, imaging of tumor proliferation with PET and SPECT has been studied with various imaging probes. In this context, thymidine and thymidine analogs are of special interest, because thymidine is the only nucleoside that is incorporated into DNA but not RNA. Radio-labeled thymidine has therefore been used for many years to study cellular proliferation *in vitro*.

By far the most extensively studied probe for imaging of cellular proliferation is 3'-deoxy-3'- $^{18}\text{F}$ -fluorothymidine ( $^{18}\text{F}$ -FLT). PET with  $^{18}\text{F}$ -FLT was first reported in 1998 by Shields et al. (1). Subsequent studies have shown that  $^{18}\text{F}$ -FLT flux as measured by dynamic PET studies and by  $^{18}\text{F}$ -FLT uptake at a fixed time after injection correlates reasonably well with histopathologic markers of tumor cell proliferation, such as the Ki-67 labeling index. Furthermore, it has been demonstrated that  $^{18}\text{F}$ -FLT uptake is significantly better than  $^{18}\text{F}$ -FDG uptake as a measure of tumor proliferation (2,3).  $^{18}\text{F}$ -FLT PET has also been shown to be more specific than  $^{18}\text{F}$ -FDG PET for cancer staging, with fewer false-positive findings in inflammatory lesions (3–5). However, some false-positive findings have been reported for  $^{18}\text{F}$ -FLT

because of proliferation of lymphocytes in reactive lymph nodes (6). Comparisons of  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FLT have also made it clear that tumor uptake of  $^{18}\text{F}$ -FLT (as measured by standardized uptake values) is only about half that of  $^{18}\text{F}$ -FDG. For tumor staging, the result is a significantly lower sensitivity for  $^{18}\text{F}$ -FLT-PET than for  $^{18}\text{F}$ -FDG PET (3–5,7,8).

## EXPERIENCE WITH $^{18}\text{F}$ -FLT PET FOR TREATMENT MONITORING

Because of these limitations of  $^{18}\text{F}$ -FLT PET for tumor staging, monitoring tumor response to therapy is perhaps the most promising clinical application of  $^{18}\text{F}$ -FLT PET. A series of animal studies has indicated that  $^{18}\text{F}$ -FLT uptake decreases rapidly in response to radiotherapy (9,10), cytotoxic chemotherapy (11,12), and various protein kinase inhibitors (13–16). In some (9,11,12,15) but not all of these studies (10,13,14), changes in  $^{18}\text{F}$ -FLT uptake better reflected the effects of therapy than did changes in  $^{18}\text{F}$ -FDG uptake. Initial patient studies have further emphasized the potential of  $^{18}\text{F}$ -FLT PET for monitoring tumor response to therapy.  $^{18}\text{F}$ -FLT uptake in untreated tumors has been shown to be stable over time, with a test–retest reproducibility of less than 10% for standardized uptake values when patients were imaged twice within a week (17,18). In 19 patients with recurrent malignant brain tumors treated with irinotecan and bevacizumab, tumor response on  $^{18}\text{F}$ -FLT PET after 1–2 wk of therapy correlated with overall survival at a high level of statistical significance. In contrast, tumor response on MRI was not a significant predictor of survival in this study (19). Pilot studies in breast cancer have also suggested that

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changes in  $^{18}\text{F}$ -FLT uptake during chemotherapy predict later clinical response (20,21). Encouraging data have been reported for epidermal growth factor receptor kinase inhibition with gefitinib in the monitoring of treatment with protein kinase inhibitors (22). In 31 patients with non-small cell lung cancer, the reduction of  $^{18}\text{F}$ -FLT uptake after 7 d of therapy with gefitinib was highly predictive of tumor response on CT at 6 wk and progression-free survival (22).

