

A prospective clinical trial of tumor hypoxia imaging with ^{18}F -fluoromisonidazole positron emission tomography and computed tomography (F-MISO PET/CT) before and during radiation therapy

Izumi TACHIBANA^{1,*}, Yasumasa NISHIMURA¹, Toru SHIBATA¹, Shuichi KANAMORI¹, Kiyoshi NAKAMATSU¹, Ryuta KOIKE¹, Tatsuyuki NISHIKAWA¹, Kazuki ISHIKAWA¹, Masaya TAMURA¹ and Makoto HOSONO²

¹Department of Radiation Oncology, Kinki University Faculty of Medicine, Osaka-Sayama, Japan

²Institute of Advanced Clinical Medicine, Kinki University Faculty of Medicine, Osaka-Sayama, Japan

*Corresponding author. Department of Radiation Oncology, Kinki University Faculty of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel: +81-72-366-0221 (ext. 3132); Fax: +81-72-368-2388; Email: izu917@med.kindai.ac.jp

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To visualize intratumoral hypoxic areas and their reoxygenation before and during fractionated radiation therapy (RT), ^{18}F -fluoromisonidazole positron emission tomography and computed tomography (F-MISO PET/CT) were performed. A total of 10 patients, consisting of four with head and neck cancers, four with gastrointestinal cancers, one with lung cancer, and one with uterine cancer, were included. F-MISO PET/CT was performed twice, before RT and during fractionated RT of approximately 20 Gy/10 fractions, for eight of the 10 patients. F-MISO maximum standardized uptake values (SUVmax) of normal muscles and tumors were measured. The tumor-to-muscle (T/M) ratios of F-MISO SUVmax were also calculated. Mean SUVmax \pm standard deviation (SD) of normal muscles was 1.25 ± 0.17 , and SUVmax above the mean $+ 2$ SD (≥ 1.60 SUV) was regarded as a hypoxic area. Nine of the 10 tumors had an F-MISO SUVmax of ≥ 1.60 . All eight tumors examined twice showed a decrease in the SUVmax, T/M ratio, or percentage of hypoxic volume (F-MISO ≥ 1.60) at approximately 20 Gy, indicating reoxygenation. In conclusion, accumulation of F-MISO of ≥ 1.60 SUV was regarded as an intratumoral hypoxic area in our F-MISO PET/CT system. Most human tumors (90%) in this small series had hypoxic areas before RT, although hypoxic volume was minimal (0.0–0.3%) for four of the 10 tumors. In addition, reoxygenation was observed in most tumors at two weeks of fractionated RT.

Keywords: tumor hypoxia; ^{18}F -misonidazole; PET/CT; reoxygenation

INTRODUCTION

Hypoxic cells of malignant tumors are considered to be radioresistant, and have been regarded as a poor prognostic factor in various cancers [1]. Misonidazole was found to be a hypoxic radiosensitizer by Asquith *et al.* in 1974 [2]. ^{18}F -fluoromisonidazole (F-MISO) was suggested as a tracer to determine hypoxic cells *in vitro* in 1983 [3]. To depict hypoxic lesions in human tumors, many clinical studies using F-MISO Positron Emission Tomography (PET) have been reported since 1991 [4]. In an animal experiment,

F-MISO image intensities were inversely correlated with measured intratumoral pO_2 [5].

Over the past 10 years, use of PET/computed tomography (CT) has grown. PET/CT provides relevant information in the staging and therapy monitoring of many tumors because it can give more accurate identification of the anatomical site than PET alone [6]. All F-MISO studies reported before the early 2000s used PET alone instead of PET/CT. For radiation therapy (RT) treatment planning, PET/CT simulation has been used clinically [7], and hypoxic imaging obtained by F-MISO PET/CT may add

some useful information. Although many investigators used the tumor-blood (T/B) ratio, tumor-cerebellum (T/C) ratio, or tumor-to-muscle (T/M) ratio to evaluate intratumoral hypoxia using F-MISO PET with cut-off points of 1.2–1.4 [8–10], no study showed a hypoxic area threshold as an absolute value of F-MISO standardized uptake values (SUV). In the present study, 10 initial patients with various tumors were analyzed to determine a threshold value of SUV_{max} of F-MISO PET for intratumoral hypoxic areas, and to visualize their reoxygenation during fractionated RT in various human tumors. Also, the appropriate timing of F-MISO PET imaging after F-MISO injection was determined.

