

¹⁸F-Fluorothymidine PET/CT as an early predictor of tumor response to treatment with cetuximab in human lung cancer xenografts

SATOSHI TAKEUCHI¹, SONGJI ZHAO^{2,3}, YUJI KUGE⁴, YAN ZHAO³, KEN-ICHI NISHIJIMA³,
TOSHIYUKI HATANO³, YASUSHI SHIMIZU¹, ICHIRO KINOSHITA¹,
NAGARA TAMAKI² and HIROTOSHI DOSAKA-AKITA¹

Departments of ¹Medical Oncology, ²Nuclear Medicine and ³Tracer Kinetics and Bioanalysis, Graduate School of Medicine, ⁴Central Institute of Isotope Science, Hokkaido University, Sapporo, Hokkaido 060-8638, Japan

Received April 26, 2011; Accepted May 17, 2011

DOI: 10.3892/or.2011.1338

Abstract. We investigated whether ¹⁸F-fluorothymidine-positron-emission tomography/computed tomography (¹⁸F-FLT-PET/CT) is useful for the evaluation of the very early response to anti-epidermal growth factor receptor (EGFR) antibody cetuximab therapy in human lung cancer xenografts. A human tumor xenograft model was established with a human non-small cell lung cancer cell line. The mice were randomly assigned to four groups: tumor growth follow-up, *ex vivo* study, PET/CT imaging and non-treated control. Mice were administered saline as control or cetuximab on day 1. An immunohistochemical study with Ki-67 was performed. Tumor volume treated with cetuximab was kept significantly smaller than control after day 8, although there was no difference on day 3. On day 3, ¹⁸F-FLT distribution was higher in the tumor than in other tissues, and was significantly decreased by treatment with cetuximab. On PET/CT imaging, ¹⁸F-FLT distribution in the tumor was clearly visualized, and the maximum standardized uptake value (SUV) was significantly decreased after treatment with cetuximab ($p < 0.01$). Ki-67 expression was also significantly decreased on day 3 ($p = 0.01$). These results suggest that ¹⁸F-FLT-PET/CT can be a useful predictor to determine the response to molecular targeted drugs such as cetuximab at an earlier time point than the change of tumor size.

Introduction

Through recent advances in molecular targeted therapy of cancer, applications targeting epidermal growth factor receptor (EGFR) are currently one of the most promising

and well advanced in the clinical setting. EGFR is a protein tyrosine kinase that plays a crucial role in signal transduction pathways, regulating key cellular functions such as proliferation, angiogenesis, metastasis, and evasion of apoptosis (1,2). EGFR targeting therapy is promising for non-small cell lung cancers (NSCLCs). The EGFR tyrosine kinase inhibitors (EGFR-TKIs) erlotinib (3) and gefitinib (4,5) are established treatments for patients with advanced NSCLCs. Anti-EGFR antibody drugs have also been developed. Cetuximab, a chimeric monoclonal antibody targeting EGFR, has recently been used for treatment of patients with advanced NSCLC in a phase III study (6).

In general, molecular targeted drugs targeting EGFR are not only more expensive than commonly used cytotoxic agents, but they may also cause unique side effects, such as dermatologic reactions. To use molecular targeted drugs effectively, it is necessary to develop early and accurate evaluation modalities for the tumor response. The X-ray computed tomography (CT) and magnetic resonance imaging (MRI) have commonly been used to evaluate the anti-tumor effect of these cytotoxic and molecular targeted drugs by measuring tumor size. However, these anatomical imaging techniques have limited value due to requiring a relatively long time to obtain tumor size shrinkage with successful drug therapy. Thus, patients may have to endure side effects (7) and high medical costs (8) during periods of desperate treatment. In addition, evaluation based on sequential measurement of tumor size may not accurately reflect viable tumor cells because of the presence of necrotic or fibrotic tissue (9).

Nuclear imaging, including positron emission tomography (PET), has recently been used for treatment evaluation, in addition to conventional methods such as CT and MRI. It can provide information about pathophysiological function, while CT and MRI evaluations are based on sequential measurements of tumor size. The thymidine analog 3'-deoxy-3'-¹⁸F-fluorothymidine (¹⁸F-FLT) has been developed as a PET tracer to image proliferation *in vivo* (10). ¹⁸F-FLT uptake has been shown to reflect the activity of thymidine kinase-1 (TK1), an enzyme expressed during the DNA synthesis phase of the cell cycle. TK1 activity is high in proliferating cells and low in

Correspondence to: Dr Yuji Kuge, Central Institute of Isotope Science, Hokkaido University, North 15, West 7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan
E-mail: kuge@ric.hokudai.ac.jp

