Hybrid Imaging PET/CT with Application of ^{18}F -Fluorothymidine in Patients with Head and Neck Carcinoma Undergoing Radiotherapy

JAN BAXA¹, JIŘÍ FERDA¹, EVA FERDOVA¹, RADOVAN VOJTISEK²,
ONDREJ TOPOLCAN³ and JINDRICH FINEK²

*Departments of ¹Imaging Methods, ²Oncology and Radiotherapy, and
³Immunochemistry, Charles University Hospital Pilsen, Pilsen, Czech Republic*

Abstract. *Aim: To introduce the possible benefits of the positron-emission tomography (PET)/computed tomography (CT) with ^{18}F -3'-deoxy-3'-fluorothymidine (^{18}F -FLT) in patients with orofacial carcinomas and its impact to patient management. Materials and Methods: Thirty-six patients with orofacial squamous cell carcinomas underwent ^{18}F -FLT-PET/CT during radiotherapy. Examinations were performed after administration of ^{18}F -FLT (1.8 MBq/kg) including full-diagnostic CT. Analysis of the radiotherapy effect was performed with possible prospect of repeated and focused irradiation. Results: Complete absence of ^{18}F -FLT uptake was found in 20 patients, thus complete response to radiotherapy was reported. Persistence of focal ^{18}F -FLT uptake was observed in 16 patients; in 11 patients, the measured activity was only mild. In five patients, a higher level of ^{18}F -FLT uptake was measured and additional irradiation was performed in defined regions. Repeated follow-up proved complete regression ^{18}F -FLT uptake. Conclusion: It was possible to assess the effect of radiotherapy with the use of ^{18}F -FLT-PET/CT and findings are suitable for radiation dose-escalation planning.*

The differentiation of residual viable tumor tissue from tissues undergoing necrotic processes during anticancer therapy is a crucial task in deciding ongoing strategies for treatment of head and neck cancer. After initial therapeutic irradiation, the radiation dose can be escalated and targeted to the tumor areas not responding sufficiently. Given the fact that reparative processes take place throughout the entire affected area, the

identification of viable tumor tissue is almost impossible by morphological imaging and also very difficult by functional methods, such as magnetic resonance imaging (MRI) or positron-emission tomography (PET)/computed tomography (CT) with ^{18}F -fluorodeoxyglucose (^{18}F -FDG). A relatively new option is the use of 3'-deoxy-3'-[^{18}F]fluorothymidine (^{18}F -FLT) in PET/CT imaging. FLT is a marker of cell proliferative activity and is therefore used to assess cell proliferation in tumor tissues (1). The highest uptake of ^{18}F -FLT was demonstrated in squamous cell carcinomas, which are the predominant type of malignant tumors of the pharynx, oral cavity, tongue and larynx, and which are also common in paranasal sinuses (2, 3).

Our work shows the examination technique and presents the possibilities of using ^{18}F -FLT PET/CT in monitoring the effects of radiotherapy.

Materials and Methods

A total of 36 examinations using ^{18}F -FLT PET/CT in patients with head and neck cancer (mean age=57.3 years, 27 males and nine females) were retrospectively included. All patients had proven orofacial squamous cell carcinoma and underwent therapeutic irradiation. Only patients who underwent complete PET/CT scan were included. The mean interval between end of radiotherapy and PET/CT was 14.3 days.

PET/CT examination. All examinations were performed 30 min after the intravenous administration of ^{18}F -FLT at 1.8 MBq/kg. A total of 21 scans were performed on a PET/CT scanner with a 16-row CT and 3-ring PET subsystems (Biograph 16; Siemens Healthcare, Knoxville, TX, USA). CT data were obtained using a 120 kV protocol and were reconstructed in diagnostic images using a filtered back projection at a 1 mm image width with a 0.7 mm reconstruction increment, with a soft-tissue reconstruction filter. PET data were reconstructed in a 256×256 matrix across the entire 70 cm axial field-of-view by means of iterative ordered subset expectation maximization and 5 mm full width at half maximum (FWHM). Acquisition was performed by the step-and-shoot technique using seven to eight positions, with an acquisition time of 3 minutes per position.

Correspondence to: Jan Baxa, Charles University Teaching Hospital in Pilsen, alej Svobody 80, 306 40 Plzen, Czech Republic. Mob: +420 775080724, Fax: +420 377103438, e-mail: baxaj@fnplzen.cz

Key Words: PET/CT, fluorothymidine, head and neck cancer, radiotherapy.

