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

Izumi TACHIBANA1,*, 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

To visualize intratumoral hypoxic areas and their reoxygenation before and during fractionated radiation therapy (RT), 18F-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 ± 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; 18F-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]. 18F-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 pO2 [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 SUVmax 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 30 min, and the mixture was neutralized with 0.3 ml of 1N sodium hydroxide. The solution was passed through a 0.45 μm filter (NITTP, ABX, Montpellier, France) and directly injected into the patient 100 min after injection of F-MISO.

**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/21fr² | 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/25fr² | 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/25fr² | 5-FU, MMC   |
| 10  | 36/M    | 1  | Nasopharynx | T4 N1 M0/Sq    | 42 mm       | 70Gy/35fr  | CDDP        |

²RT was terminated due to severe acute toxicities. ³Preoperative 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 preoperative 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

| No. | T/M ratio | SUVmax of tumor |
|-----|-----------|-----------------|
|     | 100 min   | 180 min         |
| 1   | 1.29      | 1.93            |
| 2   | 2.66      | 2.66            |
| 3   | 1.74      | 1.84            |
| 4   | 1.88      | 1.86            |
| 5   | 1.59      | 1.95            |
| 6   | 1.65      | 2.38            |
| 7   | 1.47      | 1.77            |
| 8   | 1.22      | 1.08            |
| 9   | 2.32      | 1.81            |
| 10  | 1.56      | 1.61            |

Tumor: 1.74 ± 0.45a 1.89 ± 0.42a NS 2.33 ± 0.72a 2.28 ± 0.61a NS
Muscle: 1.31 ± 0.24b 1.25 ± 0.17b p = 0.01

|  | mean ± SD of the 10 tumors. bmean ± SD of 108 normal muscle areas. |
and 180 min, the mean value of SUVmax was similar for 100 min and 180 min. On the other hand, the mean ± SD of muscle SUVmax decreased significantly 180 min after injection compared to after 100 min \( (P = 0.01) \). Because of the decrease in the SUVmax 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 SUVmax of the 108 areas of normal muscle were measured. The mean ± SD of SUVmax 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 \( \geq 1.60 \) was regarded as indicating a hypoxic area. Except for one nasopharyngeal tumor (Fig. 1), nine tumors had F-MISO SUVmax values of \( \geq 1.60 \) 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 \( \geq 1.60 \)) 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 SUVmax of F-MISO and SUVmax 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 SUVmax and/or the T/M ratio at approximately 20 Gy (Fig. 2). The remaining two tumors with increased SUVmax (cases 3 and 9) showed a decrease in HV, while one tumor with decreased SUVmax showed an increase in HV (case 6) (Table 3). Thus, all eight tumors showed a decrease in SUVmax, 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 SUVmax 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, SUVmax 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 SUVmax 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 SUVmax 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 SUVmax + 2 SD of muscles was 1.59. The range of SUVmax in tumors in this study was 1.35–3.12 with a
median of 2.1, which is similar to 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 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 SUV max 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).

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

| No. | Disease          | Treatment | 1st-FMISO SUVmax | 1st-FMISO HV(%) | 2nd-FMISO SUVmax | 2nd-FMISO HV(%) | Tumor Response |
|-----|------------------|-----------|------------------|-----------------|------------------|----------------|---------------|
| 1   | Maxillary ca.    | CRT: 42Gy/21fr | 2.10          | 2.7             | 2.50             | 24.6           | PD            |
| 2   | Esophageal ca.   | CRT: 60Gy/30fr | 3.06          | 75.6            | 2.50             | 42.3           | 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            | 0.0 PR        |
| 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             | 0.08             | 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            |

Although 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.](image)

![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.](image)
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.

**FUNDING**

This study was supported by a Grant-in-Aid for Scientific Research (22591392, 25461932) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

