

Peer Review File

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First Round Peer Review

Reviewer A

We appreciate your time spent revising our manuscript. Thank you for your constructive comments. We have revised our manuscript based on your comments.

Comment 1: STROBE checklist for cohort studies, version 4

Missing - "Indicate the study's design with a commonly used term in the title or the abstract"

Reply 1: As you have pointed out, we recognize that this study should be classified as a retrospective study. We have revised the title and the text in the abstract as follows.

Page 1 lines 3-4 in the Title page:

Feasibility and safety of percutaneous cryoablation under local anesthesia for the treatment of malignant lung tumors: a retrospective cohort study

Page 3 lines 53-55 in the Abstract section:

Demographic factors, duration of post-treatment hospitalization, and complication and mortality rates were retrospectively investigated in 366 treatment sessions targeting 609 lesions.

Comment 2: Interstitial pneumonia

The type needs to be adjudicated/determined by an expert pulmonologist (please specify training and experience level) and presented by type in the Results or a Table. Specifically, was UIP more prone to complications or was any interstitial pneumonia associated with complications?

Reply 2: Thank you for your comment. The type of interstitial pneumonia (idiopathic pulmonary fibrosis or not) was initially determined and recorded on medical charts by board-certified respiratory specialists. We have added the type of Interstitial pneumonia

in Table 2. There was no correlation between the type of Interstitial pneumonia and complications after cryoablation ($p = 0.225$). We have revised the text in the Methods section as follows.

Page 7 lines 128 to 130 in the Methods section:

The type of interstitial pneumonia (idiopathic pulmonary fibrosis or not) was initially determined and recorded on medical charts by board-certified respiratory specialists.

Comment 3: Did any patients with metastatic disease receive chemotherapy and/or immunotherapy? Was that a risk factor for complications?

Reply 3: Ninety-four out of 227 patients (41.4%) had a history of chemotherapy. On the other hand, no patients had received the immunotherapy, because the immunotherapy was not authorized during this study period in Japan. Following your comments, we have added the description below to the Methods section and characteristic as “History of chemotherapy” in Table 2 and Table 6. The history of chemotherapy was not a significant predictor of complications after cryoablation.

Page 7 lines 123 in the Methods section:

history of chemotherapy

Comment 4: Some patients contributed more than one tumor. Did some patients contribute more than one ablation session? The Statistical Analysis needs to account for clustered observations

Reply 4: Thank you for your valuable comment. Some patients had two or more ablation sessions (37.2%: 136 of 366 sessions). Following your comments, we have added the description below to the Methods section and characteristic as “No. of sessions” in Table 3 and Table 6. The No. of sessions was not a significant predictor of complications after cryoablation. All 79 complications occurred within 30 days after the cryoablation procedures were considered linkable to the individual cryoablation procedures. Therefore, we did not perform clustered observations in this study.

Page 7 lines 125 in the Methods section:

number of sessions

Comment 5: Please define "poor" PFTs, please add cut-off values in terms of FEV1 and DLCO

Reply 5: As you have pointed out, it is important to define “poor” pulmonary function. Poor pulmonary function is one of the inclusion criteria but not the necessary condition for enrollment. Therefore, no cut-off value was defined for FEV1 or DLCO. In 136 of 227 patients (60%), pretreatment FEV1 was available on medical charts. Twenty-two of 136 patients (16%) had pretreatment FEV1.0% lower than 70%. The insufficient data about pretreatment pulmonary function should be the study limitation. We clarified it in the Discussion section.

Page 17 lines 335 to 338 in the Discussion section:

Because poor pulmonary function is not the necessary condition for enrollment, pretreatment FEV1 was unavailable on medical charts in 40% of the patients. The insufficient data about pretreatment pulmonary function should be the study limitation.

Comment 6: The Endocare system is now with Siemens, please update manufacturer information

Reply 6: Thank you for your comment. As you mentioned, CRYOcare is currently manufactured and sold by Varian Medical Systems, Inc. in the Siemens Healthineers group. We added the description below to the Methods section.

Page 8 lines 143 to 144 in the Methods section:

CRYOcare is currently manufactured and sold by Varian Medical Systems, Inc (Palo Alto, CA, USA).

Comment 7: Why was Cefotiam hydrochloride administered? What dose? Antibiotics prior to lung ablation is not conform with SIR guidelines. Did the authors encounter infection as a complication in their experience?

Reply 7: As pointed out, antibiotics prior to lung ablation does not conform with SIR guidelines.

Intravenous cefotiam hydrochloride was given prophylactically to most patients with 1g three times (before/ after procedure and the POD1). Because we did not have enough evidence about the safety of cryoablation in the early 2000s, our care might have been

overmedication in retrospect. Moreover, we had encountered two sessions with infection (one wound infection and one empyema) as a complication (CTCAE \geq grade2) in our early experience. Those were the reasons for the usage of prophylactic antibiotics in this series. The discrepancy with today's SIR guidelines should be the study limitation. We added the description below as a study limitation to the Discussion section.

Page 8 lines 144 to 146 in the Methods section:

Intravenous cefotiam hydrochloride was administered prophylactically to the patients with 1g three times (before/after the procedure and the postoperative day 1).

Page 17 lines 341 to 346 in the Discussion section:

Moreover, this study included patients in the early 2000s. Because we did not have enough evidence about the safety of cryoablation at the time, our care might have been overmedication in retrospect, including prophylactic antibiotics or frequent CT checkups after the procedure. The discrepancy with today's clinical standards would be the study limitation.

