Peer Review File

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Reviewer A

The paper entitled "Bronchoscopic Deployment and Implantation of Diffusing Alpha-emitters Radiation Therapy into the Lung and Mediastinum for Treatment of Lung Cancer: A Pre-Clinical Safety and Feasibility Study" is about the implant procedure of the novel DaRT technique based on insertion of alpha particles radioactive seeds directly into the tumor. The paper novelty is very high, has merit and I will highly recommend its publication however it requires minor revision to improve its readability. Below I will detail point by point my comments:

Comment 1: Page. 6 line 208: I think that detailing the use of this accessory is confounding here.
Reply 1: We appreciate the reviewer's comment and agree that this is confusing. Therefore, the description of the experimental accessory was removed from this location.
Changes in the text: Deleted as noted above.

Comment 2: Pag.6 line 213 Figure 2 is sufficiently clear to explain the procedure however it is introduced to the reader only at page 7. I think that more figures describing the insertion technique would be of great help to improve readability, also by use of drawings.
Reply 1: Figure 1 was expanded to include more details on the insertion technique.
Changes in the text: NA (changes were made on the figure + legend)

Comment 3: Page 7 Experimental Design: this section is quite confusing and difficult to read. Dividing phases in sections doesn't help much the reader following the experimental design. Furthermore these sessions are not reported in figure 2. Try to be more clear in the experimental design description.
Reply 1: We agree that mentioning the sessions in the text but not in the figure is very confusing. We added the sessions to the figure and improved the text.
Changes in the text: We improved Fig.2 and the text on lines 263-284.

Comment 4: Page 7 line 300 "swine #5"
Reply 1: Corrected
Changes in the text: We added the # mark before the number "5" line 288.

Comment 5: Page 9 line 352 Also here one more drawing in methods will explain better the procedure and what are these clusters spaced 4 mm apart.
Reply 1: A description on the cluster configuration was added to the methods and to Figure 1. Figure 3 also demonstrates the cluster in a CT scan.
Changes in the text: explanation was added on lines 165-168. Fig 1 and 3 were updated

to include illustration of the 4 mm spaces in the cluster.

Comment 6: Page 9 line 355 Figure 3 can be improved by enlarging the details of alpha DaRT seeds because with the current size they are almost invisible. Also giving a label with the spacing details would help.
Reply 1: The figure was corrected accordingly.
Changes in the text: NA (Fig. 3 +legend were updated)

Comment 7: Page 9 line 364 Also Figure 4 must be improved. Details are too small in the left side (location) part of the figures and isodoses are unreadable in the right side (it creates also some confusion to talk about isodoses). Furthermore this figure should be used more to explain seeds movement when appropriate!
Reply 1: The isodose was added to stress that this figure is of the active case rather than the inert case and to illustrate the localized nature of treatment (which is very unique to RT) and the reason for the need in a cluster of seeds. The figure is designed such that the location is first presented (left) to be able to see the location in the whole-lung context and then a zoom in image is presented (right) to be able to see the small details. The figure was improved in the revised version as suggested by the reviewers (see also below).
Changes in the text: NA (Fig. 4 +legend were updated)

Comment 8: Page 9 line 383 this section has to be removed or introduced in the methods section
Reply 1: The description of the method (questionnaire) has been moved to the methods section. The description of the surgeon rating (result) remained in the results section.
Changes in the text: The method was moved to lines 291-292 the results were refined in lines 365-371.

Comment 9: Page 9 Migration and movement assessment: I think that you should describe more in detail this section explaining your results in detail and helping yourself with figures.
Reply 1: The text was improved and the main results were added. Additional figures of the fusion do not provide further clarification and have subsequently not been added.
Changes in the text: Lines 372-388 were updated

Comment 10: Page 10 line 414: I'm not expert in pathology so I cannot give advice for this section
Reply 1: arrows in the figure were added with labels of the findings for reader comfort.
Changes in the text: NA (Fig. 5)

Reviewer B

Interesting concept and good initial study. Further comments are included in notes in the attached PDF.