Key words: ¹⁸F-fluorothymidine, cetuximab, lung cancer, PET/CT

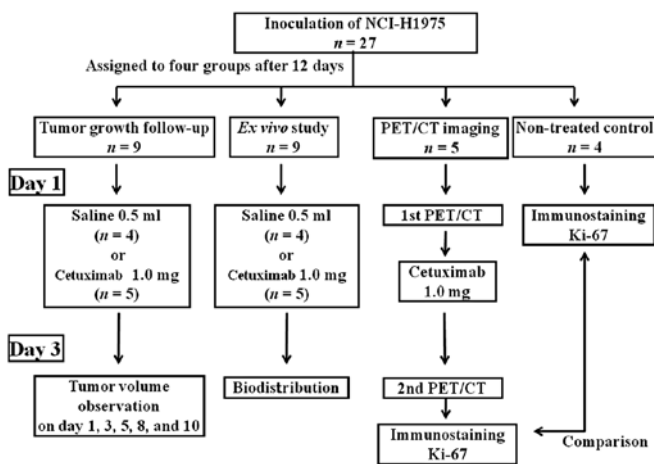


Figure 1. Experimental protocols of this study. Twelve days after inoculation of NCI-H1975 cells, mice were assigned randomly to four groups: tumor growth follow-up, *ex vivo* study, PET/CT imaging, and non-treated control groups. In the tumor growth follow-up and *ex vivo* study groups, the mice were further assigned to be administered saline or cetuximab. In the *ex vivo* study group, mice were sacrificed for a biodistribution study on day 3. In the PET/CT imaging group, mice were imaged by PET/CT pre-treatment with cetuximab on day 1. The same mice were imaged again with the same procedure post-treatment on day 3. Tumor cell proliferative activity (Ki-67 labeling index) was determined by immunohistochemistry. In the non-treated group, the Ki-67 labeling index of the tumor was determined as a control value and compared with that in the PET/CT imaging group.

dormant cells. Owing to the phosphorylation of FLT by TK1, negatively charged FLT monophosphate is formed, resulting in intracellular trapping and accumulation of radioactivity (11). Thus, this tracer is retained in proliferating cells through the activity of thymidine kinase. Measurement of tumor proliferative activity by ¹⁸F-FLT-PET/CT may be a way to provide an early and accurate assessment of the response to therapy with molecular targeted drugs (12).

The purpose of this study was to determine whether ¹⁸F-FLT-PET/CT is useful to evaluate the response to anti-EGFR antibody cetuximab therapy at very early time points in human lung cancer xenografts.

Materials and methods

Cell line. The human non-small cell lung cancer cell line NCI-H1975 (American Type Culture Collection, Manassas, VA, USA) was cultured in RPMI-1640 medium (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 0.03% glutamine at 37°C in an atmosphere of 5% CO₂. NCI-H1975 cells contain both the EGFR-TKI-sensitizing L858R and the resistant T790M point mutations (13).

Animal studies. All experimental protocols were approved by the Laboratory Animal Care and Use Committee of Hokkaido University. Nine-week-old male BALB/c athymic nude mice (supplied by Japan SLC, Inc., Hamamatsu, Japan) were used in all experiments. A human tumor xenograft model was established using this cell line (5 × 10⁶ cells/0.1 ml) by s.c. inoculation into the right flank of the mice. Twelve days after inoculation (when the tumors reached 7–10 mm in diameter)

(14), the mice were randomly assigned to four groups: tumor growth follow-up (n=9), *ex vivo* study (n=9), PET/CT imaging (n=5), and non-treated control (n=4) groups. Fig. 1 shows the experimental protocols of this study.

In the tumor growth follow-up and *ex vivo* groups, the mice were further assigned to be administered saline 0.5 ml/mouse as control or cetuximab 1.0 mg/0.5 ml/mouse given intraperitoneally. The monoclonal anti-EGFR antibody cetuximab (IMC-C225; Erbitux) was kindly provided by Merck KGaA (Darmstadt, Germany). In the tumor growth follow-up group, tumor sizes were measured by caliper on days 3, 5, 8, and 10 after the treatment on day 1. Tumor volume was calculated by the formula: $\pi/6 \times \text{larger diameter} \times (\text{smaller diameter})^2$. In the *ex vivo* study group, mice were anesthetized with isoflurane inhalation and injected with 7.4 MBq of ¹⁸F-FLT in the tail vein. After 90 min, these mice were sacrificed, and the tumor and other organs were excised. The tissue and blood samples were weighed, and radioactivity of tracers in each sample was determined using a gamma-counter (1480 Wizard 3, Wallac Oy, Turku, Finland). Tracer uptake in the tissue was expressed as the percentage of injected dose (ID) per gram of tissue after being normalized to the animal's weight (%ID/g) × kg. Uptake was calculated in the tumor and normal organs, including blood, plasma, muscle, heart, lung, spleen, liver, pancreas, stomach, small intestine, colon, kidney, skin, brown fat and brain. In the PET/CT imaging group, mice were imaged by a small animal PET/CT for 20 min pre-treatment before administration of cetuximab on day 1. The same mice were again imaged with the same procedure post-treatment with cetuximab on day 3. Tumor sizes were measured before each PET/CT imaging session. In the non-treated control group, the tumor cell proliferative activity (Ki-67 labeling index) was determined as the control value and compared with that of the PET/CT imaging group.

