The purpose of this study was to investigate the prognostic significance of average iodine density as assessed by dual-energy computed tomography (DE-CT) for lung tumors treated with stereotactic body radiotherapy (SBRT). From March 2011 to August 2014, 93 medically inoperable patients with 74 primary lung cancers and 19 lung metastases underwent DE-CT prior to SBRT of a total dose of 45–60 Gy in 5–10 fractions. Of these 93 patients, nine patients had two lung tumors. Thus, 102 lung tumors were included in this study. DE-CT was performed for pretreatment evaluation. Regions of interest were set for the entire tumor, and average iodine density was obtained using a dedicated imaging software and evaluated with regard to local control. The median follow-up period was 23.4 months (range, 1.5–54.5 months). The median value of the average iodine density was 1.86 mg/cm³ (range, 0.40–9.27 mg/cm³). Two-year local control rates for the high and low average iodine density groups divided by the median value of the average iodine density were 96.9% and 75.7% (P = 0.006), respectively. Tumors with lower average iodine density showed a worse prognosis, possibly reflecting a hypoxic cell population in the tumor. The average iodine density exhibited a significant impact on local control. Our preliminary results indicate that iodine density evaluated using dual-energy spectral CT may be a useful, noninvasive and quantitative assessment of radio-resistance caused by presumably hypoxic cell populations in tumors.

KEYWORDS: dual-energy CT, iodine density, lung cancer, oligometastases, stereotactic body radiotherapy

INTRODUCTION

Stereotactic body radiotherapy (SBRT) is considered a safe and effective treatment for medically inoperable patients with Stage I non-small-cell lung cancer (NSCLC) and lung oligometastases [1–3]. Even for medically resectable Stage I NSCLC, the use of SBRT has gradually been increasing, based on patient requests [4, 5]. Over recent years, the use of SBRT has been extended to other disease conditions and sites, such as recurrent head-and-neck cancer, renal cell carcinoma, prostate cancer, and adrenal metastases [6]. The reason for the wide use of SBRT is its excellent local control associated with a minimum of toxicity. However, some patients exhibit local recurrence after high-dose SBRT. Radioresistance as a predictor of local recurrence, in particular the presence of hypoxic cells in the tumor, has been suggested as a causal factor, and an increase in the hypoxic cell population of lung tumors may correlate with the irradiation dose required for tumor control [7].

Recently, gemstone spectral imaging analysis using dual-energy computed tomography (DE-CT) was introduced, and material

© The Author 2016. Published by Oxford University Press on behalf of The Japan Radiation Research Society and Japanese Society for Radiation Oncology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
densities became measurable [8, 9]. We previously reported a correlation between tumor size and blood volume in lung tumors assessed by dual-energy spectral CT and showed that the evaluation of relative tumor blood volume became possible by measuring iodine densities [10]. A reduction in iodine density, suggesting a decrease in blood flow, may reflect hypoxic cell populations in the tumor [11]. A hypoxic microenvironment plays an important role in tumor development and progression [12]. The purpose of this study was to investigate the prognostic significance of average iodine density assessed by DE-CT for Stage I NSCLC and lung oligometastases following high-dose SBRT.

MATERIALS AND METHODS
Patient and tumor characteristics
From March 2011 to August 2014, 93 medically inoperable patients with 74 primary lung cancers and 19 lung oligometastases who underwent DE-CT prior to SBRT of a total dose of 45–60 Gy in 5–10 fractions were retrospectively reviewed. This study included 68 males and 25 females. The median patient age was 76 years (range, 57–91 years). Of the 93 patients, nine patients had two lung tumors; thus, this study included a total of 102 lung tumors. Patient and tumor characteristics are summarized in Table 1. The median maximum tumor diameter was 20 mm (range, 9–44 mm), and the median gross tumor volume (GTV) was 4.45 cm³ (range, 0.16–25.76 cm³).

The study was approved by the institutional review board of our institution, and written informed consent was obtained from all patients.