MATERIALS AND METHODS

Patients

Between November 2009 and April 2011, 10 patients scheduled for RT for primary or recurrent tumors were enrolled in this prospective study. Eligible patients had histologically proven malignant tumors, with a performance status (PS) level of 0–1 and were aged 20–80 years. Patients without gross target volume, pregnant or lactating women, and patients with mental disorders or severe organ disorders were excluded. Patient and tumor characteristics are summarized in Table 1. Ten patients, consisting of four with head and neck cancers, four with gastrointestinal cancers, one with non-small-cell lung cancer, and one with uterine body cancer, were included. The initial tumor response for both primary tumors and metastatic lymph nodes was evaluated by CT, magnetic resonance imaging (MRI), and clinical examination 1–2 months after the end of treatment according to the RECIST criteria (version 1.1) [11]. Because some tumors regress slowly, tumor response was evaluated at the maximum tumor regression between 1 and 2 months of treatment.

The study protocol was approved by the ethical committee of the Kinki University Faculty of Medicine. All patients signed informed consent before entering the study.

F-MISO and ¹⁸F-fluoro-2-deoxyglucose PET/CT protocol

Each patient was scanned with an integrated PET/CT unit (Biograph/Somatom Emotion Duo, Siemens Medical Solutions, Hoffmann Estates, IL, USA). All PET images were acquired using a matrix of 128 × 128 pixels. The time for one bed position (162 mm in z-direction) scan was 120–150 sec. At a distance of 10 cm from the center of the field of view (FOV), the full-width at half maximum (FWHM) reached 7.4 mm × 7.4 mm × 7.1 mm, in the x, y and z directions, respectively. Voxel dimensions were 4.5 mm × 4.5 mm × 2.0 mm. CT scans were acquired in the spiral mode, with a slice thickness of 2–5 mm, a pitch of 6 mm, 130 kv and 55 mAs. The translation speed of the couch was 7.4 mm/sec. As a protocol, F-MISO PET/CT was performed twice before RT and during fractionated RT of ~20 Gy/10 fractions. Patients were injected intravenously with 7.4 MBq/kg of F-MISO. No fasting period before F-MISO injection was required. PET/CT was obtained twice, at 100 and 180 min after injection of F-MISO.

F-MISO was synthesized as described previously [12]. In short, in an automated synthesizer (F121, Sumitomo Heavy Industries, Ltd Tokyo, Japan), 5 mg of the precursor 1-(2'-nitro-1'imidazolyl)-2-O-tetrahydropyranyl-3-O-osyl-propanediol (NITTP, ABX, Montpellier, France) in 0.3 ml of acetonitrile was reacted with a mixture of dried ¹⁸F-fluoride, 7.5 mg of Kryptofix 222 (Merck, Whitehouse Station, NJ, USA), and 2.77 mg of K₂CO₃ at 110°C for 10 min. After hydrolysis with 0.3 ml of 1N HCl at 80°C for 10 min, 0.6 ml of 1N sodium acetate was added for

Table 1. Patients and tumor characteristics

No.	Age/Sex	PS	Primary site	Stage/Histology	Tumor length	RT dose	Chemotherapy
1	75/M	1	Maxilla sinus	T4a N1 M0/Sq	65 mm	42Gy/21fr ^a	CDDP, 5-FU
2	72/M	0	Esophagus	T3 N1 M0/Sq	30 mm	60Gy/30fr	CDDP, 5-FU
3	60/F	0	Uterine body	recurrence/Ad	48 mm	64.4Gy/35fr	none
4	46/M	0	Nasopharynx	T3 N2c M0/Sq	35 mm	70Gy/35fr	CDDP
5	57/M	0	Lung	T4 N3 M0/Sq	68 mm	60Gy/30fr	Nimotuzumab, CDDP, VNR
6	73/M	0	Esophagus	T3 N2 M0/Sq	28 mm	50Gy/25fr ^b	CDDP, 5-FU
7	72/F	0	Anal canal	T2 N0 M0/Sq	45 mm	59.4Gy/33fr	5-FU, MMC
8	72/M	0	Nasopharynx	T1 N2b M0/Sq	16 mm	70Gy/35fr	CDDP
9	56/M	1	Anal canal	T4 N1 M0/Ad	45 mm	45Gy/25fr ^b	5-FU, MMC
10	36/M	1	Nasopharynx	T4 N1 M0/Sq	42 mm	70Gy/35fr	CDDP

^aRT was terminated due to severe acute toxicities. ^bPreoperative chemoradiation therapy. Ad = adenocarcinoma, CDDP = cisplatin, 5-FU = fluorouracil, MMC = mitomycin C, Sq = squamous cell carcinoma, VNR = vinorelbine.

neutralization. The product was purified with HPLC chromatography (YMC PAK ODS-AM 10 mm ID × 250 mm, YMC Co., Ltd, Kyoto, Japan) using 3:97 ethanol:H₂O, and a 5.0 ml/min flow rate.

