**Peer Review File**
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**Reviewer A**

**Comment 1:**
It is a very interesting manuscript regarding a novel endobronchial injection device for sentinel lymph node detection in NSCLC. Although SLN procedure is currently the standard of care for patients with breast cancer or melanoma, these methods have not been successfully translated to lung cancer.
I think that this study showed that it is feasible to perform endobronchial injections with the use of Pioneer Plus without being able to determine the effectiveness in SLN detection.
It was mentioned that the authors tried to inject the tracer either peritumorally or intratumorally with the exception of two specimens that 99mTc-nanocolloid and indocyanine green were injected with both ways. Do you believe that if you inject the tracer both intratumorally and peritumorally, this would increase sensitivity and detection rate?
**Reply 1:**
We thank the reviewer for their time to thoroughly read this article, the kind appraisal and relevant question. We believe sensitivity and detection depends on the accessibility of the tumor endobronchially. In two instances, it was needed to inject both intra- and peritumorally to be able to cover more tumor volume. However, since in this ex vivo experiment no lymphatic drainage is present, we cannot answer this question at this stage and hope to get this answer from follow-up experiments.
**Changes in text:**
Not applicable.

**Comment 2:**
Is it required any learning curve for Pioneer plus or it can be handled successfully by everyone who is familiar with endobronchial devices
**Reply 1:**
We thank the reviewer for this comment, as it is indeed important to underline that the Pioneer Plus device is very easy to handle. We believe that even physicians who are less familiar with endobronchial devices would be able to understand its mechanisms.
**Changes in text:**
We have added two sentences in the Results section to address the handling of the Pioneer Plus and the imaging console (see Page 3, lines 220-224).

**Reviewer B**

**Comment 1:**
This is a preliminary experiment of sentinel node detection by transbronchial tracer injection using resected lung with lung cancer.
The concept of this study would be significant. However, this preliminary experiment only showed the tracer could be injected into the target lesion by using the IVUS catheter.
We could now approach the target lesion precisely by using state-of-the-art technology such as robotic bronchoscopy.
The biggest issue of exploring sentinel lymph node detection of lung cancer was very complex lymphatic drainage of the lung and difficult detection of the meaningful target lymph node.
Unfortunately, this experiment used an ex-vivo lung, and the authors could not observe the spread of tracers within the lung. The result of this study was that the authors could inject the tracers into the target, neither more nor less.
**Reply 1:**
We would like to thank the reviewer. We agree that our study is exploratory, and indeed has the goal to prepare for in-vivo use of this tool. The new robotic platforms indeed offer better control of the distal and of the catheter which may aid navigation. Yet, also robotic bronchoscopy has shown a gap between navigation success and diagnostic success. Therefore, we are convinced that also in combination with robotic platforms there will be added value of using devices that allow instrument placement under real-time ultrasound guidance for sentinel node procedures.
**Changes in text:**
Not applicable.

**Reviewer C**

This is a well written paper describing the first steps in developing a lung cancer SLN procedure which will be of interest to those working in the lung cancer field.
In the final paragraph of the discussion about limitations of SPECT-CT scanning, there are the described limitations of increased attenuation from soft tissue and the half life of Technetium. However the most important factor which is likely to affect the SPECT-CT acquisition parameters is that only a very small proportion of the injected tracer will drain to the lymph nodes. New SPECT-CT parameters (particularly increased imaging time) may need to be developed to actually detect the activity in the SLN, rather than just imaging the activity in the tumour. This should be described/explained.

**Reply 1:**

We would like to thank the reviewer for the effort and kind words and important advice. The amount of tracer draining to the lymph nodes is indeed only a small part of that injected into or surrounding the tumor; in this experimental setup we cannot determine if this would suffice for in vivo sentinel node detection and whether that will be visible on the SPECT/CT-scan with the current acquisition settings. Our hypothesis based upon current literature on SLN in lung cancer is that it will be feasible to identify SLN through drainage with these amounts in general, as it has been done trans-thoracically in other studies before. However, new in this study is the endobronchial approach and the accuracy with which we deem to inject these tracers. Our predominant aim of this exploratory study was to evaluate if we could also accurately inject with endo-bronchial devices capable of real-time image guidance for injection.

**Changes in text:**

We have added this perspective in the mentioned paragraph (Page 10, lines 308-310)

**Reviewer D**

The authors performed a fundamental study examining the feasibility of a sentinel lymph node (SLN) procedure using ex-vivo lung resection specimens. As it has already been put to practical use in breast cancer, the practical application of accurate N-staging by SLN procedures is also expected in lung cancer, and the methodology of this study is considered important in looking ahead to this. At the same time, there are some unclear statements in the text, which should be corrected.