**REFERENCES**

1. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 2007;26:225–39.
2. Asquith JC, Watts ME, Patel K et al. Electron affinic sensitization. V. Radiosensitization of hypoxic bacteria and mammalian cells in vitro by some nitroimidazoles and nitro-pyrazoles. Radiat Res 1974;60:108–18.
3. Chapman JD, Baer K, Lee J. Characteristics of the metabolism-induced binding of misonidazole to hypoxic mammalian cells. Cancer Res 1983;43:1523–8.
4. Koh WJ, Rasey JS, Evans ML et al. Imaging of hypoxia in human tumors with [18F]fluoromisonidazole. Int J Radiat Oncol Biol Phys 1991;22:199–212.
5. Zimny M, Gagel B, DiMartino E et al. FDG—a marker of tumour hypoxia? A comparison with [18F]fluoromisonidazole and pO2-polarography in metastatic head and neck cancer. Eur J Nucl Med Mol Imaging 2006;33:1426–31.
6. von Schulthess GK, Steinert HC, Hany TF. Integrated PET/CT: current applications and future directions. Radiology 2006;238:405–22.
7. Okubo M, Nishimura Y, Nakamatsu K et al. Radiation treatment planning using positron emission and computed tomography for lung and pharyngeal cancers: a multiple-threshold method for [18F]fluoro-2-deoxyglucose activity. Int J Radiat Oncol Biol Phys 2010;77:350–6.
8. Rasey JS, Koh W, Evans ML et al. Quantifying regional hypoxia in human tumors with positron emission tomography of [18F]fluoromisonidazole: a pretherapy study of 37 patients. Int J Radiat Oncol Biol Phys 1996;36:417–28.
9. Rajendran JG, Wilson DC, Conrad EU et al. [(18)F]Fluoromisonidazole uptake on oxygen delivery and tissue oxygenation in the porcine liver. Int J Radiat Oncol Biol Phys 1991;22:199–212.
10. Piert M, Machulla HJ, Becker G et al. Dependency of the [18F]fluoromisonidazole uptake on oxygen delivery and tissue oxygenation in the porcine liver. Nucl Med Biol 2000;27:693–700.
11. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–247.
12. Tang G, Wang M, Tang X et al. Fully automated one-pot synthesis of [18F]fluoromisonidazole. Nucl Med Biol 2005;32:553–8.
13. Pier M, Machulla HJ, Becker G et al. Dependency of the [18F]Fluoromisonidazole uptake on oxygen delivery and tissue oxygenation in the porcine liver. Nucl Med Biol 2000;27:693–700.
14. Yamane T, Kikuchi M, Shinohara S et al. Reduction of [18F]Fluoromisonidazole uptake after neoadjuvant chemotherapy for head and neck squamous cell carcinoma. Mol Imaging Biol 2011;13:227–31.
15. Eschmann SM, Paulsen F, Bedeshem C et al. Hypoxia-imaging with 18F-misonidazole and PET: changes of kinetics during radiotherapy of head-and-neck cancer. Radiother Oncol 2007;83:406–10.
16. Sørensen M, Horsman MR, Cumming P et al. Effect of intratumoral heterogeneity in oxygenation status on FMOISO PET,
autoradiography, and electrode Po2 measurements in murine tumors. *Int J Radiat Oncol Biol Phys* 2005; **62**:854–61.

17. Hall EJ. Radiobiology for the Radiologist. 6th edn. Philadelphia: Lippincott Williams and Wilkins, 2006, pp. 88–89.

18. Nahmias C, Wahl LM. Reproducibility of standardized uptake value measurements determined by 18F-FDG PET in malignant tumors. *J Nucl Med* 2008; **49**:1804–08.

19. Kinahan PE, Fletcher JW. Positron emission tomography–computed tomography standardized uptake values in clinical practice and assessing response to therapy. *Semin Ultrasound CT MR* 2010; **31**:496–505.

20. Park HJ, Griffin RJ, Hui S et al. Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). *Radiat Res* 2012; **177**:311–27.

21. Lee NY, Mechalakos JG, Nehmeh S et al. Fluorine-18-labeled fluoromisonidazole positron emission and computed tomography-guided intensity-modulated radiotherapy for head and neck cancer: a feasibility study. *Int J Radiat Oncol Biol Phys* 2008; **70**:2–13.

22. Choi W, Lee SW, Park SH et al. Planning study for available dose of hypoxic tumor volume using fluorine-18-labeled fluoromisonidazole positron emission tomography for treatment of the head and neck cancer. *Radiother Oncol* 2010; **97**:176–82.

23. Lin Z, Mechalakos J, Nehmeh S et al. The influence of changes in tumor hypoxia on dose-painting treatment plans based on 18F-FMISO positron emission tomography. *Int J Radiat Oncol Biol Phys* 2008; **70**:1219–28.

24. Nehmeh SA, Lee NY, Schroder H et al. Reproducibility of intratumor distribution of 18F-fluoromisonidazole in head and neck cancer. *Int J Radiat Oncol Biol Phys* 2008; **70**:235–42.

25. Dirix P, Vandecaveye V, De Keyzer F et al. Dose painting in radiotherapy for head and neck squamous cell carcinoma: value of repeated functional imaging with (18)F-FDG PET, (18)F-fluoromisonidazole PET, diffusion-weighted MRI, and dynamic contrast-enhanced MRI. *J Nucl Med* 2009; **50**:1020–7.

26. Rajendran JG, Schwartz DL, O’Sullivan J et al. Tumor hypoxia imaging with [F-18]Fluoromisonidazole positron emission tomography in head and neck cancer. *Clin Cancer Res* 2006; **12**:5435–41.