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Original Article

Visualization of tumor hypoxia and re-oxygenation after stereotactic body radiation therapy in early peripheral lung cancer: A prospective study

Masahiro Inada [a,*], Yasumasa Nishimura [a], Kohei Hanaoka [b], Kiyoshi Nakamatsu [a], Hiroshi Doi [a], Takuya Uehara [a], Mikihito Komanishi [b], Kazunari Ishii [c], Hayato Kaida [c], Makoto Hosono [a]

[a] Departments of Radiation Oncology, Kindai University Faculty of Medicine; [b] Division of Positron Emission Tomography, Institute of Advanced Clinical Medicine, Kindai University; and [c] Departments of Radiology, Kindai University Faculty of Medicine, 377-2, Onohigashi, Osakasayama-city, Osaka, Japan

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ABSTRACT

Background and purpose: In this study, fluoromisonidazole positron emission tomography (F-MISO PET/CT) was used to evaluate tumor hypoxia and re-oxygenation in patients with lung tumors treated with stereotactic body radiation therapy (SBRT).

Materials and methods: Patients with T1-2 N0 lung cancer were included in this study. The prescribed dose was 48–52 Gy in four fractions. F-MISO PET/CT was performed twice, before SBRT and 1–3 days after the first irradiation. The maximum standardized uptake value (SUVmax) and tumor/muscle ratio (TMR) were evaluated as indicators of hypoxia. The threshold for hypoxia was defined as a TMR of 1.30 or more.

Results: Between 2016 and 2021, 15 patients were included. Pre-treatment tumor hypoxia was observed in nine tumors (60 %). TMR in all six tumors without pre-treatment hypoxia rose after single high-dose irradiation. In contrast, TMR in six of nine tumors with pre-treatment hypoxia dropped after irradiation, suggesting re-oxygenation. Although no local recurrence was noted, regional and/or distant relapses were seen in four patients (27 %). Of these, three had tumors with abnormal F-MISO uptake. The remaining patient had a tumor without signs of hypoxia on pre-treatment PET/CT. The 2-year progression free survival of patients with tumors with and without pre-treatment hypoxia were 30 % and 63 %, respectively ($p = 0.319$).

Conclusion: Tumor hypoxia reduced after single high-dose irradiation. Tumor with F-MISO uptake seems to be an unfavorable prognostic factor in lung SBRT.

In radiation oncology, tumor hypoxia is a feature indicating treatment resistance and a refractory nature [1]. Numerous studies have demonstrated correlations between existing pre-treatment tumor hypoxia and poor outcomes after definitive radiation therapy [2,3]. Fractionated irradiation is a reasonable strategy to overcome this radio-resistance via the process of re-oxygenation during radiation therapy [4,5]. The releasing of a tumor from hypoxia during radiation therapy reflects the tumor's radiosensivity, and is a promising biomarker for precision medicine [3,6].

Stereotactic body radiation therapy (SBRT) is a standard treatment for early peripheral non-small cell lung cancer (NSCLC). A high single radiation dose is typically delivered in 3–8 fractions as part of SBRT [7]. Better local control can be achieved by a higher biological effective dose (BED) with a small number of high-dose fractions. However, the clinical impact of tumor hypoxia occurring as a biological effect of SBRT is unclear. A schedule with a small number of fractions seems to be disadvantageous in the perspective of re-oxygenation [8–10], and determination of the optimal irradiation schedule for hypofractionated SBRT has been challenging. This prospective study used 18F-Fluoromisonidazole (F-MISO) positron emission tomography (PET) to evaluate the tumor hypoxia of early lung cancer and its re-oxygenation during SBRT [11].

Material and methods

The trial protocol for this prospective study was approved by our institutional review board in April 2016.

* Corresponding author at: Department of Radiation Oncology, Kindai University Faculty of Medicine, 377-2 Onohigashi, Osakasayama, Osaka 589-8511, Japan.
E-mail address: im022012@med.kindai.ac.jp (M. Inada).

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Inclusion criteria

The eligibility criteria were: clinical diagnosis of NSCLC, Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0–2, and age between 20 to 84 years. Clinical diagnosis of NSCLC was defined according to the following condition (i), or conditions (ii) and (iii) together: (i) histologically or cytologically confirmed NSCLC, (ii) increase in tumor size of 1 mm or more or a consolidation/tumor ratio changing to > 50 % from $\leq$ 50 % on consecutive CT scans performed 28 days or more apart, (iii) positive accumulation on 18F-fluorodeoxyglucose-PET/CT. The exclusion criteria were: patients with a tumor of pure ground-glass opacity on CT scan, pregnancy, and patients with severe comorbidity and unsuitable for undergoing PET/CT.