¹⁸F-FLT-PET/CT studies. In the PET/CT imaging group (n=5), the mice were anesthetized with isoflurane inhalation and injected with 7.4 MBq of ¹⁸F-FLT into the tail vein. ¹⁸F-FLT was synthesized and obtained from the Hokkaido University Hospital Cyclotron facility. It was synthesized using a modification of a previously published procedure (15). The mice were placed in a small animal PET/CT scanner (Inveon; Siemens Medical Solutions USA Inc., Knoxville, TN) in a supine position 60 min after the injection of ¹⁸F-FLT. PET and CT scans were carried out for 20 and 7 min, respectively for image capture. The mice were kept using cotton bedding of our own making to maintain body temperature and anesthetized with 1.0–1.5% isoflurane inhalation during the PET/CT imaging. Following the second PET/CT imaging (90 min after the injection of ¹⁸F-FLT), the mice were sacrificed, and tumor tissues were excised for immunohistochemical staining. The data were reconstructed and corrected for attenuation and scattering using 2D filtered back-projection (FBP). The image matrix was 256 × 256 × 159, resulting in a voxel size of 0.385 × 0.385 × 0.796 mm. Images were analyzed quantitatively by drawing volumes of interest centered over the tumor without correction for partial volume effects. The standardized uptake value (SUV) was calculated using the single maximum pixel count within the region of interest and normalized to the injected dose and mouse body weight. SUV_{max} denotes the maximum SUV value within the

tumor region of interest (ROI). Using tissue samples from the tumors, formalin-fixed, paraffin-embedded specimens were prepared for subsequent histological staining.

Pathological study. Formalin-fixed, paraffin-embedded, 3- μ m thick sections of tumor were used for immunohistochemical staining. The labeled streptavidin biotin method was used after deparaffinization. Immunohistochemical staining of nuclear antigen Ki-67 was performed as a tumor cell proliferation marker. The primary antibody used was a mouse monoclonal antibody for Ki-67, clone MIB-1 (Dako, Carpinteria, CA). Tumor cells were considered positive only when clear nuclear staining was seen. All nuclei with homogeneous granular staining or multiple speckled staining were counted as positive, regardless of the staining intensity. Cells with cytoplasmic staining were excluded (16). The Ki-67 labeling index was defined as the percentage of positive cells by counting cell numbers in the entire field of the tumor.

Statistical analysis. All values are expressed as means \pm SD (standard deviation). Two-way repeated measures analysis of variance (ANOVA) and the unpaired Student's t-test were used to compare tumor volume sequentially for the tumor growth follow-up group. The unpaired Student's t-test was used to compare the biodistribution of ^{18}F -FLT between the two treatment groups. Paired Student's t-tests were used to compare the differences between different time points within one treatment group. A p-value of <0.05 was considered significant. Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Tumor volume change. Tumor volume change in the tumor growth follow-up group is shown in Fig. 2. The mice were treated by saline or cetuximab on day 1. The tumor volumes of the control group and cetuximab-treated groups were 150.9 ± 38.6 and 131.4 ± 45.9 mm^3 , respectively, on day 3. Although there was no difference in the tumor volume between these two groups on day 3, the tumor grew rapidly in the control group. A significant difference was observed on day 8. The tumor volumes of the control and cetuximab-treated groups were 381.4 ± 99.6 and 140.3 ± 37.9 mm^3 , respectively ($p < 0.01$). The difference became more obvious on day 10 ($p < 0.01$). On the other hand, tumors were maintained almost within the same size in the cetuximab-treated group. The tumor volume of the cetuximab-treated groups was 117.5 ± 46.9 mm^3 on day 1, and was kept 172.8 ± 61.3 mm^3 even on day 10. This indicated that the dose of cetuximab used was adequate to suppress tumor growth, and that cetuximab treatment had enough efficacy to suppress tumor growth. Considering this result, an *ex vivo* study and ^{18}F -FLT-PET/CT imaging were performed on day 3 for early evaluation of therapy response.