Comment 8: Please clarify “before leaving the ward”. Do the authors mean pre-ablation? At what time interval prior to the ablation? Was respiratory suppression an issue since the oldest patient was 90 (!)

Reply 8: We appreciate your comment about safety. It was difficult for patients to keep the same posture on the CT bed during the procedure for up to 2 hours without any sedation method. Therefore we administered intramuscular pentazocine (15 mg) before leaving the ward. It was about twenty minutes before the start of the procedure. Patients were carried to the CT room on the stretcher for safety. We did not use pentazocine in elderly patients. We clarified this in the Methods section.

Page 8 lines 146 to 149 in the Methods section:

We administered intramuscular pentazocine (15mg) before leaving the ward. It was about twenty minutes before the start of the procedure. Then patients were carried to the CT room on the stretcher for safety.

Comment 9: No severe pain was recorded post cryoablation. This is a key element of the paper since only local anesthetics was used in this series. Please provide details of local anesthesia (amount, drug, where, and how delivered)

Reply 9: Thank you for your invaluable suggestions regarding a key element of our study. Local anesthesia was administered by means of a subcutaneous injection of 1% lidocaine with 10-20ml from the skin down to the pleura. We added the description below to the Methods section. Moreover, we revised the description below in the Results section.

Page 8 lines 153 to 155 in the Methods section:

After the skin was cleansed with iodine, local anesthesia was administered by means of a subcutaneous injection of 1% lidocaine with 10-20ml from the skin down to the pleura.

Page 12 lines 241 to Page 13 lines 243 in the Results section:

The pain during the procedure was self-limiting, and the patients could accomplish a therapeutic procedure with local anesthesia and intramuscular pentazocine only.

Comment 10: Why was a chest CT obtained in every patient at 1 day and 1-week post ablation? Did CT at these timepoints ever change management?

Reply 10: Thank you for your comment. As pointed out, chest CT obtained at 1-week post ablation didn't affect the management. On the other hand, chest CT obtained at 1 day post ablation affect the estimation of the course of pneumothorax especially. This study included patients in the early 2000s. Because we did not have enough evidence about the safety of cryoablation at the time, we performed overmedication on the patients, including frequent CT checkups after the procedure. The discrepancy with today's clinical standards would be the study limitation.

Page 17 lines 341 to 346 in the Discussion section:

Moreover, this study included patients in the early 2000s. Because we did not have enough evidence about the safety of cryoablation at the time, our care might have been overmedication in retrospect, including prophylactic antibiotics or frequent CT checkups after the procedure. The discrepancy with today's clinical standards would be the study limitation.

Comment 11: Why were metastases from lung cancer ablated? What was the clinical rationale?

Reply 11: Thank you for your comment. The standard treatment for pulmonary metastases

from lung cancer is chemotherapy. However, some patients could not tolerate chemotherapy because of physical conditions. And they could be treated by local therapies if the number of metastases was limited. Besides, it was sometimes challenging to distinguish isolated pulmonary metastasis from lung cancer from the second primary lung cancer. In such case, we had performed cryoablation to pulmonary metastasis from lung cancer.

Comment 12: Was the same probe sometimes used to treat multiple tumors?

Reply 12: Thank you for your comment. In 12.0% (44/366) of all sessions, the same probe was used to treat multiple tumors.

Page 11 lines 218 to 220 in the Results section:

In 12.0% (44 of 366) of all sessions, the same probe was used to treat multiple tumors.

Comment 13: When, how, and by who was post-procedural pain assessed? This needs to be explained in the Methods

Reply 13: The thoracic surgeon in charge routinely assessed the post-procedural pain every day according to the Common Terminology Criteria for Adverse Events. We added the description below to the Methods section.

Page 7 lines 134 to Page 8 lines 138 in the Methods section:

Complications were described as an unanticipated problem arising within 30 days post-treatment and defined as grade 2 or higher according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5. The complications, including post-procedural pain, were prospectively graded, and recorded on medical charts every day by a thoracic surgeon in charge.

Comment 14: Who graded the complications? Retrospectively?

Reply 14: The thoracic surgeon in charge prospectively graded the complications precisely according to the Common Terminology Criteria for Adverse Events and recorded it on medical charts.

Page 7 lines 134 to Page 8 lines 138 in the Methods section:

Complications were described as an unanticipated problem arising within 30 days post-

treatment and defined as grade 2 or higher according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5. The complications, including post-procedural pain, were prospectively graded, and recorded on medical charts every day by a thoracic surgeon in charge.

Comment 15: The claim in the Discussion that cryoablation decreases the risk of bronchopleural fistula is not supported by the data provided in this study or the cited references. The authors routinely inject glue following ablation which could explain the absence of BPF in this series

Reply 15: We appreciate your constructive comment. As you pointed out, the hypothesis that cryoablation decreases the risk of bronchopleural fistula could not be proved in this study. Therefore we deleted the description comparing cryoablation and RFA on the incidence of air leakage and revised the paragraph. As you mentioned, fibrin glue might contribute to the absence of BPF in this series. We added the description about it in the Discussion section.

Page 14 lines 269 to 273 in the Discussion section:

Second, the pathway through the outer sheath can be plugged with fibrin glue, which can potentially reduce the occurrence of pneumothorax and bleeding. Wang et al. (15) reported that preserving collagenous structure in frozen tissue facilitates the rapid natural closure of needle tracts. These factors might contribute to the absence of bronchopleural fistula in this series.