An additional 15 examinations were performed following the change to a site operating PET/CT 128-row CT and 4-ring PET subsystems (Biograph mCT 128; Siemens Healthcare). CT data were obtained using a 100 kV voltage protocol with the possibility of reducing the voltage in asthenic patients to 80 kV with automatic dose correction. Data were reconstructed into diagnostic images using SAFIRE iterative data reconstruction at a 0.75 mm image width, with a 0.6 mm reconstruction increment and a soft-tissue algorithm. PET data were reconstructed in a 400×400 matrix in axial field-of-view with 46 cm diameter using the ultraHD algorithm, which combines the time of flight (TOF) and point-spread function (PSF) algorithms, and also 2 mm FWHM. Data acquisition was performed by step-and-shoot techniques using five to six positions, with an acquisition time of 1.5 minutes per position.

An intravenous iodine contrast agent was administered to all patients at a concentration of 370 mgI/ml and volume of 80-100 ml according to the patient's weight, and no contraindications for the administration of the contrast agent were found in any of the examined patients. The contrast agent was injected with an overpressure injector at a flow rate of 4 ml/s. The range of examination was limited to that from the level of the sternoclavicular junction to the upper edge of the frontal paranasal cavities - that is, 2-3 table positions for PET acquisitions. In six cases, an extension to the chest and liver was performed.

Image analysis. Evaluation of the examinations was performed in consensus by two radiologists experienced in PET/CT assessment using dedicated software application (MM Oncology, Syngo.Via; Siemens Healthcare, Forchheim, Germany). Separate images from both modalities with possibility of fusion were used (CT data with an isotropic submillimetric resolution in any direction and metabolic uptake data given by the isotropic field of PET data at a resolution of 3 mm in any direction). Signs of the presence of tissue with high proliferative activity in the primary tumor area and post-surgical site after its radical irradiation were evaluated. In the absence of increased FLT accumulation, the finding was evaluated as a complete response to treatment. If the FLT uptake was detectable in the region of tumor tissue, the finding was evaluated as partial response (Figure 1). FLT uptake was quantified using the maximal standard uptake value (SUV).

Results

Using CT, partial or complete response of the primary tumor was demonstrated in all patients. In a total of 20 patients the complete absence of increased level of ^{18}F -FLT uptake was found, thus complete response to the radiotherapy was reported. In 16 patients, persistence of low focal ^{18}F -FLT uptake was observed. In 11 patients, the measured activity was only mild (maximal SUV up to 3.0). In five patients, ^{18}F -FLT uptake was measured as high (maximal SUV over 3.0) and additional focused escalation of irradiation was decided. Repeated follow-up after targeted irradiation were performed in all five patients, all with complete regression of ^{18}F -FLT uptake.

Outside the irradiated area an increased ^{18}F -FLT uptake (mean maximal SUV of 3.8) was found in 11 lymph nodes in seven patients. Of significant findings outside of the

orofacial and cervical area associated with cancer, a brain metastasis (two patients) and pulmonary metastasis (two patients) were found. In three patients, a deep recurrent ulcer was found with the complete absence of ^{18}F -FLT uptake, visually suspicious of tumor recurrence. The subsequent histological evaluation was negative and confirmed the ^{18}F -FLT-PET/CT finding.

Discussion

^{18}F -FLT is a radiolabeled analog of thymidine, a pyrimidine nucleoside of deoxyribonucleic acid (DNA). It is a substance that passes from the bloodstream through the extracellular space to cells by facilitated diffusion when the cells are in the S-phase of the cell cycle, which includes preparation for DNA synthesis and DNA synthesis itself. Cells appearing in the S-phase are characterized by high activity of thymidine kinase 1 (TK1) which catalyzes phosphorylation of ^{18}F -FLT to ^{18}F -FLT-5-phosphate. However, ^{18}F -FLT-5-phosphate is retained, as it is no longer incorporated into the DNA strand. Studies in tissue cultures and in laboratory animal models have shown that the rate of ^{18}F -FLT accumulation is closely correlated with the rate of tissue mitotic activity (4, 5).

In the physiological state, distribution of high ^{18}F -FLT uptake is present in active hematopoietic bone marrow, liver parenchyma, and in regions with rapid regeneration of the mucosal lining, *e.g.* exocrine glands (6, 7). Increased proliferative activity is also seen as increased ^{18}F -FLT uptake in the peripheral germinative zones of activated lymph nodes, and in proliferative involvement of the lung interstitial space, such as in post-radiation fibrosis or interstitial fibrosis of non-specific interstitial pneumonia type.