For all patients, ¹⁸F-fluoro-2-deoxyglucose (FDG) PET/CT was performed before RT. The details of FDG PET/CT at our hospital have been described elsewhere [7].

Radiation therapy and chemotherapy

For all patients, F-MISO PET/CT was performed before the start of RT, but this information was not used for the treatment planning. Eight patients were treated as definitive RT with a planned total dose of 60–70 Gy/30–35 fractions, although one patient terminated RT at 42 Gy due to acute renal failure caused by chemotherapy. The remaining two patients with gastrointestinal cancer were treated with pre-operative chemo-RT (CRT) in 45–50 Gy/25 fractions, and curative resection could be done following CRT for these patients. All patients except for a patient with recurrence of uterine body cancer were treated with concurrent chemotherapy. Details of the chemotherapy are summarized in Table 1. During RT, 2–3 cycles of chemotherapy were given.

Analysis

Because normal tissues are considered to be under normoxia, F-MISO SUVs of normal muscles were measured. Data were processed with a Siemens e.soft workstation to measure SUV and hypoxic volume. In placing the volumetric regions of interest (VOIs) over the tumor and normal muscle, the SUVmax of the VOI on PET images was

adjusted by referring to CT images and PET/CT fusion images. SUVmax in 108 normal muscle areas was measured for all 18 F-MISO studies. For each study, six oval VOIs of 10–20 cm³ were measured on bilateral posterior neck muscles (multifidus muscles, semispinalis capitis muscle, and semispinalis cervicis muscle), bilateral back muscles (erector spinae muscle, rhomboid major muscle, and trapezius muscle), or bilateral buttock muscles (gluteus maximus muscle).

For each study, F-MISO SUVmax values of both primary tumors and metastatic lymph nodes of >2cm were measured, and the highest value was regarded as the SUVmax of the study. To calculate the T/M ratio for head and neck tumors, thoracic tumors, or pelvic tumors, the average SUVmax of bilateral posterior neck muscles, back muscles, or buttock muscles was calculated, respectively. For statistical analysis, the paired Student's *t*-test was used to evaluate the difference between 100 min and 180 min after injection of F-MISO.

RESULTS

Eight of the 10 patients underwent FMISO PET/CT twice: before RT and during fractionated RT. One patient refused a second F-MISO PET/CT due to the long examination time, and the other patient's second F-MISO PET/CT was cancelled due to acute renal failure caused by chemotherapy. Changes in SUVmax and the T/M ratio 100 and 180 min after injection of F-MISO in the pretreatment F-MISO study are shown in Table 2. Although large variations in F-MISO SUVmax in tumors were observed between 100 min

Table 2. Changes in SUVmax and T/M ratios at 100 and 180 minutes after injection of F-MISO in pretreatment F-MISO studies

No.	T/M ratio		SUVmax of tumor			
	100 min	180 min	100 min	180 min		
1	1.29	1.93	1.57	2.10		
2	2.66	2.66	2.79	3.06		
3	1.74	1.84	2.99	2.72		
4	1.88	1.86	2.25	2.06		
5	1.59	1.95	1.99	1.60		
6	1.65	2.38	2.33	3.12		
7	1.47	1.77	2.34	2.44		
8	1.22	1.08	1.43	1.35		
9	2.32	1.81	3.82	2.60		
10	1.56	1.61	1.75	1.71		
Tumor	1.74 ± 0.45 ^a	1.89 ± 0.42 ^a	NS	2.33 ± 0.72 ^a	2.28 ± 0.61 ^a	NS
Muscle				1.31 ± 0.24 ^b	1.25 ± 0.17 ^b	p = 0.01

^amean ± SD of the 10 tumors. ^bmean ± SD of 108 normal muscle areas.

and 180 min, the mean value of SUV_{max} was similar for 100 min and 180 min. On the other hand, the mean \pm SD of muscle SUV_{max} decreased significantly 180 min after injection compared to after 100 min ($P=0.01$). Because of the decrease in the SUV_{max} of the muscle, the mean value of the T/M ratio at 180 min increased to 1.89 compared with the T/M ratio at 100 min. This means that F-MISO PET/CT images at 180 min had more contrast than those at 100 min. Therefore, we used F-MISO PET/CT imaging 180 minutes after the injection in the present analysis.