Page 15 lines 296 to Page 16 lines 313 in the Discussion section:

Cryoablation offers several advantages over other local therapies. The first advantage is repeatability. SBRT is associated with difficulties treating several lesions with overlapping irradiation fields and retreating local progression after previous SBRT treatment. In contrast, cryoablation can be performed on neighboring lesions or repeated with disease recurrence. The second advantage is the application to the central area where larger bronchi and vessels exist. To date, there are several reports that SBRT and RFA are not suitable for lesions adjacent to hilar structures because of the risk of excessive toxicity (40). Herrera et al. reported death from massive hemoptysis after RFA of a centrally located lung tumor, which resulted in the termination of its use for central nodules (41). Several deaths as a result of massive hemothorax after RFA have also been reported (31,32). In the present study, cryoablation was performed for centrally located

tumors in 104 sessions, and no major complications were reported. Cryoablation was reported to preserve collagenous architecture such as the bronchial wall (42). Yokomise et al. reported that the bronchial wall could resist ultralow temperatures because tracheas obtained for transplantation from cadavers can be deep-frozen (43). On the other hand, the wall of large blood vessels heated by circulating blood during cryoablation resulted in preserving the vessel wall.

Comment 16: Table: Regression model is based on session, please clearly label the table to reflect this

Reply 16: Thank you for your comment. We have revised the title of table 6 as “Clinical predictors of complication according to uni- and multivariate analyses based on the session”.

Reviewer B

Comment 1: This is a retrospective analysis of 227 patients, who underwent 366 sessions of cryoablation for the treatment of malignant primary (56 patients) or metastatic lung (171 patients) in a single institution between July 2002 and December 2016. CRYOcare cryosurgical unit (Endo-Care, Irvine, CA, USA). The patients underwent two different approaches of cryoablation:

Before July 2006, the first and second freezes took 5 min, while the third one took 10 min.

After July 2006, the first freeze took 5 min, and the second and third took 10 min. Active thawing was utilized.

Of note, the authors had published the outcome cryoablation in 117 patients of their current publication before.

Just having a bigger number does not mean the paper would add to the literature. This report offers the same level of scientific evidence compared to the authors prior study on the same but smaller study population. I believe at this point, there are a lot of publication on the safety and feasibility of cryoablations and they authors need to look into something beyond this, such as efficacy compared to surgical approach or different protocols for ablation. Nowadays, there are more specialized

publications based on the underlying cancer, such as:

Comparison of Percutaneous Image-Guided Microwave Ablation and Cryoablation for Sarcoma Lung Metastases: A 10-Year Experience.

Bourgouin PP, Wrobel MM, Mercaldo ND, Murphy MC, Leppelmann KS, Levesque VM, Muniappan A, Silverman SG, Shepard JO, Shyn PB, Fintelmann FJ. *AJR Am J Roentgenol.* 2022 Mar;218(3):494-504. doi: 10.2214/AJR.21.26551. Epub 2021 Oct 6. PMID: 34612679

Or more controlled condition, such as ECLIPSE study:

The ECLIPSE Study: Efficacy of Cryoablation on Metastatic Lung Tumors With a 5-Year Follow-Up.

de Baère T, Woodrum D, Tselikas L, Abtin F, Littrup P, Deschamps F, Suh R, Aoun HD, Callstrom M.J *Thorac Oncol.* 2021 Nov;16(11):1840-1849. doi: 10.1016/j.jtho.2021.07.021. Epub 2021 Aug 9.

Reply 1: We appreciate your constructive comment suggesting further possibilities with this study. We show our opinion as follows.

1. In our previous study (Inoue M, Nakatsuka S, Yashiro H, et al. Percutaneous cryoablation of lung tumors: feasibility and safety. *J Vasc Interv Radiol* 2012;23:295-302; quiz 5), we did not report fatal complications. In the present study, there were two deaths due to acute exacerbation of interstitial pneumonia following prolonged pneumothorax. Therefore, we think the patients with interstitial pneumonia is a relative contraindication for cryoablation. The significance of the present study is that we revealed interstitial pneumonia as a risk factor for death after cryoablation. To clarify it, we added the following description in the Discussion section.

Page 15 lines 292-295 in the Discussion section:

Because we did not report mortality in our previous study on the safety of cryoablation (18), detecting interstitial pneumonia as the risk factor for cryoablation-related death is the clinical significance of this additional report on the safety of cryoablation for lung tumors.

2. In recent years, the impact of race and ethnicity has attracted attention to the safety of treatment in various cancers. The following two articles are for reference. Percutaneous cryoablation for malignant lung tumors has been certificated by FDA in

the United States of America. However, it has not been approved in Japan yet. The significance of the present study is the addition of evidence on the safety of percutaneous cryoablation for Asians to expand the indication of percutaneous cryoablation in Asia.

Pasricha S, Li N, Bulsiewicz WJ, Rothstein RI, et al. Sex and race and/or ethnicity differences in patients undergoing radiofrequency ablation for Barrett's esophagus: results from the U.S. RFA Registry. *Gastrointest Endosc* 2015;82(2):276-84.