Stereotactic body radiation therapy

The gross tumor volume (GTV) was defined as the total volume of the primary tumor. Tumor motion due to respiration was evaluated using ten-phase four-dimensional CT. The internal gross target volume (IGTV) was generated by GTV accumulation from each phase. When respiratory motion was more than 10 mm, a deep exhalation breath hold technique was used with an Abches system (APEX Medical, Tokyo, Japan). The planning target volume (PTV) was the IGTV plus a margin of at least 5 mm in all directions.

SBRT was performed using three-dimensional conformal radiation therapy (3D-CRT) or volumetric modulated arc therapy (VMAT), which used 6 MV X-rays or 6-MV flattening filter-free X-rays. The prescribed dose was 52 Gy at the isocenter in 4 fractions, or 48 Gy at the dose covering 95 % of the PTV (D95) in 4 fractions. For the isocentric method, the PTV was covered with an 86 % isodose line of 45 Gy for 3D-CRT. For the volumetric method, the maximum dose to the GTV was planned as being from 57.6 Gy to 67.2 Gy with VMAT. All treatment plans were created using an Eclipse treatment planning system (Varian Medical Systems, Palo Alto, CA, USA). The planned radiotherapy was delivered using a TrueBeam linear accelerator (Varian Medical Systems).

F-MISO PET/CT protocol and analysis

Patients were intravenously administered a 7.4 MBq/kg dose of F-MISO. All patients underwent an integrated PET/CT (Discovery 710, GE Healthcare, Milwaukee, WI, USA) examination. The PET/CT scan was started 180 minutes after the administration of F-MISO.

To reduce motion artifacts, respiratory-gated images were acquired over 20 minutes in three-dimensional time-of-flight mode. A respiratory-gating system was used for respiratory motion tracking (Real-time Position Management, Varian Medical Systems). The CT acquisition was performed using a tube voltage of 120 kVp, automated tube current with a noise index of 23, and a rotation time of 0.5 seconds.

All PET images were reconstructed with a block sequential regularized expectation maximization algorithm (BSREM). This algorithm has a regularization parameter termed the β value that controls the strength of the regularization relative to the data statistics. In this study, we adopted a β value of 800 according to our previous studies [12,13]. The PET image properties included a voxel size of 2.6 $\times$ 2.6 $\times$ 3.3 mm, matrix size of 192 $\times$ 192, and display field of view of 500 $\times$ 500 mm.

SBRT and F-MISO PET/CT were performed in three schedule patterns to assess the timing of re-oxygenation. The first F-MISO PET/CT study (pre-PET) was performed before initiation of SBRT in all treatment arms. The second F-MISO PET/CT study (post-PET) was performed 1–3 days after initiation of SBRT but before the second irradiation. The second irradiation was performed 1 day after the second F-MISO PET/CT (post-PET) in all arms. Patients were assigned to receive SBRT according to one of three irradiation and post-PET schedules: (i) a 1-day interval between first irradiation and post-PET (Arm A), (ii) a 2-day interval between first irradiation and post-PET (Arm B), and (iii) a 3-day interval between first irradiation and post-PET (Arm C). Each irradiation was performed at least 2 days apart and the overall treatment time in each arm was 7 to 10 days. Assignment into the study arms was based on the patient registration order.

The maximum standardized uptake value (SUVmax) and tumor/muscle ratio (TMR) were measured for use as hypoxic indicators. To evaluate TMR, a background measurement was obtained by generating ellipsoidal VOIs of approximately 20 cm^3 in back muscles such as rector spinae muscle, rhomboid muscle, and trapezius muscle on the same CT slice as that showing the tumor. The mean voxel value within this back muscle VOI was taken as the background measurement (SUVmean of the muscles) [14]. TMR was calculated as the value of the SUVmax of the tumor divided by the SUVmean of the muscles. The threshold for determining hypoxia was defined as a TMR of 1.30 or more. A change in value of 10 % or more was defined as a notable uptake change between pre-PET and post-PET.

Statistical analysis

To consider the impact of tumor hypoxia on the outcomes of SBRT, recurrence patterns and progression-free survival (PFS) were evaluated. The time to a specific event was defined as the interval from the start of SBRT to the date of the event. All loco-regional or distant tumor progression, and death from any cause, were considered as events. The cumulative time was calculated using the Kaplan-Meier method, and differences between two groups were demonstrated with probability curves and assessed with the log-rank test. A p-value of < 0.05 was considered statistically significant. In addition to the threshold of TMR $\geq$ 1.30 for determining hypoxia, another threshold was estimated using Youden's index and the area under the curve calculated using receiver operating characteristics (ROC) analysis. All statistical analyses were performed using BellCurve for Excel v. 3.20 (Social Survey Research Information Co., ltd. Tokyo, Japan).