In other tumor types and for other forms of therapy, however, the correlation between early changes in  $^{18}\text{F}$ -FLT uptake and later clinical or histopathologic response has been less clear. In malignant lymphomas, treatment with rituximab did not cause an early change in  $^{18}\text{F}$ -FLT uptake (23), although experimental data suggest that rituximab inhibits proliferation by interfering with cellular signaling (24). Thus, not all forms of growth inhibition may be captured by  $^{18}\text{F}$ -FLT PET. Conversely, tumor  $^{18}\text{F}$ -FLT uptake has been reported to change significantly in eventually nonresponding tumors. In 10 patients with rectal cancer treated with neoadjuvant chemoradiation,  $^{18}\text{F}$ -FLT uptake significantly decreased at day 14 in all patients. However, the reduction in  $^{18}\text{F}$ -FLT uptake was not different for histopathologically responding and nonresponding tumors (25), suggesting that inhibition of proliferation may be necessary but not sufficient for a favorable response in some tumor types or some forms of treatment. Alternatively,  $^{18}\text{F}$ -FLT uptake might be influenced by factors other than proliferation, such as changes in vascular permeability or perfusion. Finally, a temporary rise in  $^{18}\text{F}$ -FLT uptake has been reported in lung cancer patients receiving chemoradiation (26).

In summary, the findings of these pilot studies suggest that disease- and drug-specific effects need to be considered when  $^{18}\text{F}$ -FLT PET is used for treatment monitoring. A need for further and larger studies of various tumor types is indicated. Furthermore,

treatment monitoring with  $^{18}\text{F}$ -FLT PET and with  $^{18}\text{F}$ -FDG PET should be compared, since  $^{18}\text{F}$ -FDG PET has been used successfully to monitor tumor response in lymphoma and a variety of solid tumors.

#### **$^{18}\text{F}$ -FLT AND $^{18}\text{F}$ -FDG PET IN METASTATIC GCT**

In this context, Pfannenberget al. (27) report in this issue of *The Journal of Nuclear Medicine* the results of an important study comparing  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FLT PET for monitoring tumor response to chemotherapy in metastatic germ cell tumors (GCTs). GCTs are generally classified as seminomas or nonseminomas; each of these subtypes comprises approximately 50% of cases (28). Many patients with advanced GCT respond favorably to chemotherapy, but residual masses frequently remain after completion of therapy. These may represent necrotic tissue or viable tumor. In nonseminomatous GCT, metastases may also differentiate into mature teratomas. These tumors are chemotherapy-resistant but may grow over time or dedifferentiate into a malignant tumor. Therefore, complete resection represents the only curative treatment for teratomas. Differentiation of purely necrotic tissue on the one hand from viable carcinoma or teratoma on the other is an unsolved clinical problem. Patients with only necrotic tissue do not require surgery, whereas those with viable tumor tissue significantly benefit from surgical resection (28). In seminomas,  $^{18}\text{F}$ -FDG PET has been shown to accurately differentiate between viable tumor and necrotic tissue (29). In contrast, the accuracy of  $^{18}\text{F}$ -FDG PET is limited in nonseminomas, since teratomas demonstrate low and variable  $^{18}\text{F}$ -FDG uptake (30). Furthermore, inflammation in residual masses is believed to cause false findings on  $^{18}\text{F}$ -FDG PET.

Pfannenberget al. (27) performed  $^{18}\text{F}$ -FLT PET and  $^{18}\text{F}$ -FDG PET scans on a group of 11 patients with GCT (10 nonseminomas and 1 seminoma).

Patients underwent PET before the start of chemotherapy, after the first chemotherapy cycle, and after completion of chemotherapy. Changes in  $^{18}\text{F}$ -FLT and  $^{18}\text{F}$ -FDG uptake were correlated with histopathologic analysis after resection of residual masses in 7 patients and with clinical follow-up in 4 patients. Based on this reference standard, 6 patients were classified as responders (necrosis on histology or no recurrence during follow-up) and 5 as nonresponders (2 with viable carcinoma, 2 with teratoma, 1 with recurrence during follow-up).