Abdelkarem OAI, Choudhury A, Burnet NG, et al. Effect of Race and Ethnicity on Risk of Radiotherapy Toxicity and Implications for Radiogenomics. *Clin Oncol (R Coll Radiol)* 2022; 14:S0936-6555(22)00156-X.

Comment 2: Abstract *Background*: It should be shortened. One statement on background and one on aim of the study.

Reply 2: Thank you for your comment. We have revised the text in the abstract as follows.

Page 3 lines 46-49 in the Abstract Background section:

In our institution, computed tomography-guided percutaneous cryoablation has been performed in patients with malignant lung tumors under local anesthesia. This study aimed to examine the feasibility and safety of percutaneous cryoablation for the treatment of malignant lung tumors.

Comment 3: Abstract *Methods*: Please mention that this is a retrospective study. No need for stat in the abstract

Reply 3: As you have pointed out, we have revised the text in the abstract as follows. And we have deleted stat in the Abstract Methods section.

Page 3 lines 53-55 in the Abstract Methods section:

Demographic factors, duration of post-treatment hospitalization, and complication and mortality rates were retrospectively investigated in 366 treatment sessions targeting 609 lesions.

Comment 4: Abstract *Results*: 1st statement should go to method section of the abstract.

Reply 4: Following your comment, we moved one stat statement to the method section of the abstract.

Page 3 lines 51-53 in the Abstract Methods section:

From July 2002 to December 2016, 227 patients (56 with primary and 171 with metastatic lung cancer) underwent percutaneous cryoablation for the treatment of malignant lung tumors using a cryosurgical unit at our institution.

Comment 5: Abstract *Conclusion*: Should conclude about the aim of the study, “feasibility and safety”

Reply 5: Thank you for your comment. We have revised the text in the Abstract Results and Conclusions section as follows.

Page 3 lines 57-59 in the Abstract Results section:

All the cryoablation procedures were completed under local anesthesia, and the median duration of post-treatment hospitalization was two days.

Page 4 lines 68-70 in the Abstract Conclusions section:

Cryoablation is a feasible and safe treatment for malignant lung tumors with acceptable complication rates. However, the mortality risk in patients with comorbid interstitial pneumonia should be fully recognized.

Comment 6: *Keywords*: Recommend to reorganize them as following to encounter max of search: Lung, cancer, primary, metastasis, cryoablation

Reply 6: Thank you for your comment. We had reorganized Keywords as “Lung, cancer, primary, metastasis, cryoablation”.

Comment 7: Introduction

• **Page 5, Line 82: while the study population of this study composed on primary and metastatic lung cancers, the 1st and 2nd statements of the intro is only about primary cancer patients.**

Reply 7: Thank you for your comment. We revised this, as follows.

Page 5 lines 75-76 in the Introduction section:

Stage I and II non-small cell lung cancer and metastatic lung tumor without extrathoracic disease are treatable by surgery (1-6).

Comment 8: Introduction

- **Page 5, Line 94: should add percutaneous before thermal**

Reply 8: Thank you for your comment. We revised this, as follows.

Page 5 lines 82 in the Introduction section:

percutaneous thermal ablative procedures

Comment 9: Introduction

- **Page 5, Line 99 to page 6, line 102: is redundant and does not explain why this study was required.**

Reply 9: Thank you for your valuable comment. In our previous study, we did not report fatal complications. In the present study, there were two deaths due to acute exacerbation of interstitial pneumonia following prolonged pneumothorax. It is important to reveal the risk factor for complication after cryoablation based on newly accumulated data. We added the description below to the Introduction section.

Page 5 lines 94 in the Introduction section:

In our previous studies, we did not report fatal complications.

Comment 10: Introduction

- **Page 5 and 6L merge the last paragraph of page 5 with first paragraph of page 6.**

Reply 10: Thank you for your comment. We revised this, as follows.

Page 5 lines 84 to 94 in the Introduction section:

Percutaneous image-guided cryoablation is a minimally invasive treatment for cancers. And recent advancements in cryoablation devices have expanded local treatment options for various malignancies (12-15). Wang et al. first reported the percutaneous cryoablation

for lung tumors in 2005 (15). Computed tomography (CT)-guided cryoablation enables visualization of the ablation zone, defined by a consolidative or interstitial shadow or visible ice ball. It delivers complete tumor ablation, while preventing the destruction of adjacent vital structures. In addition, cryoablation can treat tumors adjacent to the visceral pleura without pain or bronchopleural fistula (16). We have performed percutaneous cryoablation for malignant lung tumors since 2002 and reported the preliminary data on its feasibility and safety in 2006 and 2012 (17,18). In our previous studies, we did not report fatal complications.

Comment 11: Introduction

- **Page 6, line 111-112: omit this statement.**

Reply 11: We added the statement following the description in “Submission Checklist”. Should I delete the information in the introduction section?

It should be noted that once your paper is accepted, the reporting checklist you provided will be published as additional information for readers. Therefore: - please indicate at the end of the Introduction section of Main Text: “We present the following article in accordance with the XXX reporting checklist.”

Comment 12: Method

- **Please include IRB approval number.**

Reply 12: Thank you for your comment. We revised this, as follows.

Page 6 lines 110-113 in the Method section:

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Keio University School of Medicine (NO.: 20210003) and the requirement for informed consent was waived owing to the retrospective nature of this study.

Comment 13: Method

- **Were any of these patients on immunotherapy?**

Reply 13: Thank you for your comment. No patients had received the immunotherapy, because the immunotherapy was not authorized during this study period in Japan. We

added the following description in the Results section.