Biodistribution of ^{18}F -FLT. The biodistribution of ^{18}F -FLT in the *ex vivo* study group on day 3 is shown in Fig. 3. The radioactivity was higher in the tumor than in other organs in the control group. A significant difference in the biodistribution of ^{18}F -FLT in the tumor was observed between the control and cetuximab-treated groups. Biodistributions in the control

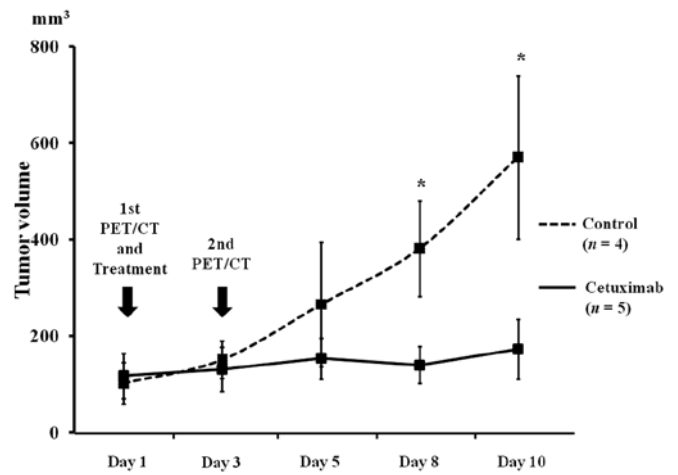


Figure 2. Change in tumor volume in the tumor growth follow-up group. Mice were treated by saline or cetuximab on day 1. There was no obvious difference in size between the two groups until day 3. Since the tumors began to grow rapidly in the control group, significant differences were seen from day 8 ($p < 0.01$). Tumors were maintained almost within the same size in the cetuximab-treated group.

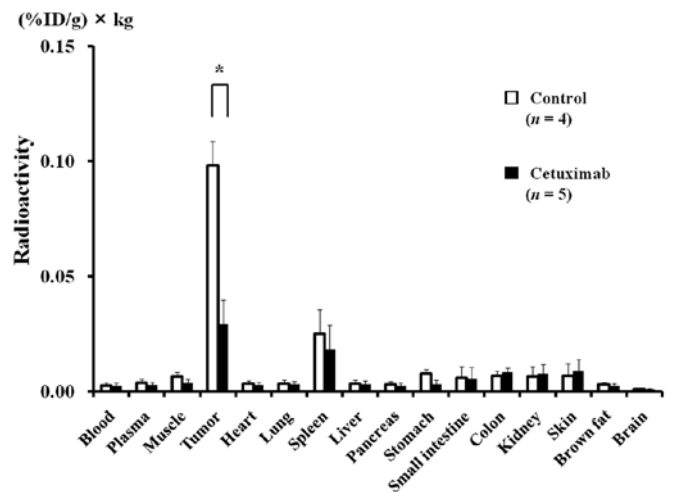


Figure 3. Biodistribution of ^{18}F -FLT in the *ex vivo* study group on day 3. The ^{18}F -FLT uptake was higher in tumor than in other normal organs. The uptake was significantly decreased in the cetuximab-treated group compared with the control group ($p < 0.001$).

and cetuximab-treated groups were 0.098 ± 0.005 (%ID/g) x kg and 0.029 ± 0.010 (%ID/g) x kg, respectively ($p < 0.001$). ^{18}F -FLT uptake was particularly high in the tumors, and it was decreased by cetuximab treatment as early as day 3. This result indicates that ^{18}F -FLT may be promising to assess the early response to therapy.

^{18}F -FLT-PET/CT image. The *in vivo* imaging by ^{18}F -FLT-PET/CT was performed on day 1 before treatment with cetuximab and then again for the same mice on day 3. A representative image of the first ^{18}F -FLT-PET/CT before treatment is shown in Fig. 4A. Since the high uptake of ^{18}F -FLT was seen only in the tumor, it can be clearly visualized with PET/CT before treatment with cetuximab. Fig. 4B shows the image of the second ^{18}F -FLT-PET/CT after treatment with cetuximab on