The results of the study are rather sobering. Briefly,  $^{18}\text{F}$ -FLT and  $^{18}\text{F}$ -FDG uptake decreased significantly with therapy, but there were no significant differences between responders and nonresponders. Neither the relative changes in tracer uptake from baseline to the first follow-up scan nor residual tracer uptake after completion of therapy allowed differentiation between responders and nonresponders. Furthermore, tumor  $^{18}\text{F}$ -FLT uptake after therapy did not correlate with Ki-67 labeling in the subgroup of patients who underwent surgical resection.

Pfannenberget al. (27) provide a thoughtful discussion of potential reasons for these unexpected results. Perhaps the findings can best be explained by considering the clinical question and the biology of GCT. Metastatic GCT is treated with curative intent. Overall, long-term cure can be achieved in more than 70% of the patients (30). Accordingly, only patients with a histopathologically complete response are considered responders. The residual tumor in nonresponders may, however, differ markedly from the primary tumor in morphology and functional state. In the 4 histopathologically verified nonresponders reported by Pfannenberget al., 2 tumors had differentiated into teratoma and 1 carcinoma demonstrated a Ki-67 labeling index of only 1%, which indicates extremely slow proliferation. Thus, these 3 tumors demonstrated a marked biologic response to chemotherapy although they did not

meet the criteria for a favorable clinical response.  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FLT uptake decreased rapidly in these tumors and thus well reflected the biologic changes in the tumor tissue. Clinically, however,  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FLT PET were false-positive for response, because viable tumor remained after therapy. Only 1 patient in the study demonstrated viable carcinoma with a high proliferation rate after therapy (Ki-67 index, 70%). This patient showed only minor changes in  $^{18}\text{F}$ -FDG and  $^{18}\text{F}$ -FLT uptake after the first chemotherapy cycle and was accordingly correctly identified as a nonresponder.

The low proliferation rate of most tumors after therapy also explains why there was no correlation between  $^{18}\text{F}$ -FLT uptake after completion of therapy and Ki-67 labeling. Because of the low proliferation rates, tumor  $^{18}\text{F}$ -FLT uptake was only minimally above background levels, making accurate quantification of  $^{18}\text{F}$ -FLT uptake challenging.

Tumor biology and clinical questions should also be considered when the results of Pfannenberget al. (27) are compared with other reports evaluating treatment monitoring with  $^{18}\text{F}$ -FLT or  $^{18}\text{F}$ -FDG PET. In malignant glioma, for example, even a relatively minor delay in tumor growth is considered a favorable response to therapy. Therefore, "response" in a patient with glioblastoma is biologically very different from "response" in a patient with GCT. Consequently, it is perhaps not surprising that the diagnostic performance of  $^{18}\text{F}$ -FLT PET in glioblastoma and GCT are different. Along these lines, one should remember that the criteria of the European Organization for Research and Treatment of Cancer for assessment of tumor response on  $^{18}\text{F}$ -FDG PET (31) were developed with the intent to detect rather minor (subclinical) responses during the development of new chemotherapeutic agents. One should therefore be careful when applying these criteria to treatment regimens that are used with curative intent. In the study by Pfannenberget

et al., all but 1 patient were classified as responders according to these criteria.

## CONCLUSION

The published data indicate that  $^{18}\text{F}$ -FLT has so far been more successful in monitoring palliative therapy with rather limited efficacy (19,21,22) than in monitoring highly effective, potentially curative treatments (25,27). These data may suggest that more effective therapeutic agents inhibit  $^{18}\text{F}$ -FLT uptake in most tumors but that a complete response is achieved in only a subset of patients. If this suggestion is correct, future research should focus on evaluating  $^{18}\text{F}$ -FLT PET in patients undergoing palliative and predominantly cytostatic treatments. However, more studies are necessary to confirm this hypothesis in a larger number of tumor types and for different forms of therapy.

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