To determine the threshold of a hypoxic region, the SUV_{max} of the 108 areas of normal muscle were measured. The mean \pm SD of SUV_{max} for normal muscle 180 min after F-MISO injection was 1.25 ± 0.17 . As the value of the mean + 2 SD was 1.59 SUV, accumulation of F-MISO ≥ 1.60 was regarded as indicating a hypoxic area. Except for one nasopharyngeal tumor (Fig. 1), nine tumors had F-MISO SUV_{max} values of ≥ 1.60 prior to RT, indicating that these tumors contained hypoxic areas before treatment. To obtain high-contrast images and to depict hypoxic areas clearly, the window of F-MISO accumulation was set between 1.6 and 2.0 SUV. In addition, hypoxic volume (HV) was calculated (Table 3). HV was defined as the percentage of hypoxic volume (F-MISO ≥ 1.60) of the primary

tumor or metastatic tumor volume. HV was minimal (0.0–0.3%) for four of the 10 tumors (Table 3). For a patient with nasopharyngeal cancer, no F-MISO accumulation was observed either in the primary tumor or metastatic lymph nodes, although strong FDG uptake was noted (Fig. 1). There was no significant correlation between SUV_{max} of F-MISO and SUV_{max} of FDG accumulation for the 10 patients ($r^2=0.037$).

Reoxygenation of tumors was evaluated in eight tumors on which F-MISO PET/CT studies were performed twice. Six of the eight tumors showed a decrease in SUV_{max} and/or the T/M ratio at approximately 20 Gy (Fig. 2). The remaining two tumors with increased SUV_{max} (cases 3 and 9) showed a decrease in HV, while one tumor with decreased SUV_{max} showed an increase in HV (case 6) (Table 3). Thus, all eight tumors showed a decrease in SUV_{max}, the T/M ratio, or HV, indicating reoxygenation. Figure 3 shows F-MISO PET/CT of a patient with anal canal squamous cell carcinoma (case 7). In this patient, both SUV_{max} and HV decreased in the second F-MISO study with 18 Gy/10 fractions.

The results of F-MISO studies and the initial tumor response of the 10 patients are shown in Table 3. In cases 2, 3, 6 and 9, SUV_{max} of the second F-MISO PET/CT still exceeded 2.5. None of the four tumors showed complete response (CR). On the other hand, four tumors with SUV_{max} of <2.5 at the second F-MISO PET/CT showed CR or partial response (PR) with long term local control (case 5). Similarly, all four tumors with HV of $<6.0\%$ in the second F-MISO study showed CR or PR with long-term local control.

DISCUSSION

In the present study, the threshold of F-MISO PET for hypoxia was determined as an absolute value of F-MISO SUV. Most previous reports used the T/M ratio or T/B ratio in the definition of tumor hypoxia [8–10]. To introduce F-MISO images to RT planning, a threshold of hypoxia as an absolute value of F-MISO SUV is easy to use. In addition, absolute values of F-MISO SUV can be translated to partial pressure for oxygen (pO_2) based on an experimental study [13]. Another advantage of using absolute values of tumor F-MISO SUV is to eliminate errors in measurements of SUV for normal tissues. We measured the absolute values of F-MISO SUV for many muscle points to determine the range of F-MISO SUV of normal muscles. PET alone has limited spatial resolution compared with PET/CT. Therefore, the PET/CT used in the present study could measure the SUV_{max} of many areas of normoxic muscle accurately. The threshold for hypoxia was determined to be SUV of F-MISO equal to 1.60 because the mean SUV_{max} + 2 SD of muscles was 1.59. The range of SUV_{max} in tumors in this study was 1.35–3.12 with a