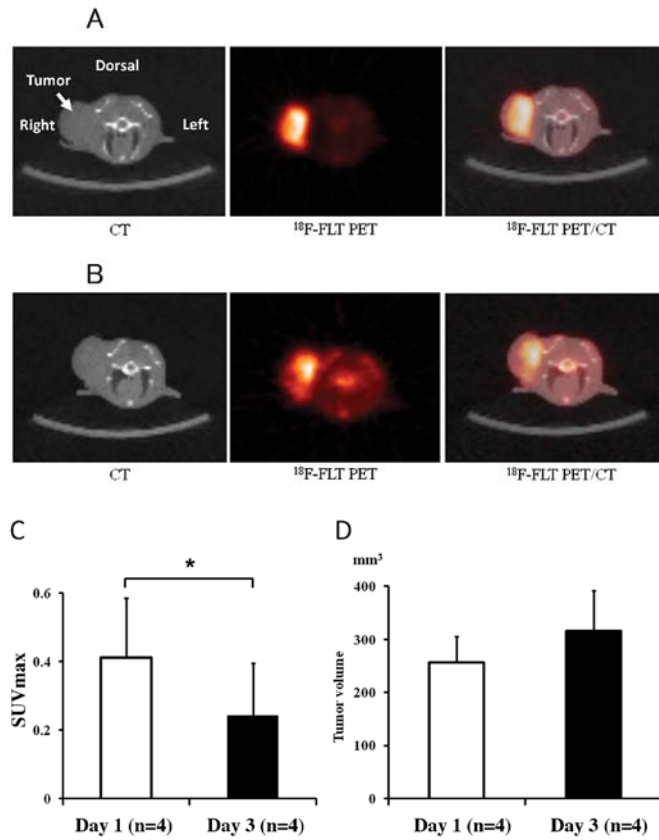


Figure 4. CT, ^{18}F -FLT PET and ^{18}F -FLT-PET/CT images and the maximum standardized uptake value (SUVmax). (A) Representative images on day 1 before treatment, and on day 3 after treatment with cetuximab (B). Tumor is seen on the right flank of the animal. The high FLT uptake is seen only in the tumor. The uptake is decreased after treatment with cetuximab as early as day 3 ($p < 0.01$). (C) The SUVmax from tumor ROIs is shown. There is a significant difference between day 1 before treatment and day 3 after treatment with cetuximab. (D) Tumor volume in the ^{18}F -FLT-PET/CT imaging group is shown. There is no significant difference in tumor volume between before and after treatment with cetuximab ($p = 0.11$).

day 3. Decreased ^{18}F -FLT uptake after treatment was visualized. SUVmax values from tumor ROIs were obtained from each ^{18}F -FLT-PET/CT (Fig. 4C). There was a significant difference between day 1 before treatment and day 3 after treatment with cetuximab (0.41 ± 0.17 before treatment on day 1 and 0.24 ± 0.15 after treatment on day 3; $p < 0.01$). However, in this ^{18}F -FLT-PET/CT imaging group, there was no significant change in tumor volume between before and after cetuximab treatment ($257.0 \pm 49.3 \text{ mm}^3$ before treatment on day 1 and $315.5 \pm 77.1 \text{ mm}^3$ after treatment on day 3; $p = 0.11$) (Fig. 4D). These results show that ^{18}F -FLT-PET/CT could visualize the change in tumor proliferation at a very early time point even without a significant change in tumor size.

Pathological study. Since ^{18}F -FLT-PET/CT visualized the change in tumor proliferation between before and after treatment with cetuximab, a pathological study was performed using tumor samples of this PET/CT imaging group. Hence, the non-treated control group was used as the control. Representative immunohistochemical staining for Ki-67 in the non-treated control group is shown in Fig. 5A as a control on day 1. Fig. 5B shows Ki-67 staining in the PET/CT imaging group post-treatment

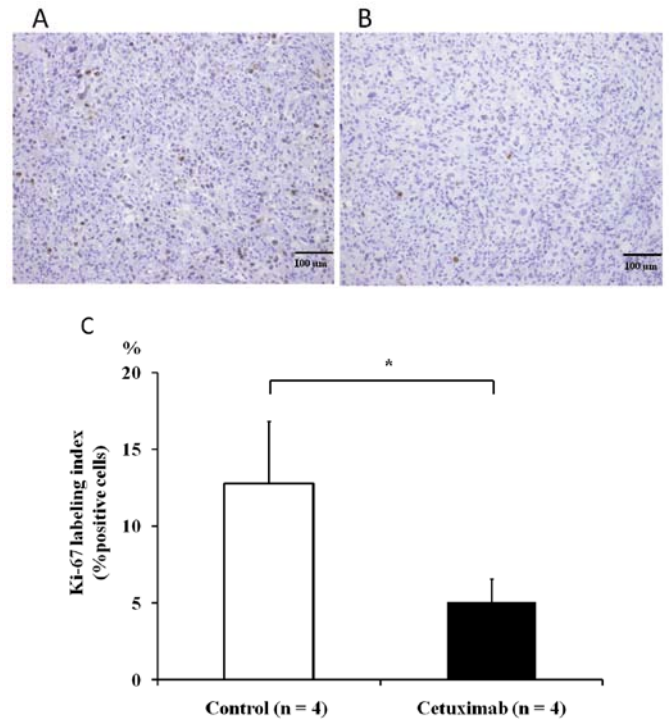


Figure 5. Representative immunohistochemical staining of Ki-67 and the Ki-67 labeling index on day 3. (A) Ki-67 staining in the non-treated control group as a control. (B) Ki-67 staining in the PET/CT imaging group post-treatment with cetuximab. Scale bar, $100 \mu\text{m}$. (C) The Ki-67 labeling index shows a significant difference between day 1 before treatment and day 3 after treatment with cetuximab ($p = 0.01$).

with cetuximab on day 3. Two days after the administration of cetuximab, there was a decrease in Ki-67-positive tumor cells. There was a significant difference in the Ki-67 labeling index between them ($12.8 \pm 4.0\%$ in the non-treated control group on day 1 vs. $5.0 \pm 1.5\%$ in the PET/CT imaging group on day 3; $p = 0.01$) (Fig. 5C). This result indicates that tumor proliferation was suppressed by cetuximab treatment as early as day 3, and this was consistent with the result of ^{18}F -FLT-PET/CT imaging.