Comment 1: Line 211 How were the DaRTs deployed?
Reply 1: Alpha DaRT is released to the tissue by using the FNA needle as introducer and its stylet as a pusher. The needle is then redrawn outside, and the Alpha DaRT remains in the tissue. This was added to the methods and to figure 1
Changes in the text: text was updated in lines 192-195 of the methods section and in the legend of figure 1.

Comment 2: Line 220 How were the DaRTs deployed?
Reply 1: Alpha DaRT is released to the tissue. The needle is then redrawn outside, and the Alpha DaRT remains in the tissue by using the FNA needle as introducer and its stylet as a pusher. This was added to the methods and figure 1
Changes in the text: text was updated in lines 192-195 of the methods section and in the legend of figure 1.

Comment 3: Line 284 How was this timing decided upon? Why day 33? Why 38?
Reply 1: The initial plan was to evaluate seed movement over a period of 30 days using fluoroscopy. However, during the experiment it was noted that the fluoroscopy did not provide sufficient image quality to evaluate seed movement, thus CT scans were introduced with a protocol modification during the initial phase of the study. Due to availability of the veterinary, technical, and study staff as well as availability of the CT scanner, day 33 was chosen as the initial CT scan date. Given the growth of the porcine model used, noting that the Vendor's well established growth rate chart, we anticipated there would be a 25-30% increase in body weight over a 30 – 38 day period thereby increasing the swine's body weight to approximately 90 kgs (198 lbs) which was the maximum size our staff could safely handle, transport and anesthetize. Therefore, Day 38 was chosen as the second time point. As noted, in phase II, which was conducted after completion of phase I, we were able to initiate a CT scan at Day 0 and then at Day 28.
Changes in the text: The text was updated on lines 376-378

Comment 4: Line 298 Was the reason for 2 phases to establish safety with inert DaRTs in phase 1, then proceed to active DaRTs in phase 2 if safety observed in phase 1?
Reply 1: Yes, this is correct. We initially wanted to optimize the entire procedure of deploying seeds, working through the process of handling the animals, familiarizing the staff with the animal husbandry etc. without the concern for radioactivity.
Changes in the text: The above explanation was added to the methods section (Experimental design) lines 263-270.

Comment 5: Line 413 While the animals clinically did well, it would be interesting to know how they did radiographically. Can images of CTs at end the of the study be described and representative images included?
Reply 1: Consistent with the final pathology report we saw lung opacities with variable degrees around the inserted sources, representing inflammatory changes, and possibly focal minor bleeding and atelectasis. This description was added to the results section and relevant images were added to Fig. 4
Changes in the text: we updated the test in lines 397-399

Comment 6: Line 453 What about higher XRT dose per DaRT? Has that been tried? What can we speculate about the number of DaRTs needed to treat, say, a 1cm lung tumor? 3cm? Maximum size for DaRT candidacy? Similarly, malignant nodes can get rather large.
Reply 1: The activity per Alpha DaRT was not increased in this experiment. As has been documented in other publications, the dose delivered by DaRT is driven by the spacing of the sources as opposed to the activity per source. This has been modeled in the referenced paper. In clinical studies, the activity per source has been increased from 2 to 3 microcuries without increased safety concerns. The number of DaRT needed to treat a given tumor depends on the geometry of the lesion, however for a 1 cc tumor, one would need to insert approximately 7 DaRT sources. The maximal size in clinical trials is currently 7 cm in longest dimension, however the specific geometry and accessibility in the case of lung insertion would be most critical.
Changes in the text: the relevant references were added to the introduction line in line 78 (refs 12-15).