Page 11 lines 207 in the Methods section:

No patients had received the immunotherapy.

Comment 14: Method

- **Did any of patients underwent simultaneous biopsy and ablation?**

Reply 14: Thank you for your comment. In the limited cases, simultaneous biopsy and ablation were performed (8.2%: 30/366 sessions). We added the following description in the Results section.

Page 11 lines 219-220 in the Results section:

Simultaneous tumor biopsy and cryoablation were performed in 8.2 % (30 of 366).

Comment 15: Method

- **Use of a customized 8- or 11-gauge stainless steel coaxial needle with an inner guiding sheath and an outer sheath (Silux, Kawaguchi, Japan) is pretty new approach. An image could help the readers.**

Reply 15: Thank you for your comment. We have added the intraprocedural figure of customized stainless steel coaxial needle with an inner guiding sheath and an outer sheath as Figure 1B. Moreover, we have revised figure legends for Figure 1A.

Legends for Figure 1A

Customized Inner sheath (upper), outer sheaths (middle), and guiding needle (lower) for percutaneous cryoablation for lung tumors.

Legends for Figure 1B

The intraprocedural figure of customized stainless steel coaxial needle with an inner guiding sheath and an outer sheath.

Comment 16: Method

- **Missing years of experience with ablation for interventionists.**

Reply 16: Thank you for your comment. The chief interventionist (SN) had ablation

experience for more than 30 years.

Page 8 lines 151-153 in the Methods section:

The chief Interventionist (SN) had ablation experience for more than 30 years and performed all the 366 sessions in this series.

Comment 17: Method

• **Since two different ablation protocol before and after 2006 have been utilized, the authors need to run sub analysis to investigate safety and efficacy fp each approach.**

Reply 17: Thank you for your comment. We have added the description below to the Methods section and characteristic as “Freezing time” in Table 2 and Table 6. Different Freezing time was not a significant predictor of complications after cryoablation.

Page 7 lines 125 in the Methods section:

cryoablation protocol

Comment 18: Results

2 first two paragraphs, tumor and population characteristics, should be moved to the method section.

Reply 18: Thank you for your comment. We have revised the text in the abstract as follows.

Page 10 lines 195 to Page 11 lines 209 in the Method section:

Tumor characteristics after cryoablation treatment

The diagnosis of 227 patients was primary and metastatic lung cancer in 56 (24.7%) and 171 (75.3%), respectively (primary focus: colon cancer, n = 48; primary lung cancer, n = 41; malignant bone and soft tissue tumor, n = 28; and others, n = 54). The tumor characteristics of 227 patients treated with cryoablation are shown in Table 1.

Patient and tumor characteristics of 366 sessions

As shown in Table 2, the demographic factors of the 366 sessions of treatment were as follows: median age, 64 (16–90) years; male, n = 236 (64.5%); smoking history, n = 193 (52.7%); comorbid diabetes, n = 45 (12.3%); comorbid interstitial pneumonia, n = 37 (10.1%); history of lung resection on the treated side, n = 134 (36.6%), and history of

radiotherapy to the tumor, n = 48 (13.1%). The median diameter of the targeted tumor was 1.3 (range, 0.2–10.2) cm, and the lesions were central (inner one-third of the lung field) in 104 (28.4%) cases.

Comment 19: Discussion

Should start with the main findings of this study.

Avoid biggest study.

Omit “This study presented satisfactory”

Page 16, line 315: SBRT or EBRT could be offered to the lesion in hilar area.

Reply 19:

Thank you for your comment. Following your comment, we have revised the beginning of the Discussion section with the main findings of this study as follows.

Page 13 lines 253-254 in the Discussion section:

In the present study, all 366 cryoablation procedures for lung tumors were completed under local anesthesia and associated with acceptable complication rates.

Following your comment, we have deleted the sentence “the biggest study” and “This study presented satisfactory”.

As you pointed out, it is not appropriate to declare that SBRT and RFA are not suitable for lesions adjacent to hilar structures. We have revised as follows.

Page 15 lines 301-303 in the Discussion section:

To date, there are several reports that SBRT and RFA are not suitable for lesions adjacent to hilar structures because of the risk of excessive toxicity (40).

Second Round Peer Review

Comment 1:

Abstract

l52 - the term "metastatic lung cancer" is confusing. The authors seem to refer to lung metastases from extrathoracic primary malignancies based on l197. Please

update the terminology throughout the manuscript and Table 1

Reply 1: Thank you for your valuable comment. We realized that the term "metastatic lung cancer" is confusing. We have categorized "malignant lung tumor" into "primary lung cancer" and "metastatic lung tumor". We have revised the text and Table 1 as follows.

Page 3 lines 51-52 in the Abstract section:

56 with primary lung cancer and 171 with metastatic lung tumor

Page 5 lines 80-81 in the Introduction section:

primary lung cancer and metastatic lung tumor

Page 10 lines 199 to Page 11 lines 201 in the Methods section:

primary lung cancer in 56 (24.7%) and metastatic lung tumor in 171 (75.3%) (primary focus: colorectal cancer, n = 48; lung cancer, n = 41; malignant bone and soft tissue tumor, n = 28; and others, n = 54), respectively.