Discussion

The present study demonstrated that ^{18}F -FLT-PET/CT may evaluate the therapeutic effect earlier than morphological measurement on CT in human cancer cell line xenografts, and that such a method could be useful to evaluate the effects of molecular targeted drugs such as cetuximab. Although there was no difference in tumor volume between the control and cetuximab-treated groups on day 3, tumor began to grow rapidly in the control group after day 3. On the other hand, the tumor was maintained almost within the same size in the cetuximab-treated group. Early changes in tumor proliferation could be visualized by non-invasive ^{18}F -FLT-PET/CT imaging, even when significant changes in tumor size were still not seen. The early change in tumor proliferation activity was confirmed by a pathological study using the Ki-67 labeling index. Thus, measurement of tumor proliferative activity by ^{18}F -FLT-PET/CT may be a way to provide an early and accurate assessment of the response to therapy with molecular targeted drugs for cancer.

Cetuximab has activity in various types of cancer, including head and neck cancer, colorectal cancer, and non-small cell lung cancer (6,17,18). Early and accurate evaluation methods for using cetuximab effectively are urgently required. Examining the K-ras mutation in advance before treatment with cetuximab is one solution (19). Patients bearing tumors with the mutated K-ras gene do not benefit from cetuximab in colorectal cancer. In contrast with colorectal cancer, the significance of K-ras mutation in non-small cell lung cancer has not yet been well clarified, even in the latest phase III study (6,20). Cetuximab-induced skin toxicity is also a predictive marker (7) and helps identify those patients for whom cetuximab is effective. The appearance of skin toxicities, however, is too variable and not a direct predictor of tumor response itself. As for nuclear imaging, the effectiveness of ^{18}F -FLT has attracted increasing interest (10,21,22).

A recent study showed that monotherapy with cetuximab was not effective in patients with advanced NSCLC who received prior EGFR-TKI treatment (23). However, in the present study, we used the human non-small cell lung cancer cell line NCI-H1975 which contains both the EGFR-TKI-sensitizing L858R and the resistant T790M point mutations (13). A previous study showed that cetuximab had antitumor activity even in this mutated cell line (24). Hence, this xenograft model using NCI-H1975 is suitable and reasonable to evaluate the early response to cetuximab therapy.

Since ^{18}F -FLT-PET/CT reflects the proliferation of tumor cells, this method is more suitable for detecting the early therapeutic effect compared with conventional modalities such as CT and MRI, which are based on sequential measurements of tumor size. This present study showed the effectiveness of ^{18}F -FLT-PET/CT in the evaluation of the therapeutic effect of cetuximab in human lung cancer xenografts at an earlier time point (day 3) than the change of tumor size. As molecular targeted drugs are used for patients with advanced-stage cancer, it is very important to know the therapeutic effect as early as possible. If the therapeutic effect can be predicted at a very early time point, it will be possible to select the clinically optimal treatment and reduce toxicity and medical costs in advance. To our knowledge, several investigators recently used ^{18}F -FLT-PET to evaluate treatment response in animal models following EGFR targeted therapy such as erlotinib and cetuximab. However, those studies performed ^{18}F -FLT-PET at later timing than our study (25), and did not use lung cancer cell line (26) or cetuximab (27).

Whether ^{18}F -FLT-PET/CT on day 3 is the best timing for early treatment evaluation remains unclear. Herrmann *et al* reported that ^{18}F -FLT-PET imaging 2 days after administration of cyclophosphamide-adriamycin-vincristine-prednisone chemotherapy with rituximab immunotherapy showed an early decrease in ^{18}F -FLT uptake in lymphoma patients, while there was no reduction in ^{18}F -FLT uptake after rituximab alone (28), though both cetuximab and rituximab are a type of the same chimeric antibody. Atkinson *et al* reported that ^{18}F -FLT-PET imaging could detect tumor response to anti-EGFR treatment using cetuximab as well as erlotinib (25). Their ^{18}F -FLT PET imaging was performed 7 days after treatment. Considering these results, the best timing for early evaluation may depend on the kind of disease and the drugs used. In any case, ^{18}F -FLT-PET/CT may be a potent modality for earlier

response evaluation than conventional modalities such as CT and MRI. However, it remains to be clarified whether ^{18}F -FLT is useful to assess the early therapeutic effect for other malignant tumors, and whether ^{18}F -FLT can be useful when other molecular targeted drugs, such as anti-angiogenesis drugs, are used.

It should be noted that cetuximab has an immune-mediated antitumor mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is supposed to contribute to the activity of cetuximab (29). In the present study, the tumor model was established in immune-deficient mice (BALB/c athymic nude mouse) that lack function of a T-cell component. Hence, the efficacy of cetuximab might be less effective than it should be. Although further investigation is needed to apply our result to immune-competent mice or humans, genetically engineered mouse models have been proven to be a powerful tool for elucidation of the biological processes and pathophysiological alterations that occur in human disease (9).