Results

Between April 2016 and December 2021, 15 patients were enrolled into this study. The patient and tumor characteristics are summarized in Table 1. All 15 patients underwent F-MISO PET/CT twice, as per the protocol. The patient characteristics and F-MISO PET data for each patient are shown in Table 2.

Although no local recurrence of the primary tumors was observed in any patient, regional nodal and/or distant metastasis were observed in four patients (Table 2). Patient 1 experienced multiple bone metastases and regional lymph node metastasis 8 months after SBRT and died 9 months after SBRT from extreme aggressive disease progression. Patient 2 experienced pleural dis-

Table 1
Patient and tumor characteristics (n = 15).

Sex	Female / Male	3 / 12
Age	Median	79 years
	range	56–83 years
Histology	Ad / Sq / Unknown	5 / 5 / 5
T Stage	T1b / T1c / T2a	9 / 4 / 2
Primary site	RU / RM / RL / LU / LL	5 / 1 / 2 / 6 / 1

Ad: adenocarcinoma, Sq: squamous cell carcinoma, RU: right upper, RM: right middle, RL: right lower, LU: left upper, LL: left lower.

Table 2
Patient and tumor characteristics and changes in F-MISO PET data, tumor outcome, and patient status at the time of last follow-up.

No.	Arm	Age	Sex	Histology	T Stage	GTV volume (cc)	Site	Pre SUVmax	Post SUVmax	Δ SUVmax	Pre TMR	Post TMR	Δ TMR	Recurrence	Last status
1	A	71	M	Ad	T1b	4.2	RU	3.41	3.04	Decrease	4.16	3.71	Decrease	Yes	DOD
2	A	66	M	Ad	T1b	1.9	RU	0.85	1.18	Increase	1.10	1.17	Stable	Yes	DOD
3	A	79	M	Sq	T1c	10.8	LU	1.27	1.55	Increase	1.34	1.37	Stable	No	NED
4	A	82	F	Unknown	T1c	5.9	RL	1.84	1.99	Stable	1.53	1.59	Stable	No	DID
5	A	73	M	Ad	T2a	30.9	RU	1.81	2.02	Increase	1.48	1.79	Increase	Yes	AWD
6	B	69	M	Unknown	T1b	1.5	RU	1.01	1.26	Increase	0.89	1.02	Increase	No	NED
7	B	79	M	Ad	T1b	1.3	RM	1.06	1.40	Increase	1.05	1.23	Increase	No	NED
8	B	79	M	Unknown	T1b	3.2	LU	0.89	1.14	Increase	1.05	1.18	Increase	No	DID
9	B	56	F	Unknown	T1b	4.5	LU	0.60	0.60	Stable	0.58	0.66	Increase	No	NED
10	B	81	M	Sq	T1c	12.7	RL	1.53	1.45	Stable	1.39	1.34	Stable	No	NED
11	C	81	M	Unknown	T1b	2.2	LU	1.84	1.40	Decrease	1.63	1.44	Decrease	No	DID
12	C	77	M	Sq	T2a	19.4	LU	1.44	1.91	Increase	1.19	1.27	Stable	No	NED
13	C	84	M	Sq	T1b	3.5	LU	1.36	1.53	Increase	1.45	1.40	Stable	No	NED
14	C	80	M	Ad	T1b	6.2	LL	3.19	2.84	Decrease	2.20	1.88	Decrease	Yes	NED
15	C	68	F	Sq	T1b	9.2	RU	1.58	1.71	Stable	1.74	1.64	Stable	No	NED

Tumors with hypoxia are underlined. Patients No 1 and No 6 were treated by 3D-CRT, whereas the other 13 patients were treated by VMAT. Patients No 10 and No 11 were treated by the flattening filter-free 6 MV X-rays, whereas the other 13 patients were treated by 6 MV X-rays.
M: male, F: female, Ad: adenocarcinoma, Sq: squamous cell carcinoma, GTV: gross tumor volume, RU: right upper, RM: right middle, RL: right lower, LU: left upper, LL: left lower, Recurrence: regional and/or distant metastasis, TMR: tumor/muscle ratio, NED: no evidence of disease, AWD: alive with disease, DID: dead of intercurrent disease, DOD: dead of disease.

semination and regional node metastasis 5 months after SBRT and died 24 months after SBRT. Patient 5 experienced a single bone metastasis 15 months after SBRT and was still alive with disease 17 months after SBRT. Patient 14 experienced an isolated regional lymph node metastasis and received definitive chemoradiation therapy for it, with no recurrence being observed thereafter.