Treatment procedure
SBRT was performed using multiple fixed coplanar and non-coplanar beams with a 6 MV linear accelerator (Varian Medical Systems, USA). Patient fixation was performed using a custom-made head rest and an immobilized system [13]. Treatment-planning CT was performed using Optima (GE Healthcare, USA) with a 1.25 mm thickness. If respiratory tumor movement was >1 cm, planning CT was performed by a breath-holding technique using Abches (APEX Medical Inc., Tokyo, Japan). If respiratory tumor movement was <1 cm, planning CT was performed with the 4D-CT technique using a real-time position management system (Varian Medical Systems, USA). A 3D treatment-planning system (XiO, version 4.8, ELECTA, Stockholm, Sweden) was applied for dose calculation. The target margins were as follows: the clinical target volume (CTV) was equal to the GTV or internal target volume (ITV) delineated on CT images displayed with a window level of −300 Hounsfield units (HU) and a window width of 1700 HU; the planning target volume (PTV) was the CTV plus a 5–10 mm margin in all directions. A 5 mm leaf margin was included around the PTV.

An isocentric dose of 50 Gy was given for the PTV in five fractions for T1 tumors (≤3 cm in maximum diameter) and 60 Gy in six fractions for T2 tumors (>3 cm in maximum diameter). For two patients with two tumors located close to each other, the isocentric dose was reduced to 45 Gy in five fractions. For three patients with tumors located near the organ at risk, the isocentric dose was changed to 56 Gy in eight fractions for one patient with a T1 tumor and 60 Gy in 10 fractions for two patients with T2 tumors. The median calculated biologic effective dose, assuming an α/β ratio of 10 Gy (BED10), was 100 Gy (range, 85.5–120 Gy).

Scanning procedure
DE-CT was performed using Discovery CT 750 HD (GE healthcare, USA) for pretreatment evaluation. A fast kilo-voltage (kV) switching method was used for CT imaging. The fast kV switching CT scanning involves the use of a new gantry crystal scintillator detector with a very fast optical response and a high-voltage generator equipped with an ultrafast tube kV switching mechanism. The dose of non-ionic, low-osmolar contrast medium was 600 mg I per kg body weight, with an iodine content of 300 or 350 mg I/ml. The total amount of contrast medium was intravenously injected within 30 s. The scan was started 25 s after initiating the injection of contrast medium. Slices used for data analysis had a thickness of 0.63 mm.

Data analyses and considerations
All CT images were transferred to a workstation (GSI Viewer, GE Healthcare, USA) and were subjected to data analyses. The region of interest (ROI) area was set at the maximum cross-sectional diameter of the tumor, surrounding the whole tumor on the CT image using a pulmonary window (window width, 1000 HU; window level, −700 HU) and the image was converted to an iodine (water) image, as shown in Fig. 1. The average iodine density obtained by gemstone spectral imaging software was evaluated with regard to variables (diagnosis, histology, tumor diameter, and BED10) and local control.

Statistical analysis
The study endpoint was local control. Local control rates were calculated by the Kaplan–Meier method, from the first date of treatment until the date of local recurrence. Local recurrence was diagnosed on the basis of local tumor enlargement on CT, which continued for at least 6 months. 18Fluoro-2-deoxyglucose positron emission tomography and/or histological confirmation was recommended when local recurrence was suspected, but this was not mandatory. Local

Table 1. Characteristics of patients and tumors

| Patient characteristics (n = 93) |
|----------------------------------|
| Age in years, median (range)     | 76 (57–91) |
| Sex (male/female)                | 68/25      |
| Diagnosis                        |            |
| Primary lung cancer              | 74         |
| Lung metastasis                  | 19         |
| Number of targets (1/2)          | 84/9       |
| Tumors (n = 102)                 |            |
| Type (Solid/GGO)                 | 86/16      |
| Tumor diameter in mm, median (range) | 20 (9–44) |
| Histology                        |            |
| Adenocarcinoma                   | 47         |
| Squamous cell carcinoma          | 28         |
| Unknown                          | 27         |