^{18}F -fluorodeoxyglucose (FDG) is the most widely used tracer for tumor imaging with PET and can image viable tumor cells by reflecting glucose metabolism. Avril *et al* reported a significant correlation between ^{18}F -FDG uptake and proliferative activity in breast cancer (30), but the correlation coefficient was low (0.41). On the other hand, Brepoels *et al* reported that ^{18}F -FLT is a more accurate measurement of tumor response than ^{18}F -FDG after the administration of cyclophosphamide (31). It is well known that ^{18}F -FDG is not tumor specific, since its uptake occurs in inflammatory lesions where glucose is utilized (32). ^{18}F -FDG-PET may not clearly distinguish between residual disease and post-treatment inflammation (33). ^{18}F -FDG shows considerable accumulation in macrophages and granulation tissues (34). These characteristics of ^{18}F -FDG suggest that careful assessment is needed when using ^{18}F -FDG to evaluate treatment, though further studies, including comparative studies between ^{18}F -FLT and ^{18}F -FDG, and also with various tumor biomarkers are necessary to demonstrate the advantages of ^{18}F -FLT-PET. Although we injected ^{18}F -FDG into this xenograft model, tracer uptake in the tumor was not high compared to other organs, and was not decreased by cetuximab treatment (data not shown).

In conclusion, the results of this study suggest that ^{18}F -FLT-PET/CT can be useful for evaluating the response to molecular targeted drugs such as cetuximab at an earlier time point than the evaluation of tumor size in human lung cancer cell line xenografts. In the future, clinical studies evaluating ^{18}F -FLT-PET/CT as an early predictor of tumor response to anti-EGFR antibody drugs will be required. In the clinical setting, ^{18}F -FLT-PET/CT is expected to optimize clinical outcomes and reduce exposure to an ineffective treatment. It is also expected to avoid toxicity, to decrease duration of treatment, and to reduce medical costs in advance.

Acknowledgements

This study was performed through special coordination funds for promoting science and technology, provided by the Ministry of Education, Culture, Sports, Science, and Technology of the Japanese Government. This study was also supported in part by grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science. The authors would like to thank

the staff members of the Department of Nuclear Medicine, Department of Medical Oncology, Central Institute of Isotope Science and Institute for Animal Experimentation, Hokkaido University, and Hokkaido University Hospital for supporting this study.