Comment 7: Line 455 What do we know about DaRT effects on other organs?
Reply 1: Since DaRT is a local alpha radiation-based treatment no direct effect is expected at distant organs due to the extremely short range of the radiation in the tissue. A minimal non-clinically significant leakage of Pb-212 to the blood is expected, according to the biokinetic model of Alpha DaRT that was confirmed in measurements performed in squamous cell carcinoma patients (skin and H&N). This was added to the discussion.
Changes in the text: text and the relevant references (refs 12-15) were added to the introduction and discussion line in line 78, 438-443.

Comment 8: Line 461 Being so small, could these theoretically embolize if they migrated into vasculature?
Reply 1: The Alpha DaRT could theoretically embolize if placed into the vasculature, however, we did not experience this phenomenon. We used the endobronchial ultrasound (EBUS) to assure the sources are placed into a soft tissue and not in the vasculature. The soft tissue was expected to hold onto the source. This was proved to be true with negligible seeds movement seen in serial CT scans. EBUS was used to detect any sizeable vessel in the area of seed placement, and none of the seed was seen in a vessel either immediately after placement or in the follow up imaging. This explanation was added to the discussion.
Changes in the text: text was added in lines 444-454

Comment 9: Line 478 Agreed; longer-term studies to follow effects of radiation (eg, more delayed pneumonitis) would be helpful.
Reply 1: Thank you. Additional limitations were added to the discussion.
Changes in the text: a limitation paragraph is in lines 459-464

Comment 10: Fig. 4 The non-magnified images are too "zoomed-out" to be useful; almost nothing relevant can be seen. It would be more helpful to have magnified

images with and without the green outline.
Reply 1: Corrected
Changes in the text: NA (Fig. 4)

Comment 11: Fig 5. As with the previous figure, it would be more helpful to have magnified images with and without the outline/highlight. The left image is too zoomed-out to be instructive.
Reply 1: After changes in figure 4 (Adding a cluster example) we decided that fig 5 is redundant and we removed it from the manuscript.

Changes in the text: NA

Reviewer C

The manuscript attempts to validate the feasibility and safety of a novel procedure for the bronchoscopic implantation of Alpha DaRT seeds into the lungs of a porcine model and the outcomes of the implantation.

Comment 1: -Highlight Box mentions "This is the first study to demonstrate the feasibility.... to the lung and mediastinum". I am unclear if the study provided that insight. I believe that the feasibility and safety of local alpha particle delivery would also involve removal of the seeds and at the very least an evaluation of if the seeds can be safely removed without detrimental outcomes.
Reply 1: Thank you for the comment. The only clinical situation whereby Alpha DaRT would be removed from either the mediastinum or lung parenchyma would be in the setting of a planned neo-adjuvant study. Further, the type of surgical resection would be an en-bloc resection as it typically performed for oncologic resection, thus the need to assess the ability to specifically remove the Alpha DaRT while not disturbing the surrounding tissue appears mute. See also response to comment 13 below.
Changes in the text: No change

Comment 2: -Line 171 - "...anesthesia consisted of isoflurane (2-2%)..." uncertain if authors made a typo here or are trying to indicate that it was only 2% that was used.
Reply 1: This was indeed a typo. Thank you. It should be 2-2.5%
Changes in the text: the correction was in line 146

Comment 3: -Can authors address the reason why the mediastinal and distal regions were chosen for the implantation? And why the specific parameters for implantation (e.g 1-2cm from pleural surface for distal and paratracheal and subcarinal areas for mediastinal)?
Reply 1: Lung tumors are expected to invade the lung parenchyma and mediastinal areas and we developed the mechanism to be able to place the sources in both areas. Since we do not have a live large animal model for lung cancer, we have no choice to place the sources but in the normal mediastinum and lung parenchyma. We carefully selected the areas of the lung and mediastinum that had minimal soft tissue to give enough space for placement of the source without any injury to the surrounding normal

structures. This explanation was added to the paper
Changes in the text: text was added in lines 180-185