In tumor tissues, high ^{18}F -FLT uptake has been observed in tissues generally showing high mitotic activity, but the highest FLT accumulation has been shown in squamous cell carcinoma with a high level of epithelial growth factor (EGFR) activity. For this reason, most studies evaluated the use of ^{18}F -FLT-PET or ^{18}F -FLT-PET/CT imaging of squamous cell tumors of the orofacial region, of the lung and of the esophagus (3, 8, 9). In addition to squamous cell tumors, data related to the assessment of brain tumor proliferative activity have also been published showing ^{18}F -FLT-PET contributes to the differentiation of low- from high-grade neuroepithelial tumors. However, ^{18}F -FLT is not able to cross the intact blood-brain barrier in the early phase of distribution (10-12).

In terms of optimal choice, it is very important to compare ^{18}F -FDG and ^{18}F -FLT in tumor tissue assessment because these are two markers of different physiological processes (3, 13). ^{18}F -FLT uptake is a measure of processes in the cell that lead to preparation for cell division, a process that reflects the mitotic activity of tissues. In contrast, ^{18}F -FDG is a substance that reflects the level of oxidative glycolysis in tissues, and

Figure 1. Evolution of the findings in a patient with squamous cell carcinoma of the left piriform recess before (A), during (B), and at 3 (C) and 6 (D) months after completion of radiation therapy. From the interim examination, the highly proliferating tissue of the tumor had disappeared, and proliferation of the bone marrow of the cervical spine stopped in the same time. Upper row: Computed tomographic images, middle row: 3'-deoxy-3'- ^{18}F -fluorothymidine (^{18}F -FLT PET) images, lower row: maximum intensity projection of the head and neck in lateral view using ^{18}F -FLT PET.

thereby the rate and kind of energy metabolism of cells and tissues. These two differences help in understanding the difference in quality and intensity of tissue imaging using ^{18}F -FLT-PET and ^{18}F -FDG-PET. ^{18}F -FLT achieves a lower total uptake rate even in tissues with a high level of proliferation, the maximum level of the uptake being reached in bone marrow and in the liver parenchyma. On the other hand, it usually does not penetrate into the brain or muscles. At the same time, due to a low serum thymidine level, there is virtually no competition with any natural equivalent such as with ^{18}F -FDG. Different physiological processes, thus, lead to differences in the preparation of patients for imaging of orofacial tumors. When using ^{18}F -FLT, it is not necessary to limit the patient's intake of food or sweetened drinks, and there is also no need to restrict speaking and oral hydration during the accumulation phase.

Previously published studies reported a lower sensitivity of ^{18}F -FLT in staging of orofacial and lung tumors, that is caused

particularly by a lower signal-to-noise ratio due to the lower level of uptake in primary tumors and in lymph node metastases (3, 13). Therefore, ^{18}F -FLT-PET/CT is not a suitable method for determining primary staging. When assessing nodal metastases in patients with orofacial squamous cell tumors, ^{18}F -FLT does not address the limited accuracy of ^{18}F -FDG (14). In both methods, false-positive results may be obtained in activated lymph nodes, although through a different principle. It should be noted, however, that ^{18}F -FLT offers a more specific assessment. In the case of a slightly increased FLT uptake in the peripheral layer of smaller nodes, these are more likely nodes with an active germinative zone. Conversely, if a focally increased uptake is detected only in a small central part of the node, it is highly probably focal proliferative activity of metastatic infiltration.

The most significant benefit of ^{18}F -FLT is the early assessment of the response to anticancer therapy (15-18). The prognostic value of reduced ^{18}F -FLT uptake was demonstrated

as early 2 weeks after the initiation of targeted therapy for non-small cell lung cancer. Conversely, an increase of ^{18}F -FLT uptake indicates insufficient therapeutic effect (9, 18). Furthermore, it appears that a similar significance can be expected in the assessment of radiotherapy effect.