The F-MISO PET data are summarized in Table 2. Pre-treatment hypoxia was seen in nine patients. After single high-dose irradiation, a notable change in SUVmax and lower TMR were seen in three tumors (patients 1, 11, and 14). These three tumors showed a relatively high TMR (1.63, 2.20, and 4.16) compared with the other 12 tumors (0.58–1.74, median: 1.26). Of the nine patients with tumor hypoxia, regional and/or distant metastases were seen in three (patients 1, 5, and 14). In the remaining six patients without tumor hypoxia, distant metastasis was seen in one (patient 2). As an example, the F-MISO image of patient 11, who showed a notable F-MISO uptake reduction after single high-dose irradiation, is shown in Fig. 1. Of note, the F-MISO uptake values in patients 1 and 14, who experienced disease progression, showed strong pre-PET uptake (TMR = 4.16 and 2.20) and residual uptake after first irradiation (TMR = 3.71 and 1.88). The change in the F-MISO uptake of patient 1 is shown in Fig. 2.

The changes in TMR from pre-PET to post-PET in each arm are shown in Fig. 3. After high dose irradiation, TMR values tended to increase in Arms A and B, whereas they tended to be stable or show a decrease in Arm C. The TMR values in all six tumors that were classified as non-hypoxic were higher after single high-dose irradiation. Although the changes in TMR in the nine tumors with pre-treatment F-MISO uptake showed variation, the TMR values in all five hypoxic tumors enrolled in Arms B and C dropped after single high-dose irradiation.

The 2-year PFS was 30 % (95 % confidence interval: 0 %–65 %) in patients who had tumors with pre-treatment hypoxia and 63 % (95 % confidence interval: 21 %–100 %) in patients who had tumors without hypoxia. The ROC analysis identified an alternative threshold for hypoxia as a pre-PET TMR $\geq$ 1.48. The 2-year PFS was 0 % in patients who had tumors with a pre-PET TMR $\geq$ 1.48, and 74 % in patients who had tumors with pre-PET TMR < 1.48 (95 % confidence interval: 43 %–100 %). Kaplan-Meier curves of PFS according to pre-PET TMR of $\geq$ 1.30 versus TMR < 1.30, and TMR $\geq$ 1.48 versus TMR < 1.48, are shown in Fig. 4. There was no statistically significant difference in PFS between patients who had tumors with pre-PET TMR $\geq$ 1.30 and patients who had tumors with pre-PET TMR < 1.30 (p = 0.319), although there was a statistically significant difference in PFS between patients who had tumors with pre-PET TMR $\geq$ 1.48 and those who had tumors with pre-PET TMR < 1.48 (p = 0.0097). In the terms of post-PET TMR, Kaplan-Meier curves of PFS according to post-PET TMR of $\geq$ 1.30 versus TMR < 1.30 were equivalent to those of according to pre-PET TMR of $\geq$ 1.30 versus TMR < 1.30 (Fig. 4a). The ROC analysis performed in post-PET TMR identified a threshold for PFS as a post-PET TMR $\geq$ 1.44. Similarly, Kaplan-Meier curves of PFS according to post-PET TMR of $\geq$ 1.44 versus TMR < 1.44 were equivalent to those of according to pre-PET TMR of $\geq$ 1.48 versus TMR < 1.48 (Fig. 4b).

Discussion

In the present study, tumor hypoxia and re-oxygenation were evaluated using F-MISO PET/CT in 15 patients with early lung cancer who were treated with SBRT. Nine of the 15 patients had pre-treatment tumor hypoxia according to the definition of TMR $\geq$ 1.30. Patients who had tumors with hypoxia showed a tendency for poorer PFS than patients who had tumors without hypoxia.

One of the problems with evaluating small lung tumors with PET is the deterioration in image quality caused by respiratory motion artifacts [15]. However, the respiratory motion artifacts occurring during PET can be rather useful for determining the margin for the internal target volume (ITV) in radiation planning [16,17], although the respiratory motion has a negative effect on GTV size and SUV measurements. In the present study, the F-MISO PET/CT data were acquired using a respiratory gated method instead of conventional three-dimensional PET. Respiratory gated PET/CT is reported to improve staging accuracy in the assessment of early lung cancer [15], and we believe that inaccuracy due to respiratory motion was minimal in our study.

We found that a notable decrease which corresponding to a reduction in TMR of more than 10 % in F-MISO uptake was observed in three patients. These three patients had relatively high TMR on pre-PET compared with the other 12 patients who showed stable or increasing TMR. We think that this phenomenon can be