Comment 4: -Line 205 - Implantation methodology suggests that distal implantations occur in a region "...without an adjacent blood vessel". However, both Figure 6 A and B appear to show implantation sites near blood vessels. Can the authors provide an explanation for this? Also, as a continuation of the previous point, can the authors justify why implantation sites have to be specifically not near a blood vessel.
Reply 1: As described in another comment, we avoided blood vessels, to assure no seeds will be placed in a vessel. But as it is obvious, in healthy animal, there is minimal soft tissue in the mediastinum and the bronchoscopist was able to carefully place the sources in a tiny space between the vital organs without invading the vessels. Same in the lung parenchyma, no source was invaded a blood vessel, but the vessels are seen around the implanted sources. Placement in the blood vessel has the risk of migration of the source.
Changes in the text: text was added in lines 180-185

Comment 5: -Figure 1: Resolution is really poor given the presented size in the manuscript.
Reply 1: Corrected
Changes in the text: NA (Fig. 1)

Comment 6: -Figure 3: Yellow arrows are difficult to visualize in both images.
Reply 1: The figure was revised and improved
Changes in the text: NA (Fig. 3 + legend)

Comment 7: -Figure 4: Suggest the use of well visible indicators to identify where the location of the implanted seeds are in the images on the left. Currently it is difficult to visually identify them despite the right series of images being close ups of the seeds.
Reply 1: the figure was revised and improved
Changes in the text: NA (Fig. 4 + legend)

Comment 8: -Table 2: No mention of how this questionnaire was designed or chosen in the methods. Unclear what the purpose of the qualitative assessment of the difficulty of implantation has to do with the overall purpose of the study.
Reply 1: The questionnaire provided a semi quantitative assessment of the physicians comfort with using the Alpha DaRT deployment technique. The questionnaire was built from other previously designed Likert questionnaires on feasibility. We wanted to assess that if there were errors in the deployment, whether they were related to the comfort or with the deployment methodology itself. This is preliminary but critical to device design. This was added to the Results and Methods section and the revised manuscript.
Changes in the text: text was added in lines 291-292 and 365-371

Comment 9: -Line 406 - What is meant by significant here?
Reply 1: The meaning is clinically significant. the text was corrected accordingly.
Changes in the text: Corrected in line 390

Comment 10: -Is there a reason why the study does not offer a control group? Or alternatively, at least a comparison between the animals receiving inert seeds and the animals receiving the active seeds? Because at present, it is difficult to ascertain if the claim that this novel bronchoscopic implantation method is safe is true. For example, given current reported outcomes, there is no way to ascertain that the rise in serum creatine phosphokinase is due to intramuscular injections of the preanesthetic as there is no other group to compare this outcome to. Similarly, the reported outcome indicating a lack of alteration beyond reference ranges for all other aspects of the serum biochemistry, CBC and UA become difficult to make conclusions about because it is unclear how a non-treated animals (or the animals receiving inert seeds) respond given the experimental conditions.
Reply 1: This is a valid concern. A control group could have clarified if the changes in CPK (creatine phosphokinase) are due to Alpha-DaRT source placement vs. other injections or manipulations. We agree with the reviewer that further studies are needed. Furthermore, we do not expect any systemic effect from Alpha-DaRT that may cause a rise in CPK as none of the current human studies of Alpha-DaRT has raised that concern.
Changes in the text: the expected systemic effects are discussed in lines 439-443.

Comment 11: -Figure 6: Some indicators should be used to identify the elements suggested by the image description. In addition, clearer text on the scale bar is necessary, currently I am unable to make out the scale value indicated in the images.
Reply 1: Corrected
Changes in the text: NA (Fig. 5)

Comment 12: -Table 3: There is mention of an Alpha DaRT Mean Movement (mm) metric but it is unclear how this metric was determined. Can the authors indicate how the Alpha DaRT Mean Movement (mm) metric is calculated?
Reply 1: the legend was updated to include the calculation.

Changes in the text: Table 3 legend was updated.