In our study, we demonstrated the feasibility of ^{18}F -FLT PET/CT in assessment of an early response to radiotherapy. In the majority of patients, complete regression of proliferative activity was achieved. In the case of residual ^{18}F -FLT uptake, the threshold for sufficient response is still questionable. We used the threshold of 3 mSv and after escalated irradiation, complete response was observed. In a few days, the cumulative dose has such an effect on the tumor tissue that it stops cell preparation for division and leads to a decrease or disappearance of TK1 activity, reducing the ability of the tissue to accumulate ^{18}F -FLT. This phenomenon was also used in the procedure for testing the response to therapy considered in our study. Compared to ^{18}F -FDG, the complete absence of ^{18}F -FLT uptake is beneficial in the early evaluation of response to therapy. The assessment of ^{18}F -FDG uptake can be affected by inflammatory and reparative changes in the tissues after irradiation. During the post-radiotherapy period, activated lymphocytes, macrophages, activated fibroblasts and endothelial progenitor cells occur in the irradiated area, showing a high level of ^{18}F -FDG accumulation due to oxidative glycolysis-dependent energy metabolism. At the same time, however, these cells would not undergo active division at the site of their tissue activity (13). A minimal increase in ^{18}F -FLT uptake is only found where re-epithelization or epithelial regeneration occurs after a radiation insult.

In ^{18}F -FDG-PET/CT, detection of the response to radiotherapy is reported to be delayed for at least 6 weeks after its completion. At the same time, a homogeneous sensitivity of the tumor tissue to radiotherapy cannot be expected and the dose may not be distributed homogeneously in the tissue. Targeted irradiation of areas with a worse response to the first therapy emerges as an effective option. Focused escalated radiotherapy, therefore, is used to treat resistant or insufficiently covered tissues. Dose escalation should target only the residual viable tumor tissue that requires additional irradiation, while sparing the surrounding tissues. To avoid revitalization of the tumor tissue and development of resistance, it is advisable to detect residual activity within the shortest possible time after the end of the irradiation series. Thus, ^{18}F -FLT-PET/CT helps to detect residues of viable tumor tissue and deliver targeted therapy against it during a vulnerable phase of the cell cycle and is more effective in comparison to ^{18}F -FDG (18, 19).

Our study is limited in the number of patients included in our study. Furthermore, the interval between irradiation and PET/CT was not consistent. No ^{18}F -FLT PET/CT was performed prior to irradiation.

In conclusion, our results confirm the existing positive experience with the use of ^{18}F -FLT-PET/CT in patients with head and neck tumors as a promising method in monitoring an early response to radiotherapy of squamous cell carcinoma. This method can be used for targeted focal escalation of radiotherapy and can make an important contribution to the planning of treatment for orofacial tumors.

Acknowledgements

This study was supported by the grant of Ministry of Health of the Czech Republic - Conceptual Development of Research Organization (Faculty Hospital in Pilsen - FNPI, 00669806) and the project of the Charles University, Prague - Progress Q39.

References

- 1 Dimitrakopoulou-Strauss A and Strauss LG: The role of ^{18}F -FLT in cancer imaging: Does it really reflect proliferation? *Eur J Nucl Med Mol Imaging* 35: 523-526, 2008.
- 2 Yue J, Chen L, Cabrera AR, Sun X, Zhao S, Zheng F, Han A, Zheng J, Teng X, Ma L, Ma Y, Han D, Zhao X, Mu D, Yu J and Li Y: Measuring tumor cell proliferation with ^{18}F -FLT PET during radiotherapy of esophageal squamous cell carcinoma: A pilot clinical study. *J Nucl Med* 51: 528-534, 2010.
- 3 Hoshikawa H, Nishiyama Y, Kishino T, Yamamoto Y, Haba R and Mori N: Comparison of FLT-PET and FDG-PET for visualization of head and neck squamous cell cancers. *Mol Imaging Biol* 13: 172-177, 2011.
- 4 Pan MH, Huang SC, Liao YP, Schae D, Wang CC, Stout DB, Barrio JR and McBride WH: FLT-PET imaging of radiation responses in murine tumors. *Mol Imaging Biol* 10: 325-334, 2008.
- 5 Lee TS, Ahn SH, Moon BS, Chun KS, Kang JH, Cheon GJ, Choi CW and Lim SM: Comparison of ^{18}F -FDG, ^{18}F -FET and ^{18}F -FLT for differentiation between tumor and inflammation in rats. *Nucl Med Biol* 36: 681-686, 2009.
- 6 Agool A, Slart RH, Thorp KK, Glaudemans AW, Cobben DC, Been LB, Burlage FR, Elsinga PH, Dierckx RA, Vellenga E and Holter JL: Effect of radiotherapy and chemotherapy on bone marrow activity: A ^{18}F -FLT-PET study. *Nucl Med Commun* 32: 17-22, 2011.
- 7 Agool A, Dierckx RA, de Wolf JT, Vellenga E and Slart RH: Extramedullary haematopoiesis imaging with ^{18}F -FLT PET. *Eur J Nucl Med Mol Imaging* 37: 1620, 2010.
- 8 Linecker A, Kermer C, Sulzbacher I, Angelberger P, Kletter K, Dudczak R, Ewers R and Becherer A: Uptake of (^{18}F)-FLT and (^{18}F)-FDG in primary head and neck cancer correlates with survival. *Nuklearmedizin* 47: 80-85, 2008.
- 9 Menda Y, Boles Ponto LL, Dornfeld KJ, Tewson TJ, Watkins GL, Gupta AK, Anderson C, McGuire S, Schultz MK, Sunderland JJ, Graham MM and Buatti JM: Kinetic analysis of 3'-deoxy-3'-(^{18}F)-fluorothymidine [(^{18}F)-FLT] in head and neck cancer patients before and early after initiation of chemoradiation therapy. *J Nucl Med* 50: 1028-1035, 2009.
- 10 Backes H, Ullrich R, Neumaier B, Kracht L, Wienhard K and Jacobs AH: Noninvasive quantification of (^{18}F)-FLT human brain PET for the assessment of tumour proliferation in patients with high-grade glioma. *Eur J Nucl Med Mol Imaging* 26: 1960-1967, 2009.