<u>Major comments</u>

**Comment 1:**

It does not appear to specify by what route the IVUS catheter was guided toward intra- or peritumoral area. It is presumed to be through the bronchus, but it should be clearly stated.

**Reply 1:**

We would like to thank the reviewer for the time and thorough evaluation of our

manuscript. Indeed, we have used the endobronchial route for this device designed as IVUS (intravascular) catheter. We have changed the text to emphasize this.
**Changes in text:** We have added 'endobronchially' to line 139 to emphasize the use in this study. We replaced 'endobronchially' by 'through the bronchial tree' in line 154 and 155.

**Comment 2:**
On page 3, lines 80–81: The authors mentioned that they used a dose escalation protocol by varying injection volume. However, it is not clear how that was actually changed and reflected in the results. This also makes the discussion paragraph beginning on line 221 difficult to interpret.
**Reply 1:**
We thank the reviewer for their comments. Indeed, it is not a true dose escalation study as would be expected from pharmaceutical trials and might therefore be prone to misinterpretation. We merely varied the total volume we injected from 0.3 up to 1.2 ml total injected volume, while keeping the tumor volume per tissue in mind. Our original intention was to more formally administer increasing volumes, but we unfortunately soon noticed that heterogeneous tumor density and size prevented this formalization. As indeed deemed now incorrect, we therefore changed the phrasing of 'dose escalation' to 'varied injection volume' to avoid confusion with systematic dose escalation studies.
**Changes in text:**
We have added explanation in line 117-120 on Page 4.

**Comment 3:**
On page 3, lines 89–91: The authors hypothesized that lesions with GGO would allow for better retention of tracer volume compared to solid tumors. However, the reason was not indicated and should be clarified.
**Reply 1:**
Thank you for pointing this out. Soft tissue as well as solid lung lesions in human consist predominantly out of water. Water retention is enabled through cellular and extracellular barriers. In soft tissue, injection of fluids therefore often causes separation of the ECM and/or different tissue types. GGO's are different in their origins. In GGO – as also with normal lung parenchyma – there is place for air through the anatomical architecture of the lungs. Air resides outside the cells, but within the tissue structure. Whereas water is a relatively incompressible material and harbored even within cells, air is easily compressible and is also not harbored within the individual cells but within an anatomical (open) structure allowing for air intake. A GGO consists of parts solid tissue and parts anatomical spaces which harbor air. As a consequence, this air can be easily displaced for lesser volatile compounds such as liquids. As air is more easily compressible, an injection within a GGO can more easily absorb the expansion of the total volume when compared to a full soft tissue lesion. In solid lesions this pressure will translate into potential leakage back alongside the needle tract. In response to this important remark of the reviewer we have revised the

text to better explain the decision to allocate non-solid lesions to intratumoral injections and the reasoning behind intratumoral pressure, mentioned later in the discussion.

**Changes in text:**

The hypothesis and its reason have been explained in these lines by description of both lesion types (see Page 4, lines 126-132).

**Comment 4:**

The table number in the text is incorrect and needs to be corrected.

**Reply 1:**

This is indeed a mistake, and we would like to thank the reviewer for pointing this out.

**Changes in text:**

Table number has been changed (see Page 7, line 207).

Minor comments

**Comment 5:**

Dashes, not hyphens, should be used for numeric ranges.

**Reply 1:**

Thank you for pointing this out, we have changed it accordingly.

**Changes in text:**

A hyphen has been replaced with a dash in line 194, Page 6.

**Comment 6:**

Since GGO indicates the appearance of the pulmonary shadow and does not by itself suggest a lesion, a modification of the terminology should be considered.

**Reply 1:**

We agree that this term refers to the appearance on CT only part of the range of subsolid lesions and may not be accurate in all cases.

**Changes in text:**

We have removed the term GGO altogether and have replaced GGO by non-solid lesion throughout the manuscript and we have changed ‘solid with GGO’ to sub-solid in Table 1 (see Page 7, line 201).

**Comment 7:**

On page 5, lines 140–141: “Reconstructed with an iterative reconstruction…”is not a sentence, and its relation to the previous sentence should be clarified.

**Reply 1:**

We agree with the reviewer that this sentence is not in line with the sentence before and that the message is not clear by using reconstruction twice.

**Changes in text:**

The sentence has been adjusted so it follows the sentence before better and states what was exactly performed and why that was done (see Page 6, lines 184-186).

**Comment 8:**
On page 7, line 185: Arabic numerals should not be listed at the beginning of a sentence.
**Reply 1:**
This should indeed not have been phrased this way and we thank the reviewer for pointing this out.
**Changes in text:**
The sentence has been rewritten to start with a letter (see Page 8, lines 136-237).