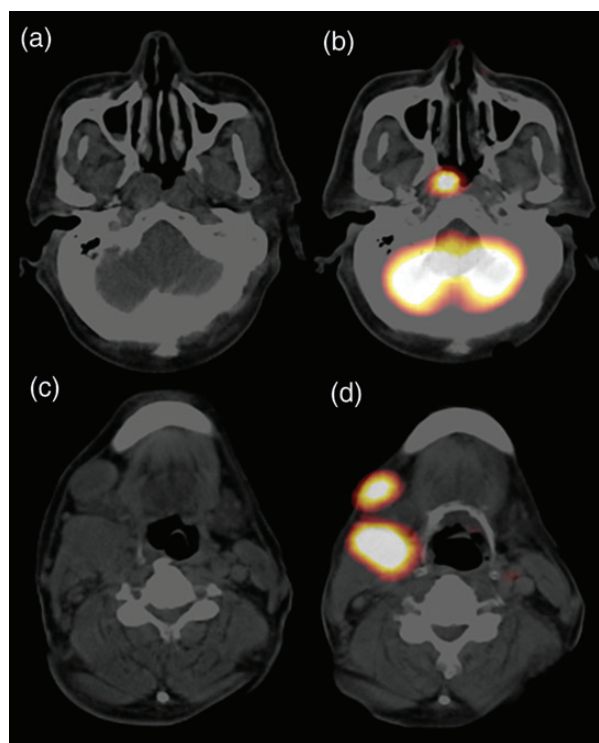


Fig. 1. F-MISO PET/CT (a, c), and FDG PET/CT (b, d) for a patient with nasopharyngeal cancer (case 8: T1N2bM0). In this patient, no significant F-MISO accumulation was observed in the primary tumor (SUV_{max}; 1.20) or metastatic lymph nodes (1.35), although strong FDG uptake was noted.

Table 3. Changes in F-MISO SUVmax and hypoxic volume (HV) and the initial tumor response

No.	Disease	Treatment	1st-FMISO		2nd-FMISO		Tumor Response
			SUVmax	HV(%)	SUVmax	HV(%)	
1	Maxillary ca.	CRT: 42Gy/21fr	2.10	2.7			PD
2	Esophageal ca.	CRT: 60Gy/30fr	3.06	75.6	2.50	24.6	PR
3	Uterine body ca.	RT: 64.4Gy/35fr	2.72	42.3	3.32	36.5	PR
4	NPC	CRT: 70Gy/35fr	2.06	0.3	1.43	0.0	CR
5	Lung ca.	CRT: 60Gy/30fr	1.60	0.0	1.70	0.0	PR ^a
6	Esophageal ca.	CRT: 50Gy/25fr	3.12	6.7	2.69	7.9	NC
7	Anal canal ca.	CRT: 59.4Gy/33fr	2.44	7.6	.08	2.2	CR
8	NPC	CRT: 70Gy/35fr	1.35	0.0			CR
9	Anal canal ca.	CRT: 45Gy/25fr	2.60	65.8	3.16	6.1	PR
10	NPC	CRT: 70Gy/35fr	1.71	0.1	1.32	0.0	CR

^aAlthough the tumor response evaluated by the RECIST criteria was PR, local control was achieved for >2 years.

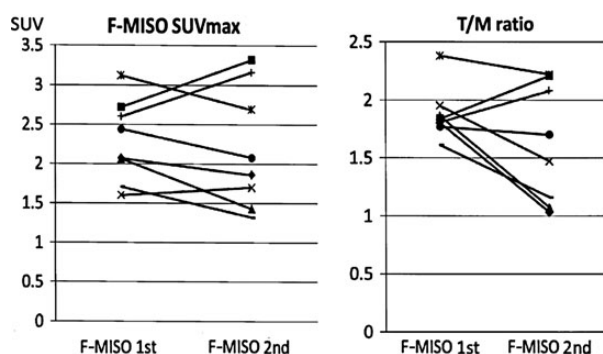


Fig. 2. Changes in F-MISO SUVmax and T/M ratios for eight patients. Six of the eight tumors showed a decrease in SUVmax and/or the T/M ratio after approximately 20 Gy of fractionated RT.

median of 2.1, which is similar to other previous reports [14, 15].

SUVmax of muscles decreased significantly at 180 min after injection of F-MISO compared with that at 100 min. This may be attributable to the fact that F-MISO in tumor hypoxic areas exhibits slow clearance due to its high lipophilicity. Because of the difference in accumulation and clearance of F-MISO between tumors and normal tissues, it has been reported that the T/M ratio increased up to 3 h after injection of F-MISO [10, 16]. This means that F-MISO PET/CT images at 180 min had more contrast than those at 100 min. Therefore, we used F-MISO PET/CT imaging 180 min after the injection in the present analysis.