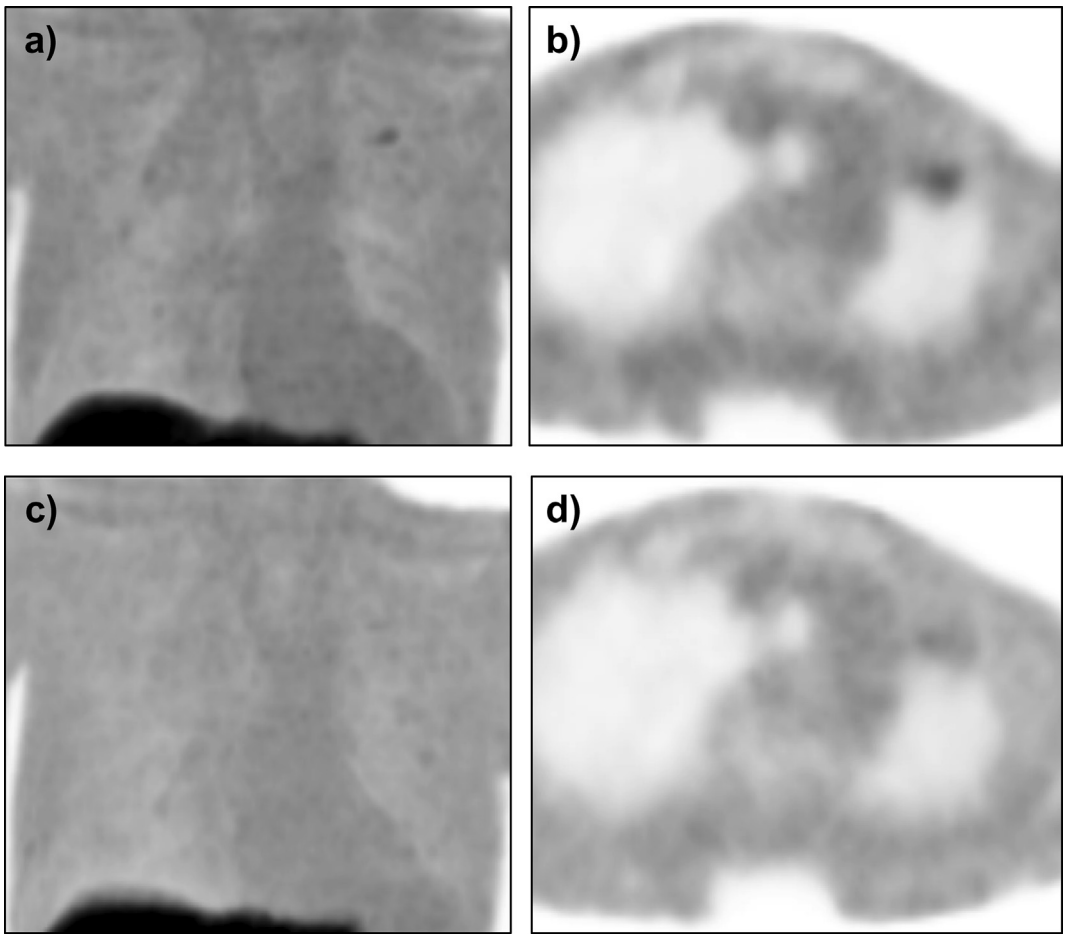

Fig. 1. F-MISO PET image of patient 11 who showed abnormal pre-treatment F-MISO uptake that decreased after single high-dose irradiation; a) pre-treatment maximum intensity projection (MIP) image, b) pre-treatment axial image, c) post-treatment MIP image, d) post-treatment axial image.

explained by re-oxygenation after single high-dose irradiation. A decrease in F-MISO uptake was seen 1 day after single high-dose irradiation in the first patient (patient 1); the tumor re-oxygenation phenomenon seems to occur within 1 day after single high-dose irradiation.

As shown in Fig. 3, F-MISO uptake changes after single high-dose irradiation seemed to show features associated with the post-PET schedule. In Arms A and B, most of the tumors showed increased or stable F-MISO uptake after single high-dose irradiation. We believe the phenomenon of an elevated level of hypoxia after single high-dose irradiation is due to radiation-induced vascular damage [17–19]. Song et al. reported that after 1 to 3 days of 20-Gy irradiation, many murine tumor blood vessels were occluded and levels of HIF-1α (hypoxia induced factor) increased [18]. They also reported that a collapse of the tumor vasculature of murine tumors was observed at an earlier time (<6 hours), and that re-perfusion was seen at 2 days after irradiation [19]. In the present study, F-MISO uptake tended to increase in Arm A and B patients, whereas post-treatment TMR was stable or decreased in Arm C patients (3 days after irradiation). These F-MISO uptake data seem to indicate both the incidence of radiation-induced hypoxia and the release from radiation-induced hypoxia. Our clinical data also seem to be consistent with animal studies [18,19]. The timing of tumor microenvironment changes after single high-dose irradiation may differ between tumors with pre-treatment hypoxia and tumors without pre-treatment hypoxia.