GGO = ground-glass opacity.
recurrence was confirmed by biopsy in three patients. The log-rank test was used to evaluate statistically significant differences among Kaplan–Meier curves in the univariate analyses. Average iodine density (high vs low), diagnosis (primary lung cancer vs lung metastasis), histological type (adenocarcinoma vs squamous cell carcinoma vs unknown), tumor diameter ($\leq 3\, \text{cm} \text{ vs } >3\, \text{cm}$), and BED$_{10}$ ($<100\, \text{Gy} \text{ vs } 120\, \text{Gy}$) were all entered into the log-rank test. The tumor characteristics in the two groups (high and low groups) divided by average iodine density were compared using the chi-square test. Differences were regarded as statistically significant at a probability ($P$) value of $<0.05$. All statistical analyses were performed using SPSS Statistics version 22.0 (IBM, Tokyo, Japan).

## RESULTS

### Treatment outcome

All patients were treated without any case of acute toxicity. The median follow-up period for all patients was 23.4 months (range, 1.5–54.5 months). Thirteen patients had died by the last follow-up because of disease progression (5 patients), progression of other chronic illness (5 patients), other types of cancer (2 patients) and suicide (1 patient). Local recurrence was observed in 11 patients at the last follow-up. The 2-year local control rate for all tumors was 87.4% (95% confidence interval, 79%–95%).

### Evaluation of average iodine density

The median value of the average iodine density was $1.86\, \text{mg/cm}^3$ (range, 0.40–9.27 mg/cm$^3$). The relationships between the high and low average iodine density groups divided by the median value of the average iodine density and the variables (diagnosis, tumor diameter, histology and BED$_{10}$) are summarized in Table 2. No significant differences were observed between the two groups in terms of diagnosis and histology. In contrast, a significant difference was observed in terms of tumor diameter ($P = 0.004$) and BED$_{10}$ ($P = 0.029$).

### Average iodine density and outcome

The 2-year local control rates for the high and low average iodine density groups were 96.9% and 75.7% ($P = 0.006$), respectively, as shown in Fig. 2. Tumors with lower values of average iodine density ($\leq 1.86\, \text{mg/cm}^3$) had a statistically significant worse prognosis.

| Average iodine density                        | $P$ value |
|-----------------------------------------------|-----------|
| High $(n)$ vs Low $(n)$                       |           |
| (Threshold: median)                           |           |

### Table 2. Tumor characteristics divided by average iodine density

| Diagnosis                      | High $(n)$ | Low $(n)$ | $P$ value |
|-------------------------------|------------|-----------|-----------|
| Primary lung cancer           | 38         | 37        | NS        |
| Lung metastasis               | 13         | 14        |           |

| Histological type             | High $(n)$ | Low $(n)$ | $P$ value |
|-------------------------------|------------|-----------|-----------|
| Adenocarcinoma                | 23         | 24        | NS        |
| Squamous cell carcinoma       | 11         | 17        |           |
| Unknown                       | 17         | 10        |           |

| Tumor diameter in mm          |            |           |           |
|-------------------------------|------------|-----------|-----------|
| $\leq 30$                     | 49         | 39        | 0.004     |
| $>30$                         | 2          | 12        |           |

| BED$_{10}$ in Gy              |            |           |           |
|-------------------------------|------------|-----------|-----------|
| $\leq 100$                    | 47         | 39        | 0.029     |
| 120                            | 4          | 12        |           |

NS = not significant, BED = biologically effective dose.

---

Fig. 1. Location of the lung tumor regions of interest (red circle): computed tomography image of the pulmonary window (A); iodine (water) image (B).
We also analyzed differences in local control stratified by diagnosis, histological type, tumor diameter, and BED10 for local control. However, diagnosis, histological type, tumor diameter, and BED10 did not exhibit significant differences with regard to local control (Table 3).