Comment 2

Abstract

l60 - according to the 2021 SIR guideline, pneumothorax is no longer considered a "complication" following lung ablation

Reply 2: Thank you for your comment. Two patients died of acute exacerbation of interstitial pneumonia following prolonged air leakage in this study. We consider pneumothorax as a significant complication, especially in patients with interstitial pneumonia. Therefore we included pneumothorax (grade 2 or higher) as a complication in this study, although it is not a complication in the SIR guideline 2021.

Comment 3:

Methods

l115 - how was "poor pulmonary function" defined?

Reply 3: As you have pointed out, it is important to define "poor" pulmonary function. Poor pulmonary function is one of the inclusion criteria but not the necessary condition for enrollment. Therefore, no cut-off value was defined for FEV1 or DLCO. In 136 of 227 patients (60%), pretreatment FEV1 was available on medical charts. Twenty-two of

136 patients (16%) had pretreatment FEV1.0% lower than 70%. The insufficient data about pretreatment pulmonary function should be the study limitation. We have clarified it in the Discussion section.

Page 18 lines 347-350 in the Discussion section:

Because poor pulmonary function is not the necessary condition for enrollment, pretreatment FEV1 was unavailable on medical charts in 40% of the patients. The insufficient data about pretreatment pulmonary function should be the study limitation.

Comment 4:

Methods

1116 - how was "advanced age" defined?

Reply 4: Thank you for your valuable comment. We defined “advanced age” as 75 years or older. It meets the definition of “latter stage elderly” by World Health Organization. We have revised the text as follows.

Page 7 lines 116 in the Methods section:

advanced age (75 years or more)

Comment 5:

Methods

1134 - since the authors grade according to CTCAE suggest replacing "complication" with "adverse event" throughout

Reply 5: We appreciate your comment. We have replaced "complication" with "adverse event" throughout the manuscript and Tables.

Comment 6:

Methods

1137 - what scale was used to grade pain?

Reply 6: Thank you for your comment. The adverse events, including post-procedural pain, were prospectively graded according to the Common Terminology Criteria for Adverse Events, and recorded on medical charts every day by a thoracic surgeon in charge. We have clarified the description below in the Methods section.

Page 7 lines 134 to Page 8 lines 139 in the Methods section:

Adverse events were defined as an unanticipated problem arising within 30 days post-treatment, of grade 2 or higher according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5. The adverse events, including post-procedural pain, were prospectively graded, and recorded on medical charts every day by a thoracic surgeon in charge.

Comment 7:

Methods

I157 - was the 8 or 11-gauge needle advanced across the pleura into the lung?

Reply 7: Yes it was. We used the 8 or 11-gauge co-axial needle to place the cryoprobe into hard tumors following the insertion of a 21-gauge guiding needle. And we plugged fibrin glue into the needle tract before removing the 8 or 11-gauge needle to prevent pneumothorax or hemorrhage. We reported the method in our previous literature [1,2]. We explained the detail in the Discussion section.

Page 14 line 278 to page 15 line 285 in the Discussion section.

The ablation needle used in this study (8–11 gauges) was thicker than an RFA needle (17 gauge) and, thus, could create a larger needle hole on the visceral pleura. However, this modified coaxial system offers two advantages. First, it enables a more precise penetration of the targeted tumor. Specifically, because the lung parenchyma is soft and lung tumors are easily moved, the cutting ability of the cryoprobe is inadequate to penetrate solid or small tumors. Second, the pathway through the outer sheath can be plugged with fibrin glue, which can potentially reduce the occurrence of pneumothorax and bleeding.

Reference:

[1] Kawamura M, Izumi Y, Tsukada N, Asakura K, Sugiura H, Yashiro H, Nakano K, Nakatsuka S, Kuribayashi S, Kobayashi K. Percutaneous cryoablation of small pulmonary malignant tumors under computed tomographic guidance with local anesthesia for nonsurgical candidates. *J Thorac Cardiovasc Surg.* 2006 May;131(5):1007-13. doi: 10.1016/j.jtcvs.2005.12.051. PMID: 16678583.

[2] Inoue M, Nakatsuka S, Yashiro H, Ito N, Izumi Y, Yamauchi Y, Hashimoto K, Asakura K, Tsukada N, Kawamura M, Nomori H, Kuribayashi S. Percutaneous cryoablation of

lung tumors: feasibility and safety. *J Vasc Interv Radiol*. 2012 Mar;23(3):295-302; quiz 305. doi: 10.1016/j.jvir.2011.11.019. Epub 2012 Jan 20. PMID: 22265246.

Comment 8:

Methods

1170 - did the authors inject the glue into the tract? please explain. this is a relevant detail because the 8G introducer needle used to puncture the pleura is HUGE

Reply 8: We appreciate your comment. As we described above, we used the 8 or 11-gauge co-axial needle to place the cryoprobe into hard tumors following the insertion of a 21-gauge guiding needle. And we plugged fibrin glue into the needle tract before removing the 8 or 11-gauge needle to prevent pneumothorax or hemorrhage. We reported the method in our previous literature [1,2]. We explained the detail in the Discussion section.

Page 14 line 278 to page 15 line 285 in the Discussion section.

The ablation needle used in this study (8–11 gauges) was thicker than an RFA needle (17 gauge) and, thus, could create a larger needle hole on the visceral pleura. However, this modified coaxial system offers two advantages. First, it enables a more precise penetration of the targeted tumor. Specifically, because the lung parenchyma is soft and lung tumors are easily moved, the cutting ability of the cryoprobe is inadequate to penetrate solid or small tumors. Second, the pathway through the outer sheath can be plugged with fibrin glue, which can potentially reduce the occurrence of pneumothorax and bleeding.

