Submission ID #: 61593
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18785913

Title: Near Infrared Photoimmunotherapy for Mouse Models of Pleural Dissemination

Authors and Affiliations: Hirotoshi Yasui[1], Yuko Nishinaga[1], Shunichi Taki[1], Kazuomi Takahasi[1], Yoshitaka Isobe[1], and Kazuhide Sato[1,2,3]

[1]Respiratory Medicine, Nagoya University Graduate School of Medicine
[2]Nagoya University Institute for Advanced Research, S-YLC
[3]Nagoya University Institute for Advanced Research, B3-Unit, Advanced Analytical and Diagnostic Imaging Center (AADIC)/Medical Engineering Unit (MEU

Corresponding Author:
Kazuhide Sato
k-sato@med.nagoya-u.ac.jp

Co-Authors:
yh0814@med.nagoya-u.ac.jp
ynishinaga@med.nagoya-u.ac.jp
shuntaki@med.nagoya-u.ac.jp
kazuomi@med.nagoya-u.ac.jp
yisobe@med.nagoya-u.ac.jp

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen capture files provided, do not film

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interview Statements are read by JoVE's voiceover talent.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **21**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **JoVE's Voiceover Talent**: This method can be used to evaluate the therapeutic effects of NIR-PIT on thoracic tumors in a clinically relevant tumor environment **[1]**.

1.1.1. Use 4.4.1. Cavity being irradiated

REQUIRED:

1.2. **JoVE's Voiceover Talent**: The NIR-PIT procedures using the pleural disseminated cancer model are easy to understand and to perform **[1]**.

1.2.1. Use 2.5.2. Mouse being injected

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Nagoya University Animal Care and Use Committee.

Protocol

2. Pleural Dissemination Model Generation

2.1. To set up a mouse dissemination model, use polystyrene foam to make a stopper **[1]** and disinfect it with 70% ethanol **[2-added]**.

2.1.1. WIDE: Talent making stopper

2.1.2. Added shot: Disinfect the stopper by placing it in 70% ethanol.

2.2. Press a 30-gauge needle against a hard object to bend the tip **[1-TXT]** and fill a syringe with 1×10^6 target tumor cells in 100 microliters of PBS **[2]**. Attach the needle to the stopper with the tip extending 5 millimeters out of the stopper **[3-added]**.

2.2.1. Needle being pressed/bent **TEXT: Bending helps prevent pneumothorax**

2.2.2. Talent filling syringe with cells

2.2.3. Added shot: attaching needle to syringe, and then, attaching stopper to needle.

2.3. and confirm a lack of response to pedal reflex in an anesthetized, 19-21-gram, 8-12-week-old, female, homozygote, athymic nude mouse **[2-TXT]**.

~~2.3.1.~~

2.3.2. Toe being pinched **TEXT: Anesthesia: 4-5% -> 2-3% isoflurane**

2.4. Insert the needle into the chest through the intercostal space, moving the needle up and down to avoid contacting the ribs **[1-TXT]**.

2.4.1. Chest being pierced/needle being moved up and down *Videographer: Important/difficult step*

2.5. When the tip has passed through the intercostal space, position the syringe so that it is pressed against the mouse **[1]** and inject the entire volume of target cells **[2-TXT]**.

2.5.1. Syringe being pressed against mouse *Videographer: Important/difficult step*

2.5.2. Cells being injected *Videographer: Important step* **TEXT: Mouse will breathe deeply when cells enter chest cavity**

2.6. After the injection, roll the mouse 2-3 times to spread the cells throughout the thoracic cavity **[1]** and return the mouse to its cage with monitoring until full recovery **[2]**.

2.6.1. Mouse being rolled *Videographer: Avoid mouse head in shot*
2.6.2. Talent placing mouse into cage *Videographer: More Talent than mouse in shot*

3. Bioluminescence (BLI) Measurement

3.1. Twenty-four hours after cell injection and every day thereafter, inject the anesthetized, tumor cell-injected mice with 200 microliters of 15-milligrams/milliliter of D-luciferin **[1]**.

3.1.1. WIDE: Talent injecting mouse *Videographer: More Talent than mouse in*

3.2. After 10 minutes, place the mice into a bioluminescence imager **[1]** and open the **Acquisition Control Panel** in the imager software **[2]**. Select **Luminescent**, **Photograph**, and **Overlay [3]**.

3.2.1. Talent placing mice into imager
3.2.2. Talent opening panel, with monitor visible in frame
3.2.3. SCREEN: screenshot_1: 00:02-00:10

3.3. Set the exposure time to **Auto**, the **Binning** to small, the **f-stop** to 1 for luminescent and to 8 for photograph, and the **Field of View** to C. Click **Acquire** to image the bioluminescence **[1]**.