In terms of oncologic outcomes, patients who had tumor with pre-treatment hypoxia showed a trend for poorer PFS than those patients who had tumor without pre-treatment hypoxia. The recurrence pattern in the patient with strong F-MISO uptake (patient 1, TMR = 4.16) was relatively aggressive. Patient 14, who showed high abnormal F-MISO uptake (TMR = 2.20), also experienced early metastasis after SBRT. In these two patients, a substantial decrease in F-MISO uptake was observed, although F-MISO uptake still remained high after single high-dose irradiation. No local recurrence was observed in the present study, and it seems that SBRT can deliver a sufficient BED for local control, including in hypoxic tumor [20]. Adjuvant chemotherapy or immunotherapy may be indicated for F-MISO positive patients, to prevent regional and distant metastasis [21,22].

In the present study, the threshold for defining tumor hypoxia was defined as a TMR $\geq$ 1.30. In previous papers that evaluated lung tumor hypoxia using F-MISO PET, the definition of hypoxic area varied, being defined by absolute SUV, TMR, or TBR (tumor/blood ratio) [23–32]. The ROC analysis performed in the present study suggested a TMR of $\geq$ 1.48 as a promising threshold for PFS. An optimal F-MISO threshold for determining the prognosis of early lung cancer should be investigated in future studies.

Only two previous studies have evaluated F-MISO PET in patients who received lung SBRT [28,33]. Kelda et al. acquired three F-MISO PET acquisitions per patient: before irradiation, 2 days after first irradiation, and 4 days after first irradiation [28]. They defined the hypoxic volume as that with a TBR > 1.2, and evaluated the hypoxic volume as an indicator of hypoxia. They reported that pre-treatment hypoxia was seen in three of five tumors (60 %). Similar to their finding, we found that 60 % of

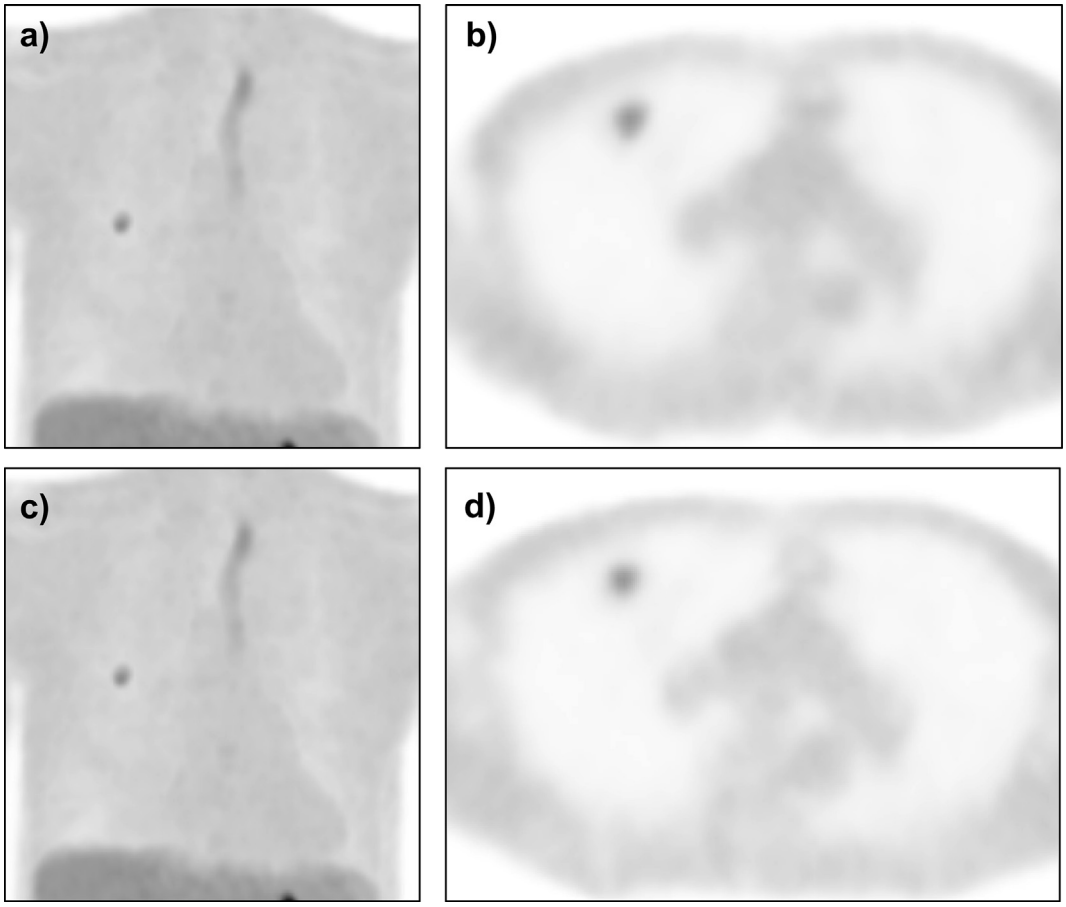

Fig. 2. F-MISO PET image of patient 1 who showed abnormal pre-treatment F-MISO uptake and notable residual uptake after single high-dose irradiation; a) pre-treatment MIP image, b) pre-treatment axial image, c) post-treatment MIP image, d) post-treatment axial image.