- 11 Hatakeyama T, Kawai N, Nishiyama Y, Yamamoto Y, Sasakawa Y, Ichikawa T and Tamiya T: ^{11}C -Methionine (MET) and ^{18}F -fluorothymidine (FLT) PET in patients with newly diagnosed glioma. *Eur J Nucl Med Mol Imaging* 35: 2009-2017, 2008.
- 12 Tripathi M, Sharma R, D'Souza M, Jaimini A, Panwar P, Varshney R, Datta A, Kumar N, Garg G, Singh D, Grover RK, Mishra AK and Mondal A: Comparative evaluation of F-18 FDOPA, F-18 FDG, and F-18 FLT-PET/CT for metabolic imaging of low grade gliomas. *Clin Nucl Med* 34: 878-883, 2009.
- 13 Vera P, Bohn P, Edet-Sanson A, Salles A, Hapdey S, Gardin I, Ménard JF, Modzelewski R, Thiberville L and Dubray B: Simultaneous positron emission tomography (PET) assessment of metabolism with (^{18}F)-fluoro-2-deoxy-D-glucose (FDG), proliferation with (^{18}F)-fluoro-thymidine (FLT), and hypoxia with (^{18}F)-fluoro-misonidazole (F-miso) before and during radiotherapy in patients with non-small-cell lung cancer (NSCLC): A pilot study. *Radiother Oncol* 98: 109-116, 2011.
- 14 Troost EG, Bussink J, Oyen WJ and Kaanders JH: ^{18}F -FDG and ^{18}F -FLT do not discriminate between reactive and metastatic lymph nodes in oral cancer. *J Nucl Med* 50: 490-491, 2009.
- 15 De Saint-Hubert M, Brepoels L and Mottaghy FM: Can evaluation of targeted therapy in oncology be improved by means of ^{18}F -FLT? *J Nucl Med* 51: 1499-1500, 2010.
- 16 Weber WA: Monitoring tumor response to therapy with ^{18}F -FLT PET. *J Nucl Med* 51: 841-844, 2010.
- 17 Everitt S, Hicks RJ, Ball D, Kron T, Schneider-Kolsky M, Walter T, Binns D and Mac Manus M: Imaging cellular proliferation during chemo-radiotherapy: a pilot study of serial ^{18}F -FLT positron-emission tomography/computed tomography imaging for non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 75: 1098-1104, 2009.
- 18 Troost EG, Bussink J, Hoffmann AL, Boerman OC, Oyen WJ and Kaanders JH: ^{18}F -FLT PET/CT for early response monitoring and dose escalation in oropharyngeal tumors. *J Nucl Med* 51: 866-874, 2010.
- 19 Been LB, Hoekstra HJ, Suurmeijer AJ, Jager PL, van der Laan BF and Elsinga PH: [^{18}F]FLT-PET and [^{18}F]FDG-PET in the evaluation of radiotherapy for laryngeal cancer. *Oral Oncol* 45: e211-215, 2009.

Received April 18, 2018

Revised May 16, 2018

Accepted May 21, 2018