In terms of oxygen tension in F-MISO-accumulated areas, one animal experiment using a pig liver showed that F-MISO preferentially binds when pO_2 is < 15 mmHg [13]. In that study, the F-MISO SUV of 1.57 corresponded to a pO_2 of 20 mmHg. Therefore, F-MISO-accumulated areas

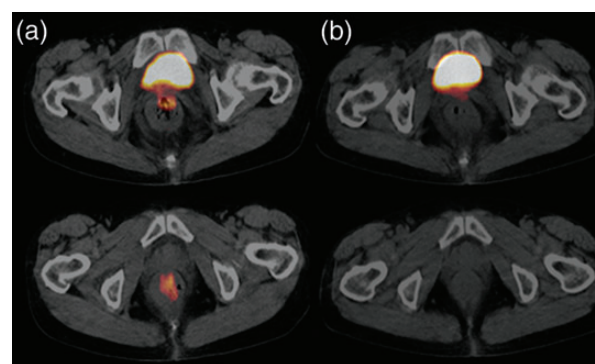


Fig. 3. F-MISO PET/CT (a) before RT, and (b) during RT (18 Gy/10 fractions), for a patient with anal canal squamous cell carcinoma (case 7: T2N0M0). In this patient, F-MISO accumulation in the primary tumor (SUVmax; 2.44) decreased to 2.08 in the second F-MISO study at 18 Gy/10 fractions.

with a threshold of 1.6 SUV in the present study may represent hypoxic areas with a pO_2 of <20 mmHg. The oxygen enhancement ratio (OER) was approximately 3 under well-oxygenated conditions and it decreased for pO_2 below 20 mmHg [17]. The OER decreased to 2.0–2.8 at an oxygen tension of 3–20 mmHg. Thus, the F-MISO-accumulated areas are radioresistant compared with other normoxic regions.

In the present study, SUVmax, the T/M ratio, or HV decreased in all eight tumors after approximately 20 Gy, indicating reoxygenation. It is reported that errors in measurements on SUVmax are approximately 10%, so a decrease in SUVmax of >10% may be significant [18, 19]. Except for case 5, the decrease in SUVmax was >10% for the eight tumors (Table 3). Notably, for case 9, SUVmax increased in the second F-MISO study (Table 3), although HV decreased to 6% from the initial value of 66%. HV may be a more appropriate indicator for reoxygenation than

SUVmax or the T/M ratio because both SUVmax and the T/M ratio are calculated based on the maximum point SUV. The decrease in F-MISO SUVmax may be related to a decrease in blood flow following vascular damage due to RT. However, it has been demonstrated that the functional vascularity in human tumors remains unchanged or improves slightly during the early period of conventional fractionated RT with 1.5–2.0 Gy daily doses [20]. Therefore, we consider that the decrease in F-MISO accumulation indicates reoxygenation of tumor cells.

The information on tumor hypoxia from F-MISO PET/CT can be used for dose painting intensity modulated radiation therapy planning. Although some reports showed dose painting RT plans up to a maximum tumor dose of 84–105 Gy using F-MISO PET/CT [21, 22], no clinical trials for dose escalation to hypoxic areas have been reported. One reason for not performing such clinical trials is that the reproducibility of intratumor distribution of F-MISO is unclear [23]. Nehmeh *et al.* [24] found that only six of 13 human tumors showed well-correlated intratumor distributions of F-MISO after a three-day interval without treatment. The remaining seven tumors showed different distributions of F-MISO after three days. The other factor is reoxygenation of hypoxic areas during fractionated RT. Therefore, dose escalation to the hypoxic areas in the initial PET/CT before RT may be inappropriate. In the near future, if frequent imaging of F-MISO PET/CT is available, adaptive RT for tumor hypoxia may be used clinically.

F-MISO PET/CT obtained before or during fractionated RT can be used as an independent prognostic measure and has implications for treatment strategy. If tumor response can be predicted at the initial period of RT, the treatment strategy can be changed to a more intensive one or a substitute therapy can be started. In the present study, tumors with an SUVmax of >2.5 or an HV of >6.0% in the second F-MISO study showed poor local response. Similar results were shown in various reports on head and neck cancer and lung cancer using pretreatment hypoxia imaging [25, 26]. The predictive value of hypoxic imaging should be evaluated in future studies.

In conclusion, accumulation of F-MISO of ≥ 1.60 SUV was regarded as an intratumoral hypoxic area in our F-MISO PET/CT system. Most human tumors (90%) in this small series had hypoxic areas before RT, although HV was minimal (0.0–0.3%) in four of the 10 tumors. In addition, reoxygenation was observed in most tumors at two weeks of fractionated RT.

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