Comment 13: -Authors indicate that seeds planted for both phases of the study were removed at euthanasia. Is there a reason why the authors did not include observation or data of the animal model after removal of the DaRTs? Similar to the first comment about the contents of the highlight box, if the purpose of this study was to demonstrate the feasibility and safety of local alpha particle delivery to the lung and mediastinum then shouldn't the study also present the outcomes of seed removal and demonstrate long term well being post-seed removal?
Reply 1: The removal of the DaRTs in this experiment was purely to allow for pathologic assessment. The DaRT is made from stainless steel and thus can not be cut using a microtome. In order to assess the pathologic effect of the DaRT, the physical seeds had to be removed.
As mentioned above, in a clinical scenario where DaRT would be removed from a patient, for example a neoadjuvant study, the type of resection would remove not only

the DaRT but also the surrounding tissue to preserve the tissue architecture as is standard in oncologic operations.
Finally, the half life of Radium is 3.7 days, thus after 37 days, the DaRT is effectively inert and thus should behave similarly to a inert fiducial seed, thus not necessitating removal.

Changes in the text: No change

Reviewer D

The authors are conducting a safety study in animals for the lung and mediastinum as a preliminary step for the use of alpha radiation as a brachytherapy in humans. Alpha radiation therapy is a very active area of research that has recently been used in BNCT and Radionuclide therapy, and this study is an important paper proposing a new therapy. However, there are a few areas that require discussion and revision.

Comment 1: There is a lack of description of the basic physics in the paper. For example, a 10 mm needle is used as the source of radiation for this study, but the structure is not clarified. The attached Figure 1 is a rough image and cannot be inferred. The range of α-rays in the tissue should be extremely small, as in water, and since α-rays cannot pass through the part covered with stainless steel, the radiation dose around the 10-mm needle should be extremely different in various parts of the needle. There is no description of the fission reaction process and the respective energy levels. This information should be clearly disclosed and further discussed by calculating a dose distribution diagram and matching it to the histopathological images.
Reply 1: All this information was previously published. A text was added to the introduction with the proper references.
Changes in the text: text was added to the introduction in lines 75-79 with the proper references.

Comment 2: In Line 117, when you mention that multiple clinical trials are being conducted, which clinical trials are you referring to specifically? The authors should provide specific citations for each organ, such as citations to protocol papers or ClinicalTrials.gov.
Reply 1: The clinical trials numbers were added.
Changes in the text: text was added to lines 96-97

Comment 3: There is no mention of the time taken for each of the insertions using EBUS. A full discussion should be added with a detailed review of whether it differed from surgeon to surgeon and the difference between individual insertion and insertion in clusters.
Reply 1:
We did not specifically count the time for insertion of the sources, as we were testing the applicators and the proposed mechanisms for the first time. However, we noticed

that the speed of the implantation of the source increased significantly towards the end of the procedure. Of note, there was only one bronchoscopist with expertise in using EBUS in central and peripheral airways who performed the implantation. We added the reviewers comment as a subject for further investigation.
Changes in the text: text was added line 460

Comment 4: The authors state that the insertion and subsequent treatment of Alpha DaRTs is feasible and safe, but the present study is very small in number and falls far short of being feasible and safe. In addition, although migration did not occur in this study, it is rare but improbable for marker insertion for radiotherapy to be carried by the bloodstream to a different site. If safety is to be considered, Alpha DaRTs should be inserted into small blood vessels, such as the heart or intestinal tract, to examine the safety of the markers on the blood vessels, blood flow, and tissues. Either additional experiments should be performed in this regard, or the lack of consideration should be clearly stated in the paper.
Reply 1: Further limitations of the study were added to the discussion.
Changes in the text: text was added in lines 461-464

Comment 5: The authors define EBUS in Line 445, but defines in Line201 already and it is not necessary.
Reply 1: Corrected
Changes in the text: the definition was deleted.