### Table 3. Two-year local control data for all tumors

| Characteristics                | n  | % 2-year LC | P value |
|-------------------------------|----|-------------|---------|
| Average iodine density        |    |             |         |
| High group                    | 51 | 96.9        | 0.006   |
| Low group                     | 51 | 75.7        |         |
| Diagnosis                     |    |             |         |
| Primary lung cancer           | 75 | 85.6        | 0.690   |
| Lung metastasis               | 27 | 93.3        |         |
| Histological type             |    |             |         |
| Adenocarcinoma                | 47 | 93.5        | 0.062   |
| Squamous cell carcinoma       | 28 | 73.9        |         |
| Unknown                       | 27 | 91.1        |         |
| Tumor diameter in mm          |    |             |         |
| ≤30                           | 88 | 90.7        | 0.141   |
| >30                           | 14 | 64.8        |         |
| BED10 in Gy                   |    |             |         |
| ≤100                          | 86 | 90.5        | 0.236   |
| 120                           | 16 | 68.6        |         |

BED = biologically effective dose, LC = local control.

**DISCUSSION**

In subgroup analyses by tumor diameter of ≤3 cm, BED10 of ≤100 Gy, and primary lung cancer, the 2-year local control rates for the high and low average iodine density groups were 96.8% and 81.2% (P = 0.018), 96.7% and 81.2% (P = 0.022) and 96.3% and 72.1% (P = 0.020), respectively (Table 4). In contrast, in subgroup analyses by histological type, no significant difference was noted between adenocarcinoma and squamous cell carcinoma.

**Table 4. Two-year local control data according to average iodine density by subgroup**

| Subgroups                  | n  | % 2-year LC | P value |
|----------------------------|----|-------------|---------|
| Tumor diameter ≤30 mm      |    |             |         |
| High group                 | 49 | 96.8        | 0.018   |
| Low group                  | 39 | 81.2        |         |
| BED10 ≤100 Gy              |    |             |         |
| High group                 | 47 | 96.7        | 0.022   |
| Low group                  | 39 | 81.2        |         |
| Primary lung cancer        |    |             |         |
| High group                 | 38 | 96.3        | 0.020   |
| Low group                  | 37 | 72.1        |         |
| Adenocarcinoma             |    |             |         |
| High group                 | 23 | 100         | 0.125   |
| Low group                  | 24 | 85.9        |         |
| Squamous cell carcinoma    |    |             |         |
| High group                 | 11 | 87.5        | 0.269   |
| Low group                  | 17 | 60.6        |         |

BED = biologically effective dose, LC = local control.

In this study, we found that the average iodine density is a prognostic factor for local control in patients with early-stage NSCLC and lung oligometastasis treated with SBRT. The reduction of average iodine density to a value <1.86 mg/cm³ had a significant negative impact on local control. The distribution of the number of patients divided by average iodine density signifi- cantly differed for tumor size and BED10; however, the average iodine density was also significantly associated with local control in the subgroup with a tumor size of ≤3 cm and a BED10 of ≤100 Gy.

The reduction in average iodine density, suggestive of a decrease in blood flow, may reflect hypoxic cell population activity in the tumor. Rodallec et al. [11] reported an association between tumor enhancement (assessed by contrast-enhanced helical CT) and vascular density (assessed by light microscopy) for endocrine pancreatic tumors. Similar findings using contrast-enhanced helical CT were reported for pulmonary nodules by Yi et al. [14] and for head-and-neck cancer by Ash et al. [15]. A hypoxic microenvironment plays an
important role in tumor development and progression. Jiang et al. [12] accounted for the potential molecular mechanisms underlying the hypoxia-dependent regulation of the epithelial-to-mesenchymal transition (EMT) in cancer, and the hypoxic microenvironment characteristic of cancer cells emerges as an important factor in the induction of a pathological EMT, which is a key feature in cancer progression. We believe that the reduction in average iodine density plays an important role in radioresistance and cancer progression caused by a hypoxic tumor microenvironment.

The association between tumor size and hypoxia has been well documented. Shibamoto et al. [16] investigated the variation in the hypoxic fraction among mouse tumors of different types, sizes and sites and reported that the hypoxic fraction increased by tumor size in EMT6/KU tumors. Ping et al. [17] investigated the expression and significance of hypoxia-inducible factor 1α (HIF-1α) in NSCLC and reported that HIF-1α expression was associated with tumor size. Tumors >3 cm in diameter show significantly higher HIF-1α expression in comparison with those <3 cm. In our study, the average iodine density was significantly associated with tumor size, and tumors >3 cm in diameter showed significantly lower average iodine density. However, reduction in average iodine density was also observed, even in tumors ≤3 cm in diameter, suggesting that hypoxia may occur in small tumors. Various dose fractionation schedules have been used in SBRT for lung cancer [18], and optimum schedules have been sought. In addition, dose escalation studies have been performed depending on tumor size [19] and tumor location [20]. Our results suggest the need for dose escalation studies that take average iodine density into account.