References

1. Wieduwilt MJ and Moasser MM: The epidermal growth factor receptor family: biology driving targeted therapeutics. *Cell Mol Life Sci* 65: 1566-1584, 2008.
2. Dassonville O, Bozec A, Fischel JL and Milano G: EGFR targeting therapies: monoclonal antibodies versus tyrosine kinase inhibitors. Similarities and differences. *Crit Rev Oncol Hematol* 62: 53-61, 2007.
3. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, *et al.*: Erlotinib in previously treated non-small cell lung cancer. *N Engl J Med* 353: 123-132, 2005.
4. Mitsudomi T, Morita S, Yatabe Y, *et al.*: Gefitinib versus cisplatin plus docetaxel in patients with non-small cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 11: 121-128, 2010.
5. Maemondo M, Inoue A, Kobayashi K, *et al.*: Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR. *N Engl J Med* 362: 2380-2388, 2010.
6. Pirker R, Pereira JR, Szczesna A, *et al.*: Cetuximab plus chemotherapy in patients with advanced non-small cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* 373: 1525-1531, 2009.
7. Lynch TJ Jr, Kim ES, Eaby B, Garey J, West DP and Lacouture ME: Epidermal growth factor receptor inhibitor-associated cutaneous toxicities: an evolving paradigm in clinical management. *Oncologist* 12: 610-621, 2007.
8. Mittmann N, Au HJ, Tu D, *et al.*: Prospective cost-effectiveness analysis of cetuximab in metastatic colorectal cancer: evaluation of National Cancer Institute of Canada Clinical Trials Group CO.17 trial. *J Natl Cancer Inst* 101: 1182-1192, 2009.
9. Spaepen K, Stroobants S, Dupont P, *et al.*: [(18)F]FDG PET monitoring of tumour response to chemotherapy: does [(18)F] FDG uptake correlate with the viable tumour cell fraction? *Eur J Nucl Med Mol Imaging* 30: 682-688, 2003.
10. Shields AF, Grierson JR, Dohmen BM, *et al.*: Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat Med* 4: 1334-1336, 1998.
11. Toyohara J, Waki A, Takamatsu S, Yonekura Y, Magata Y and Fujibayashi Y: Basis of FLT as a cell proliferation marker: comparative uptake studies with [3H]thymidine and [3H]arabinothymidine, and cell-analysis in 22 asynchronously growing tumor cell lines. *Nucl Med Biol* 29: 281-287, 2002.
12. Yang DJ, Kim EE and Inoue T: Targeted molecular imaging in oncology. *Ann Nucl Med* 20: 1-11, 2006.
13. Pao W, Miller VA, Politi KA, *et al.*: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2: e73, 2005.
14. Zhao S, Takeuchi S, Kuge Y, *et al.*: FLT PET can early predict antiproliferative response to anti-EGFR molecular targeting therapy (cetuximab) in human lung cancer xenograft. *J Nucl Med* 51 (Suppl 2): 399, 2010.
15. Oh SJ, Mosdzianowski C, Chi DY, *et al.*: Fully automated synthesis system of 3'-deoxy-3'-[18F]fluorothymidine. *Nucl Med Biol* 31: 803-809, 2004.
16. Spyrtos F, Ferrero-Pous M, Trassard M, *et al.*: Correlation between MIB-1 and other proliferation markers: clinical implications of the MIB-1 cut-off value. *Cancer* 94: 2151-2159, 2002.
17. Bonner JA, Harari PM, Giralt J, *et al.*: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 354: 567-578, 2006.
18. Goldberg RM, Rothenberg ML, Van Cutsem E, *et al.*: The continuum of care: a paradigm for the management of metastatic colorectal cancer. *Oncologist* 12: 38-50, 2007.
19. Karapetis CS, Khambata-Ford S, Jonker DJ, *et al.*: K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 359: 1757-1765, 2008.
20. Khambata-Ford S, Harbison CT, Hart LL, *et al.*: Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small cell lung cancer. *J Clin Oncol* 28: 918-927, 2010.
21. Vesselle H, Grierson J, Muzi M, *et al.*: In vivo validation of 3'-deoxy-3'-[(18)F]fluorothymidine ([18F]FLT) as a proliferation imaging tracer in humans: correlation of [(18)F]FLT uptake by positron emission tomography with Ki-67 immunohistochemistry and flow cytometry in human lung tumors. *Clin Cancer Res* 8: 3315-3323, 2002.
22. Salskov A, Tammisetti VS, Grierson J and Vesselle H: FLT: measuring tumor cell proliferation in vivo with positron emission tomography and 3'-deoxy-3'-[18F]fluorothymidine. *Semin Nucl Med* 37: 429-439, 2007.
23. Neal JW, Heist RS, Fidiya P, *et al.*: Cetuximab monotherapy in patients with advanced non-small cell lung cancer after prior epidermal growth factor receptor tyrosine kinase inhibitor therapy. *J Thorac Oncol* 5: 1855-1858, 2010.
24. Steiner P, Joynes C, Bassi R, *et al.*: Tumor growth inhibition with cetuximab and chemotherapy in non-small cell lung cancer xenografts expressing wild-type and mutated epidermal growth factor receptor. *Clin Cancer Res* 13: 1540-1551, 2007.
25. Atkinson DM, Clarke MJ, Mladek AC, *et al.*: Using fluoro-deoxythymidine to monitor anti-EGFR inhibitor therapy in squamous cell carcinoma xenografts. *Head Neck* 30: 790-799, 2008.
26. Manning HC, Merchant NB, Foutch AC, *et al.*: Molecular imaging of therapeutic response to epidermal growth factor receptor blockade in colorectal cancer. *Clin Cancer Res* 14: 7413-7422, 2008.
27. Ullrich RT, Zander T, Neumaier B, *et al.*: Early detection of erlotinib treatment response in NSCLC by 3'-deoxy-3'-[F]-fluoro-L-thymidine ([F]FLT) positron emission tomography (PET). *PLoS One* 3: e3908, 2008.
28. 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* 13: 3552-3558, 2007.
29. Mendelsohn J and Baselga J: The EGF receptor family as targets for cancer therapy. *Oncogene* 19: 6550-6565, 2000.
30. Avril N, Menzel M, Dose J, *et al.*: Glucose metabolism of breast cancer assessed by 18F-FDG PET: histologic and immunohistochemical tissue analysis. *J Nucl Med* 42: 9-16, 2001.
31. Brepoels L, Stroobants S, Verhoef G, De Groot T, Mortelmans L and De Wolf-Peeters C: (18)F-FDG and (18)F-FLT uptake early after cyclophosphamide and mTOR inhibition in an experimental lymphoma model. *J Nucl Med* 50: 1102-1109, 2009.
32. Yamada S, Kubota K, Kubota R, Ido T and Tamahashi N: High accumulation of fluorine-18-fluorodeoxyglucose in turpentine-induced inflammatory tissue. *J Nucl Med* 36: 1301-1306, 1995.
33. Shreve P, Anzai Y and Wahl R: Pitfalls in oncologic diagnosis with FDG PET imaging: physiologic and benign variants. *Radiographics* 19: 61, 1999.
34. Kubota R, Yamada S, Kubota K, Ishiwata K, Tamahashi N and Ido T: Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. *J Nucl Med* 33: 1972-1980, 1992.