Dear Dr. Vineeta Bajaj, Review Editor, JoVE

We were gratified to receive the request for a revision of our review**, Near Infrared photoimmunotherapy for mouse models of pleural dissemination**, by Yasui H, et al. We are glad the reviewers and editors found sufficient merit in our work to justify further revision and we wish to express our appreciation to the reviewers for insightful comments. We believe we can address all the points raised and below, we respond on a point-by-point basis to the reviewers' comments.

Your manuscript, JoVE61593R1 "Near Infrared photoimmunotherapy for mouse models of pleural dissemination," has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually.

Your revision is due by **Oct 19, 2020**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

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Please note that novelty is not a requirement for publication and reviewer comments questioning the novelty of the article can be disregarded.

Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please revise the manuscript to thoroughly address these concerns. Additionally, please describe the changes that have been made or provide explanations if the comment is not addressed in a rebuttal letter. We may send the revised manuscript and the rebuttal letter back to peer review.

Editorial comments:

1. Please revise the following lines to avoid previously published text: 42-44, 51-53, 64-69

We revised.

2. Additional details are needed:
1.2: What type of column is used?

We revised to Sephadex column.

1.4: What standard concentrations are used here?

The standard concentration of IR700 is recommended to be 0.5 – 5 μM.

1.4.4: How is this determined? Please provide an equation here.

We added equations to 1.3.2, 1.3.4.

2.1: How are the cells prepared?

Luciferase-expressing cells were prepared by luciferase gene transfection and confirmed high expression of luciferase after performing more than 10 cell passages.

Cells are cultured in medium supplemented with 10% fetal bovine serum and penicillin (100 IU/mL) and streptomycin (100 mg/mL).

2.3: What concentration of isoflurane is used here? How is sufficient depth of anesthesia determined?

Added to protocol about how to check anesthesia concentration and anesthesia depth. “When performing the procedure to mice, anesthetize them with isoflurane (introduction: 4-5%, maintenance 2-3%) and pressed the tail with tweezers to confirm that there was no reaction.

”

2.8: What happens to the mouse after?

The mouse will wake up from anesthesia and walk normally.

3. Please highlight up to 3 pages of essential protocol steps to be included in the protocol section of the video.

The next section is an important protocol step. (Page 2.5)

2. Creating a pleural dissemination model (1.5 pages)

5. In vivo NIR-PIT for pleural dissemination model. (1 page)

4. Please consolidate or remove some of the figures (especially the menu screenshots). If we are filming those steps, the menu screenshots would not be needed.

We integrated some of the screenshot content.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The MS has been greatly improved since the original version. The protocol is more detailed and the additional reference and context are appreciated.

Minor Concerns:

It is stated in the rebuttal that any mAb can be used; but presumably it should be directed to a cell surface/transmembrane protein?

Yes, that's right. Although not published in the paper due to negative data, NIR-PIT has no effect when targeting proteins “distant” from the cell membrane.

I am not clear on the response to question 23 in the rebuttal letter. Why is it not necessary to show the effect of the mAb alone as compared to the control and PIT-treated (Fig. 18B-C)?

We evaluate the group with dorsal tumor xenografted model, in advance. The result is no effect compared to the control. Therefore, the therapeutic effect with the disseminated pleural model was shown by comparing the two groups of control and NIR-PIT.

コメントの追加 [べ1]: 反論の内容は大丈夫でしょうか？

Fig.1: Should show the bent needle so the reader can see the degree to which the needle is bent. Suggestion for safety (line 148): Use forceps to bend the needle.

Thank you very much. We added a figure and added a description.

Line 145: Toe pinch to test depth of anesthesia prior to injection?

Yes, we anesthetize and check the depth of anesthesia before treatment. Added after the anesthesia concentration of the protocol.

Line 194-195: Do you mean that if the BLI image is not diffused in the thorax then the tumor may be mistakenly transplanted subcu?

Yes, that's right. As far as we experimented, all mice that showed luminescence only at the insertion site were subcutaneously transplanted. Even after continuing the observation, no tumor was formed in the thoracic cavity, and a subcutaneous tumor developed at the insertion site.

Line 312: Do you mean "pierce the lung with the needle angled towards the lung"?

No, it sticks the needle towards the lungs, but not pierce the lungs.

I changed it to the following expression to avoid misunderstanding.

Turn the mouse sideways and stick the needle into the mouse toward the lung. Since the stopper and needle tip are bent, the needle enters the thoracic cavity without sticking to the lungs. Inject target cells while pressing the needle against the mouse. Thank you.

Reviewer #2:

This is a study for which the PDT-related details remain sparse, poorly described and will not hold up to scrutiny from experts in the field.

The main issue is that the authors seem averse to constructive critiques and are more interested in insisting that they are right than in improving the manuscript.

The light dosimetry parameters, the photosensitizer dose, and numerous details related to background in the field remain missing or poorly considered. This is not a high quality submission and is not an article that, in current form, will reflect well on JoVE. It is also not evident that the authors will take criticism in the spirit of the peer review process that is designed to help their study hold up to scrutiny in the scientific community.

コメントの追加 [べ2]: なんて返事したらいいかわからないです・・・

We responded your comments with honest. I'm sorry for such a terrible reply. Also, our study is totally different from the PDT tratment. The mechanism of NIR-PIT is completely different from the conventional PDT.

Please see below.

Near-Infrared Photoimmunotherapy of Cancer.
Kobayashi H, Choyke PL. Acc Chem Res. 2019 Aug 20;52(8):2332-2339. doi: 10.1021/acs.accounts.9b00273.

Reviewer #3:

Manuscript Summary:

The paper described a protocol using NIR irradation for photo-immunological treatment to cancer.

Major Concerns:

No

Minor Concerns:

1. The introduction should be more informative on the Photoimmunotherapy towards cancers. A review paper can be referred, Near Infrared Light Triggered Photo/Immuno-Therapy Toward Cancers. The paragraphs in Introduction section should be more coherent. Last paragraph needs provide more details of the work.

Thank you for introducing the references.

We organized paragraphs and added consideration about photoimmunotherapy.

2. The way to determine the ratio of antibody to IR700 dyes in the APC after they bounded using UV-Vis is not very direct. When there are free antibody or IR700 dyes

molecules in the solution, the UV-Vis results would not be accurate. Can author explain how much accuracy the measurement can be achieved.

Previous reports of NIR-PIT have examined the purity of mAb-IR700 by multiple methods such as mass spectrometry (Cancer Cell-Selective In Vivo Near Infrared Photoimmunotherapy Targeting Specific Membrane Molecules; Makoto M, Nat Med. 2011; 17 (12): 1685-91). Thus the accuracy is already confirmed.

3. The irradiation power of the NIR light is not consistent, 40-100 mW/cm2 and 30-150 J/cm2.

Yes, that's right. Irradiation energy and total irradiation amount vary depending on the APC used. Most reports irradiate at 100 mW/cm^2, but in this paper it is treated at 40 mW/cm^2, so the conditions are broader.

4. The protocol can be described in a more quantitative way. It is a bit too general.

Specific values are described in the protocol, and the conditions to be adjusted are described in NOTE. Please refer to the section. Thank you.