Although DE-CT was first conceived in the 1970s, it has not been widely used clinically [21, 22]. The basic principle of DE-CT is the acquisition of two datasets from the same anatomic location using different kV (usually 80 kV and 140 kV). Recently, two types of DE-CT systems have been introduced: multidetector CT with two X-ray tubes and rapid kV switching [23, 9]. Regardless of the device, DE-CT is based on two distinct capabilities: material differentiation and material identification as well as quantification [24]. DE-CT has a number of advantages compared with conventional helical CT. First, DE-CT can quantify the iodine density, using only contrast-enhanced CT because DE-CT does not always require comparison with plain CT, which enables a reduction in patient radiation exposure. Second, the estimation of average iodine density with DE-CT is not affected by the air in the tumor, unlike enhanced conventional CT [25]. Finally, DE-CT enables the evaluation of material composition, including water, fat, iron, sodium chloride, uric acid, muscle, cystine, copper and calcium content in tumors, and also the effective atomic number of tumors [26]. Therefore, DE-CT is a promising technique with potential clinical applications in the field of radiation oncology.

This study has several limitations. First, it was a retrospective review with a limited number of patients and limited follow-up; hence, further studies with a larger number of study individuals and longer follow-up are warranted to confirm these results. Second, there are many factors that influence the average iodine density of a tumor, including scan delay, total amount of contrast agent, injection rate, the manner in which ROIs have been set with reference to the tumor, and patient characteristics. Third, patient characteristics differed between high and low average iodine density groups divided by average iodine density. Finally, SBRT dosage is subject to variation because the SBRT dose is changed according to tumor size and location. However, this study indicates the potential of DE-CT for predicting radioresistance and also the malignant potential of tumors associated with low blood flow and, presumably, hypoxic conditions.

**CONCLUSIONS**

A reduction in average iodine density had a significant negative impact on local control. Our preliminary results indicate that iodine density assessed by dual-energy spectral CT may offer a useful, non-invasive and quantitative assessment of radioresistance caused by a presumably hypoxic cell population in the tumor. Further studies are required to confirm these results in larger populations with longer follow-ups.

**ACKNOWLEDGEMENTS**

This work was presented at 15th International Congress of Radiation Research, 25–29 May 2015, Kyoto, Japan.

**FUNDING**

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 24591830. Funding to pay the Open Access publication charges for this article was provided by Hirosaki University.

**REFERENCES**

1. Uematsu M, Shiода A, Suda A. Computed tomography-guided frameless stereotactic radiography for stage I non-small-cell lung cancer: 5-year experience. *Int J Radiat Oncol Biol Phys* 2001;51:666–70.
2. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. *JAMA* 2010;303:1070–6.
3. Norihisa Y, Nagata Y, Takayama K, et al. Stereotactic body radiotherapy for oligometastatic lung tumors. *Int J Radiat Oncol Biol Phys* 2008;72:398–403.
4. Onishi H, Shirato H, Nagata Y, et al. Stereotactic body radiotherapy (SBRT) for operable stage I non-small-cell lung cancer: can SBRT be comparable to surgery? *Int J Radiat Oncol Biol Phys* 2011;81:1352–8.
5. Shibamoto Y, Hashizume C, Baba F, et al. Stereotactic body radiotherapy using a radiobiology-based regimen for stage I non-small cell lung cancer: a multicenter study. *Cancer* 2012;118:2078–84.
6. Lo SS, Loblaw A, Chang EL, et al. Emerging applications of stereotactic body radiotherapy. *Future Oncol* 2014;10:1299–310.
7. Fowler JF, Tome WA, Fenwick JD, et al. A challenge to traditional radiation oncology. *Int J Radiat Oncol Biol Phys* 2004;60:1241–56.
8. Matsumoto K, Jinzaki M, Tanami Y, et al. Virtual monochromatic spectral imaging with fast kilovoltage switching: improved image quality as compared with that obtained with conventional 120-kVp CT. *Radiology* 2011;259:257–62.
9. Zhang D, Li X, Liu B. Objective characterization of GE Discovery CT750 HD scanner: gemstone spectral imaging mode. *Med Phys* 2011;38:1178–88.
10. Aoki M, Takai Y, Narita Y, et al. Correlation between tumor size and blood volume in lung tumors: a prospective study on dual-energy gemstone spectral CT imaging. *J Radiat Res* 2014;55:917–23.
11. Rodallec M, Vilgrain V, Couvelard A, et al. Endocrine pancreatic tumors and helical CT: contrast enhancement is correlated with microvascular density, histoprognostic factors and survival. *Pancreatology* 2006;6:77–85.