3.3.1. SCREEN: screenshot_1: 00:10-00:29 *Video Editor: please speed up*

3.4. Set the **Display format** to Radiance and select the **Circle** to from the **Region of Interest Tools** in the **Tool Palette** panel **[1-TXT]**.

3.4.1. SCREEN: screenshot_2: 00:01-00:20 *Video Editor: please speed up* **TEXT: Suitable pleural dissemination model shows strong luminescence in ventral diffused chest site view**

3.5. Click **Measure Regions of Interest** to measure the surface bioluminescent intensity and use **Configure Measurement** to select the values relevant to the experiment. Export this data table as a .csv file **[1]**.

3.5.1. SCREEN: screenshot_2: 00:20-00:40 *Video Editor: please speed up*

3.6. Then use the **Total Flux** values for the bioluminescent intensity quantification in the file **[1-TXT]**.

3.6.1. SCREEN: screenshot_3: 00:02-00:11 **TEXT: Include only mice with sufficient luciferase activity in study**

4. Near-Infrared Photoimmunotherapy (NIR-PIT)

4.1. Before performing near-infrared phototherapy of the tumor-injected mice, use a power meter to measure the light dose of a 690-nanometer wavelength laser **[1]** and adjust the output to 100 milliwatts/square-centimeter **[2]**.

4.1.1. WIDE: Talent measuring laser
4.1.2. Talent adjusting output NOTE: the measured output was xx mW/3cm^2

4.2. Twenty-four hours before the treatment, intravenously inject 100 micrograms of antibody photosensitizer conjugate in 50-200 microliters of PBS via the tail vein of the tumor-injected animal **[1]**.

4.2.1. APC being injected, with APC container visible in frame

4.3. On the day of the phototherapy treatment, place the anesthetized, conjugate-injected, tumor-laden mouse in the supine position **[1]** and shield the non-target sites with aluminum foil **[2]**.

4.3.1. Mouse being placed in supine position
4.3.2. Foil being placed *Videographer: Important step*

4.4. When all of the shields have been placed, use a 100 joules/square-centimeter laser to irradiate the thoracic cavity with near infrared light for about 30 seconds **[1-TXT]**.

4.4.1. Cavity being irradiated **TEXT: If tumor disseminated back to belly, divide NIR dose in multiple directions**

4.5. When the irradiation is complete and the mouse has awoken, return the animal to its cage **[1]** and measure the bioluminescence daily as demonstrated **[2]**.

4.5.1. Talent placing mouse into cage *Videographer: More Talent than mouse in shot*
4.5.2. LAB MEDIA: Figure 11A

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.4.1., 2.5.1., 2.5.2., 4.3.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
2.4.1., 2.5.1.

Results

5. Results: Representative NIR-PIT Thoracic Mouse Tumor Treatment

5.1. In this representative analysis **[1]**, the conjugation of anti-podoplanin antibody with IR700 (eye-R-seven hundred) was confirmed by SDS-PAGE (S-D-S-page) analysis **[2-TXT]**.

5.1.1. LAB MEDIA: Figure 8

5.1.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize NZ-1-IR700 band in both gel images* **TEXT: SDS-PAGE: sodium dodecyl sulfate-polyacrylamide electrophoresis**

5.2. After tumor cell injection **[1]**, bioluminescence imaging and diffuse luminescence imaging tomography should be performed to determine which mice express sufficient luciferase activity in the chest cavity for further study **[2]**.

5.2.1. LAB MEDIA: Figure 9

5.2.2. LAB MEDIA: Figure 9 *Video Editor: please emphasize fluorescence in at least whole body image*

5.3. At day 5 after injection **[1]**, anti-podoplanin antibody-IR700-injected mice demonstrate high IR700 fluorescence and luciferase activity in thoracic tumors, indicating that intravenously injected IR700-conjugated antibody reaches disseminated pleural tumor sites **[2]**.

5.3.1. LAB MEDIA: Figure 10

5.3.2. LAB MEDIA: Figure 10 *Video Editor: please emphasize NZ-1-IR700 Bioluminescence and 700 mm Fluorescence signals*

5.4. Notably, pleural disseminated mice treated with near-infrared photoimmunotherapy **[1]** demonstrate a decreased luciferase activity **[2]**, while the relative light units in the control group exhibit a gradual increase in intensity **[3]**.

5.4.1. LAB MEDIA: Figure 11

5.4.2. LAB MEDIA: Figure 11 *Video Editor: please emphasize day 3 PIT image and PIT data line*

5.4.3. LAB MEDIA: Figure 11 *Video Editor: please emphasize day 3 control image and control data line*

Conclusion

6. Conclusion Interview Statements

6.1. **JoVE's Voiceover Talent**: Since the required NIR irradiation energy depends on the cell line and antibodies, be sure to check the conditions in advance in vitro **[1]**.

6.1.1. Use 4.1.1. and/or 4.1.2. Laser and output being adjusted