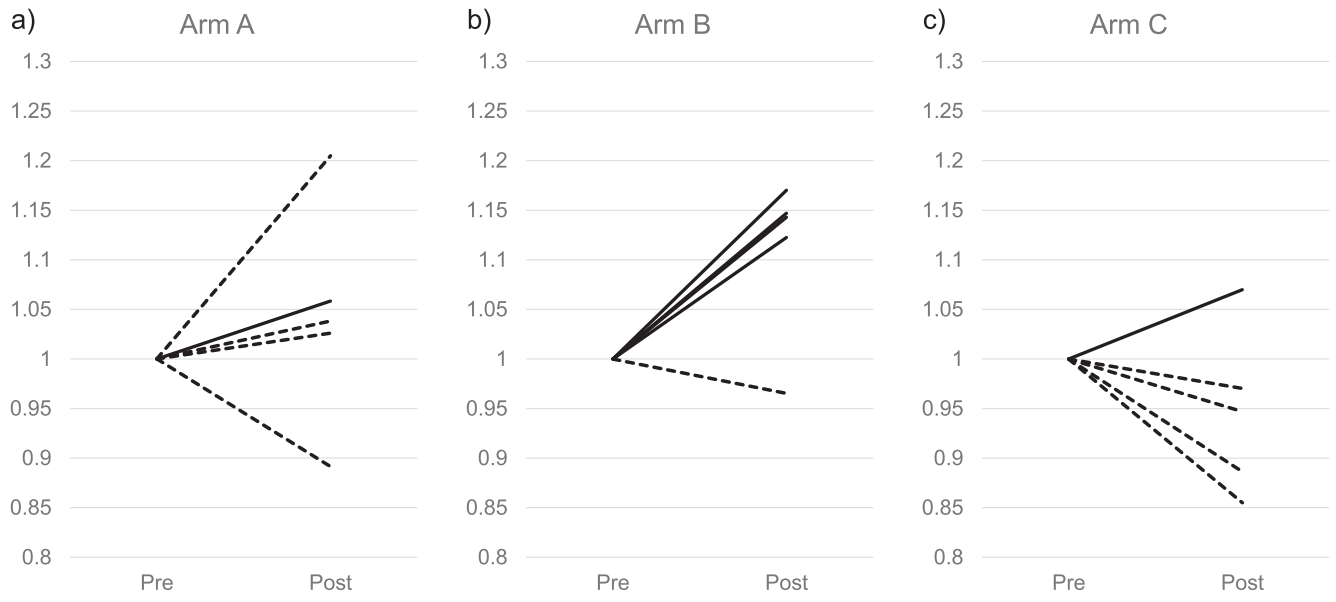

Fig. 3. The rate of change in TMR from pre-PET to post-PET in each tumor according to the treatment schedule arms. Pre-PET values were converted to 1.00 and post-PET values were converted into post-PET TMR divided by pre-PET TMR. a) in Arm A, b) in Arm B, c) in Arm C. Dotted lines indicate tumors with pre-treatment hypoxia, and solid lines tumors without pre-treatment hypoxia.

tumors showed pre-treatment hypoxia in our study. Kelda et al found that the hypoxic volume was higher 2 days after single high-dose irradiation in three of five tumors, with responses on day 4 varying. However, there was no tumor showing re-oxygenation after single high-dose irradiation in their study. Watanabe et al. tested pre-treatment FDG PET and F-MISO PET in