12. Jiang J, Tang YL, Liang XH. EMT: a new vision of hypoxia promoting cancer progression. *Cancer Biol Ther* 2011;11:714–23.

13. Aoki M, Abe Y, Kondo H, et al. Clinical outcome of stereotactic body radiotherapy of 54 Gy in nine fractions for patients with localized lung tumor using a custom-made immobilization system. *Radiat Med* 2007;25:289–94.

14. Yi CA, Lee KS, Kim EA, et al. Solitary pulmonary nodules: dynamic enhanced multi-detector row CT study and comparison with vascular endothelial growth factor and microvessel density. *Radiology* 2004;233:191–9.

15. Ash L, Teknos TN, Gandhi D, et al. Head and neck squamous cell carcinoma: CT perfusion can help noninvasively predict intratumoral microvessel density. *Radiology* 2009;251:422–8.

16. Shibamoto Y, Yukawa Y, Tsutsui K, et al. Variation in the hypoxic fraction among mouse tumors of different types, sizes, and sites. *Jpn J Cancer Res* 1986;77:908–15.

17. Ping W, Jiang WY, Chen WS, et al. Expression and significance of hypoxia inducible factor-1α and lysyl oxidase in non-small cell lung cancer. *Asian Pac Cancer Prev* 2013;14:3613–8.

18. Chi A, Liao Z, Nguyen NP, et al. Systemic review of the patterns of failure following stereotactic body radiation therapy in early-stage non-small-cell lung cancer: clinical implications. *Radiother Oncol* 2010;94:1–11.

19. Baba F, Shibamoto Y, Ogino H, et al. Clinical outcomes of stereotactic body radiotherapy for stage I non-small cell lung cancer using different doses depending on tumor size. *Radiat Oncol* 2010;5:81.

20. Bral S, Gevaert T, Linthout N, et al. Prospective, risk-adapted strategy of stereotactic body radiotherapy for early-stage non-small-cell lung cancer: results of a Phase II trial. *Int J Radiat Oncol Biol Phys* 2011;80:1343–9.

21. Rutherford RA, Pullan BR, Isherwood I. X-ray energies for effective atomic number determination. *Neuroradiology* 1976;11:23–8.

22. Alvarez RE, Macovski A. Energy-selective reconstructions in X-ray computerized tomography. *Phys Med Biol* 1976;21:733–44.

23. Flohr TG, McCollough CH, Bruder H, et al. First performance evaluation of a dual-source CT (DSCT) system. *Eur Radiol* 2006;16:256–68.

24. De Cecco CN, Darnell A, Rengo M, et al. Dual-energy CT: oncologic applications. *AJR Am J Roentgenol* 2012;199:598–105.

25. Kawai T, Shibamoto Y, Hara M, et al. Can dual-energy CT evaluate contrast enhancement of ground-glass attenuation? *Acad Radiol* 2011;18:682–9.

26. Karçaaltınçab M, Aktaş A. Dual-energy CT revisited with multidetector CT: review of principles and clinical applications. *Diagn Interv Radiol* 2011;17:181–94.