Reference:

[1] Kawamura M, Izumi Y, Tsukada N, Asakura K, Sugiura H, Yashiro H, Nakano K, Nakatsuka S, Kuribayashi S, Kobayashi K. Percutaneous cryoablation of small pulmonary malignant tumors under computed tomographic guidance with local anesthesia for nonsurgical candidates. *J Thorac Cardiovasc Surg*. 2006 May;131(5):1007-13. doi: 10.1016/j.jtcvs.2005.12.051. PMID: 16678583.

[2] Inoue M, Nakatsuka S, Yashiro H, Ito N, Izumi Y, Yamauchi Y, Hashimoto K, Asakura K, Tsukada N, Kawamura M, Nomori H, Kuribayashi S. Percutaneous cryoablation of lung tumors: feasibility and safety. *J Vasc Interv Radiol*. 2012 Mar;23(3):295-302; quiz 305. doi: 10.1016/j.jvir.2011.11.019. Epub 2012 Jan 20. PMID: 22265246.

Comment 9:

Methods

1187 - two points for the statistical analysis.

a) please clearly state which parameters were considered for the multivariate analysis and why. was it only the ones with a p-value <0.05 in the univariate analysis like in a stepwise selection?

b) since some patients underwent more than one session, the data is clustered which needs to be accounted for in the statistical analysis. strongly recommend considering generalized estimating equations and review by a PhD-level statistician

Reply 9: Thank you for your splendid comment. Based on your comment, we had a consultation about statistics with a PhD-level statistics specialist Yasunori Sato (Associate Professor, Department of Preventive Medicine and Public Health, Keio University School of Medicine).

a) All tests were two-sided, and $p < .05$ were considered statistically significant. And factors that were significant ($p < .05$) in the univariate analysis were entered into the multivariate analysis. We have revised the text in the Methods section as follows.

Page 10 lines 192-194 in the Methods section:

All tests were two-sided, and $p < .05$ were considered statistically significant. Factors that were significant ($p < .05$) in the univariate analysis were entered into the multivariate analysis.

b) In the previous version of the manuscript, we indexed the adverse events on a “per-session basis”. As you pointed out, the impact of multiple sessions on the same patient was not taken into account. Based on your comment, we did a consultation with a chief statistician in our statistics unit (Professor Yasunori Sato, Department of Preventive Medicine and Public Health, Keio University School of Medicine). Following his proposal, we performed additional analyses of the adverse events on a per-patient basis. He considered it possible to assess the impact of multiple sessions on the same patient by this method. As a result, significant predictors of adverse events on a per-patient basis analysis were the same as those on a per-session basis analysis. We had added the per-patient basis data as supplemental files (Supplemental table1-4). We have revised the text in the Results section and added Acknowledgments as follows.

Page13 lines 260 to page14 lines 264 in the Results section:

We performed additional analysis on a per-patient basis to consider the impact of multiple sessions on the same patient. The data was shown in *Supplemental Table 1-4*. The predictors for adverse events identified by both analyses were the same (*Supplemental Table 4*).

Page 19 lines 375-377:

Acknowledgments

The authors thank Yasunori Sato, Department of Preventive Medicine and Public Health, Keio University School of Medicine, for his help with statistical analysis.

Comment 10:

Methods

I203 - is 16-90 the age range?

Reply 10: Thank you for your comment. As you pointed out, the age range was 16 to 90 years old. Clinical diagnosis of teenagers (3 patients) was metastatic lung tumor from malignant bone and soft tissue tumor. And clinical diagnosis of a 90-year-old patient was metastatic lung tumor from primary lung cancer. They had met the inclusion criteria of the present study.

Comment 11:

Methods

I204 - how many of the interstitial pneumonias were classified as UIP pattern?

Reply 11: Thank you for your comment. In the present study, there were 13 of 227 (5.7%) patients who were diagnosed as idiopathic pulmonary fibrosis. And all 13 patients with idiopathic pulmonary fibrosis were diagnosed as having UIP patterns by diagnostic radiologists. We have revised the text in the Methods section as follows.

Page 11 lines 210 in the Methods section:

All patients with IPF had usual interstitial pneumonia (UIP) patterns on chest CT.

Comment 12:

Results

I223 - how is "severe pain" defined? the discussion refers to "significant" pain on

I317. please unify the language

Reply 12: Thank you for your comment. The Common Terminology Criteria for Adverse Events (CTCAE) defines Grade 2 and 3 pains as “moderate pain, pain or analgesics interfering with function but not interfering with ADL” and “severe pain, pain or analgesics severely interfering with ADL”, respectively. We have revised the terminology “severe pain” to “moderate to severe (grade 2 or higher) pain” according to the CTCAE, version 5 as follows.

Page 17 lines 331 in the Discussion section:
moderate to severe (grade 2 or higher) pain

Comment 13:

Results

I225 - the incidence of complications may be best indexed to ablation sessions, not patients. language in the discussion I255 it may actually relate to sessions? please clarify

Reply 13: Thank you for your comment. As you pointed out, adverse events were collected on a per-session basis in the present study. Thus, we have revised the text in the Discussion section as follows.

Page 14 lines 268-269 in the Discussion section:
Adverse events were observed in 79 (21.6%) sessions

Comment 14:

Results

I229 - what type of interstitial pneumonia did the patients have who died?