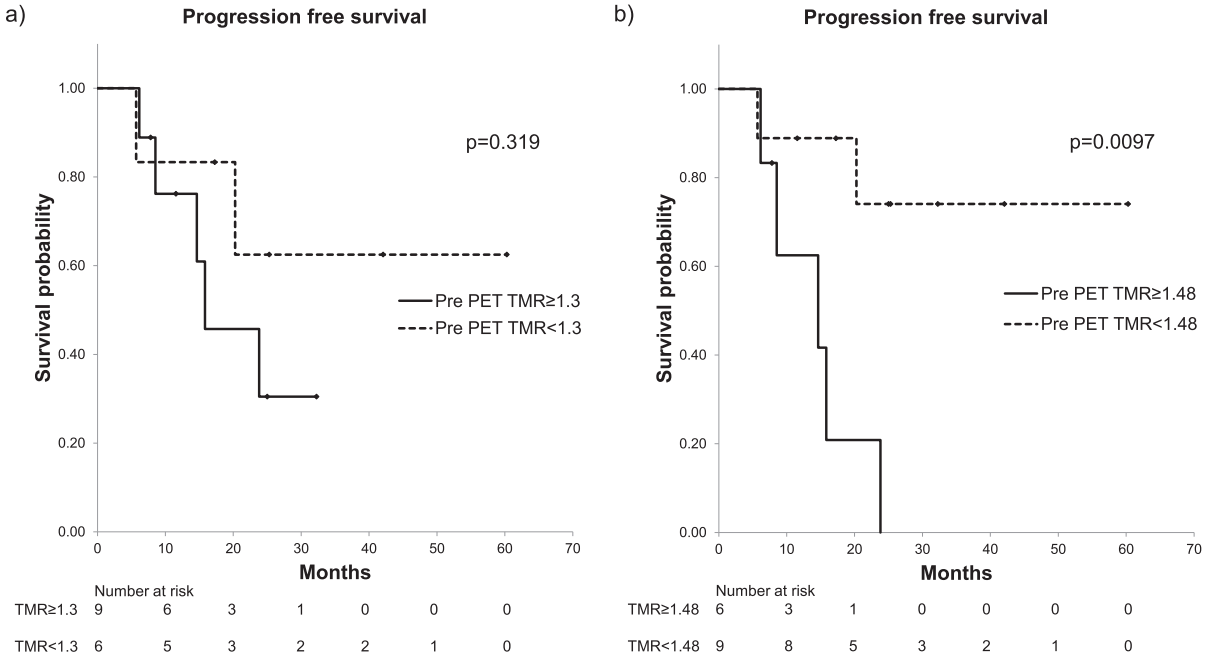

Fig. 4. A) kaplan-meier curves of pfs according to pre-pet tmr ≥ 1.3 versus pre-PET TMR < 1.3. b) Kaplan-Meier curves of PFS according to pre-PET TMR ≥ 1.48 versus pre-PET TMR < 1.48.

32 patients with lung cancer treated with SBRT. They reported that higher uptake of FDG and F-MISO was associated with worse PFS [33]. However, unlike our study, they did not acquire repeated F-MISO PET after SBRT.

Several limitations to the present study should be discussed. The first is the small number of patients; a larger number of patients is needed to verify the effect of the SBRT schedule. However, our pilot data showing the re-oxygenation phenomenon during SBRT are important in that they suggest a novel prognostic factor for patients with early lung cancer. Further prospective studies with a larger number of patients are warranted to demonstrate the efficacy of adjuvant therapy according to tumor hypoxia. In addition, prognostic factors other than hypoxia could not to be sufficiently investigated. As shown in Table 2, the tumor with pre-treatment F-MISO uptake seemed to have a large volume compared with the tumor without pre-treatment F-MISO uptake. However, the two tumors with the highest TMR (patients 1 and 14) had a relatively small volume (4.2 and 6.2 cm^3). In our previous study, we found that higher clinical T Stage was a significant prognostic factor for poor local control and survival [34,35]. Another limitation is that the SBRT technique and prescribed dose were changed during the present trial. In a previous report, a higher maximum dose to the PTV was associated with better local control of lung SBRT [34]. A volumetric method using the VMAT was more effective for escalating the maximum dose compared with 3D-CRT. However, no local recurrence was observed in our study, and we therefore believe that any different effects resulting from the different SBRT techniques were minor. Lastly, one-third of our patients did not receive a histological diagnosis. In further studies, the hypoxic status should be examined histologically.

Conclusions

This study investigated tumor hypoxia and the re-oxygenation phenomenon in human lung tumors using repeated F-MISO PET/CT following SBRT. The F-MISO uptake reduced after single high-dose irradiation, suggesting tumor re-oxygenation. Abnormal F-MISO uptake may be associated with a poor prognosis after SBRT. Further prospective studies with a larger number of patients are warranted to demonstrate how the efficacy of adjuvant therapy is associated with tumor hypoxia.

Ethics statement

This study was approved by the institutional review board of Kindai University Hospital (approval number 28-053) on 16 March 2017.

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CRediT authorship contribution statement

Masahiro Inada: Investigation, Formal analysis, Data curation, Methodology, Resources, Writing – review & editing, Writing – original draft. **Yasumasa Nishimura:** Investigation, Methodology, Conceptualization, Resources, Writing – review & editing, Writing – original draft. **Kohei Hanaoka:** Data curation, Investigation, Resources, Writing – review & editing, Writing – original draft. **Kiyoshi Nakamatsu:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Hiroshi Doi:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Takuya Uehara:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Mikihito Komanishi:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Kazunari Ishii:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Hayato Kaida:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Makoto Hosono:** Investigation, Resources, Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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