Reply 14: Thank you for your comment. In the present study, there were two deaths due to acute exacerbation of interstitial pneumonia following prolonged pneumothorax. Type of interstitial pneumonia of these patients was “idiopathic pulmonary fibrosis”. We added the patients’ information in the Results section to clarify this.

Page 12 lines 231-233 in the Results section:
There was no 30-day mortality; however, there were two 60-day mortalities (0.5%) due

to acute exacerbation of interstitial pneumonia following prolonged air leakage. Both patients had IPF with UIP pattern on chest CT.

Comment 15:

Results

I230 - again, if you use CTCAE, please use the term "adverse event" instead of complications

Reply 15: We appreciate your comment. We have replaced "complication" with "adverse event" throughout the manuscript and Tables.

Comment 16:

Results

I234 - were all six instances of hypoxemia in the same patient?

Reply 16: Thank you for your comment. There were six patients (not the same patient) who had hypoxemia. They did oxygen inhalation until the next morning. We added the description below to the Results section to clarify this.

Page 13 lines 243-245 in the Results section:

Hypoxemia occurred after six (1.6%) of the 366 sessions. However, all six patients recovered with oxygen inhalation within 24 hours.

Comment 17:

Results

I234 - how is "transient" defined? hours? days? weeks? months?

Reply 17: Thank you for your comment. "transient" means "within a span of twenty-four hours". The patients with hypoxemia did oxygen inhalation until the next morning . We added the description below to the Results section to clarify this.

Page 13 lines 243-245 in the Results section:

Hypoxemia occurred after six (1.6%) of the 366 sessions. However, all six patients recovered with oxygen inhalation within 24 hours.

Comment 18:

Results

I235 - when in relation to the ablation did the onset of acute exacerbation occur? how was it diagnosed and defined?

Reply 18: Thank you for your comment. We had two 60-day mortalities from acute exacerbation. Both patients had received home oxygen therapy due to IPF with UIP pattern and developed prolonged air leakage after the cryoablation. A 64-year-old man with primary lung cancer was diagnosed as developing an acute exacerbation on the 30 post-treatment day and died on the 38 post-treatment day. An 85-year-old man with metastatic lung tumor from esophageal cancer developed an acute exacerbation on the 18 post-treatment day and died on the 44 post-treatment day. To clarify this, we added the description below to the results section.

Page 12 lines 233-239 in the Results section:

They had received home oxygen therapy due to IPF with UIP pattern and developed prolonged air leakage after the cryoablation. A 64-year-old man with primary lung cancer was diagnosed as developing an acute exacerbation on the 30 post-treatment day and died on the 38 post-treatment day. An 85-year-old man with metastatic lung tumor from esophageal cancer developed an acute exacerbation on the 18 post-treatment day and died on the 44 post-treatment day.

Comment 19:

Discussion

I273 - the incidence of bronchopleural fistula may be related to treating peripheral lesions. does this bear out in your data?

Reply 19: As you mentioned, the incidence of the bronchopleural fistula may be related to cryoablation for subpleural lesions. However, we had no cases that underwent surgical intervention for prolonged air leakage. Moreover, two patients who died of acute exacerbation of interstitial pneumonia following prolonged air leakage did not have a subpleural lesion. Therefore, we can not show the correlation between bronchopleural fistula and treating a subpleural lesion in this study.

Comment 20:

Discussion

I323 - intercostal nerve injury is a complication that needs to be included in Table 5.

this aspect was not previously presented. new data is not admissible in the discussion

Reply 20: Thank you for your comment. Post-procedural pain, including pain from intercostal nerve injury, of grade2 or higher was recorded as adverse events. However, there were no cases with grade2 or higher post-procedural pain, so we did not have sufficient data about grade1 post-procedural pain. Thus, we have deleted the following inappropriate sentence in the Discussion section.

“However, some patients complained of dull pain in the anterior chest after treatment, which was likely related to freezing of the intercostal nerves, and typically disappeared within a few months. This may explain why the intercostal nerve pain in most patients in the present study was recoverable.”

Comment 21:

Discussion

I356 - please quote the actual incidence of adverse events grade 3 and higher

Reply 21: Thank you for your comment. We have revised the text in the Discussion section as follows.

Page 19 lines 369-371 in the Discussion section:

The grade 3 or higher adverse event rate was 1.4%, suggesting that it can be safely performed.

Comment 22:

Tables

tables 2 and 6 - how is "history of chemotherapy" defined?

Reply 22: Thank you for your comment. "chemotherapy" means “cytotoxic chemotherapy and molecular targeted therapy”. We have revised the text in the Methods section as follows.

Page 7 lines 123-124 in the Methods section:

history of chemotherapy (cytotoxic chemotherapy and molecular targeted therapy)

Comment 23:

Tables

table 5 - specify in the title that this analysis is per session

Reply 23: Thank you for your comment. We revised the title as “Adverse events (grade 2 or higher) of cryoablation for malignant lung tumors of 366 sessions”.

Comment 24:

Tables

table 6 - was the test looking for association with adverse events grade 2 or higher? I did not see any grade 1 adverse events listed.

Reply 24: Thank you for your comment. We recorded grade 2 or higher adverse events only. To clarify this, we revised the title as “Clinical predictors of adverse event (grade 2 or higher) according to uni- and multivariate analyses based on the sessions”.