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Title: Intradermal Tumor Implantation as a Robust Model for Murine Cancer Intratumoral Immunotherapy Studies

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**
-our laboratory on the 6th floor in Rubin

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 46

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Kevine Kamga**: We develop intradermal murine tumor models to optimize intratumoral immunotherapy and investigate mechanisms driving local and systemic (abscopal) responses.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7.1*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Kevine Kamga**: Subcutaneous models lack visualization and precise delivery, causing variability. Intradermal implantation enables direct visualization, accurate injections, reproducibility, and stronger immunogenic responses.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Alicia Santos**: This allows researchers to study treatments for intratumoral immunotherapy, which is gaining more interest.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 4.5.1*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Alicia Santos:** The most important consideration is practice, finding the right technique, and perfecting it.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Alicia Santos:** Future directions following this work would be new intratumoral treatments that can be applied to current cancer treatments.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Dartmouth College.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Animal Preparation and Intradermal Injection Training

Demonstrators: Kevine Silihe Kamga and Alicia M. Santos

- 2.1. To begin, position the mouse on a stable surface [1] and scratch the mouse by the skin of the nape with the left thumb and index finger [2]. Gently tuck the left side of the body into the palm to expose the right side [3]. Hold the left leg and tail with the ring and or pinky fingers to fully expose the right flank [4].

~~2.1.1. Talent puts mouse on the cage wire (stable surface). NOTE: This shot is modified~~

2.1.2. Talent scruffing the mouse at the nape using thumb and index finger. NOTE: 2.1.2- 2.2.1 are combined in a single shot

2.1.3. Talent tucking the left side of the mouse into the palm to expose the right side.

2.1.4. Talent securing the left leg and tail while fully exposing the right flank.

- 2.2. Then, shave the fur from the hind leg up to the lower ribs to create a clear area for injection [1].

2.2.1. Talent shaving the fur from the hind leg to the lower ribs of the mouse.

- 2.3. ~~After shaving, monitor all mice until they are fully recovered from anesthesia [1].~~

~~2.3.1. Talent observing mice in a recovery area until full recovery. NOTE: This is not filmed as the procedure was performed on awake mice~~

- 2.4. To practice the injections, mix food coloring with PBS in a 1.5-milliliter Eppendorf tube until the solution appears dark [1].

2.4.1. Talent added food coloring into a 1.5 milliliter Eppendorf tube containing PBS and mixing the solution.

- 2.5. Place the anesthetized practice mouse under a nose cone [1], tilt the body to expose the right flank, and [2] gently pinch the footpad and confirm no response to ensure adequate anesthesia [3].

2.5.1. Talent positioning the anesthetized mouse under a nose cone.

2.5.2. Talent tilting the mouse to expose the right flank. NOTE: 2.5.2 and 2.5.3 are filmed in a single shot

2.5.3. Talent pinching the mouse footpad to check for lack of response.

- 2.6. Load 30 microliters of dye solution into a 0.3 milliliter insulin syringe with a 29-gauge, half-inch needle [1] and remove all air bubbles from the syringe [2].
 - 2.6.1. Talent drawing dye solution into a 0.3 milliliter insulin syringe.
 - 2.6.2. Talent expelling air bubbles from the syringe.
- 2.7. Using the non-dominant hand, gently pinch and stretch the leg and skin to create a smooth, taut surface [1]. Insert the needle bevel up into the dermis at a shallow parallel angle to the skin [2]. Keep the needle visible beneath the surface to confirm dermal placement [3].
 - 2.7.1. Talent pinching and stretching the mouse leg and skin to create a taut surface.
NOTE: 2.7.1 to 2.8.1 are filmed in a single shot
 - 2.7.2. Talent inserting the needle bevel up at a shallow angle into the dermis.
 - 2.7.3. Shot of the needle visible beneath the skin surface indicating correct dermal placement.
- 2.8. Slowly inject the full 30 microliters of dye until a small, distinct intradermal bubble forms. Continue practicing until consistent bleb formation is achieved [1].
 - 2.8.1. Talent slowly injects the dye solution and forms a visible bubble under the skin.
- 2.9. After completing practice injections, euthanize the mouse according to institutional guidelines [1].
 - 2.9.1. Shot of the mouse after completion of the practice injections.

3. Preparation of Tumor Cells and Intradermal Injection Procedure in Mice

- 3.1. Thaw tumor cells several days before intradermal injections in a water bath maintained at 37 degrees Celsius [1].
 - 3.1.1. Talent places a vial of tumor cells in a 37 C water bath for a few seconds to thaw.
- 3.2. Culture the cells in a T-150 culture flask to 70 percent to 80 percent confluence while maintaining adequate fresh media [1].
 - 3.2.1. Talent plating the thawed cells in a T-150 culture flask.
- 3.3. Harvest the cells by centrifugation and prepare the cells at 7×10^6 cells per milliliter in PBS-serum-free Roswell Park Memorial Institute or RPMI (*R-P-M-I*) medium, or other media used for growing the cells [1]. Keep the cells on ice after harvest and proceed quickly to injection [2].
 - 3.3.1. Talent showing cell pellet, resuspending in medium, and adding it into an Eppendorf tube to the specified concentration.
 - 3.3.2. Talent placing the Eppendorf tube of harvested cells on ice.

- 3.4. After anesthetizing the shaved mouse, place it under a nose cone [1] and position the body to fully expose the right flank [2].
 - 3.4.1. Talent positioning the anesthetized mouse under a nose cone.
 - 3.4.2. Talent adjusting the mouse to expose the right flank. NOTE: 3.4.2 to 3.5.3 are filmed in a single shot
- 3.5. Gently invert the cell suspension to ensure even mixing [1]. Draw 30 microliters of cell suspension into a 0.3 milliliter syringe with a 29-gauge, half-inch needle [2] and remove air bubbles from the syringe [2].
 - 3.5.1. Talent gently inverting a tube containing the cell suspension.
 - 3.5.2. Talent drawing cell suspension into the syringe.
 - 3.5.3. Talent expelling air bubbles from the syringe.
- 3.6. Using the non-dominant hand, gently stretch the leg and skin to create a smooth, tight surface without lifting it upward [1]. Insert the needle bevel up into the dermis at a shallow parallel angle [2]. Keep the needle visible under the skin to confirm proper placement [3].
 - 3.6.1. Talent stretching the mouse leg and skin to create a taut surface.
 - 3.6.2. Talent inserting the needle bevel up at a shallow angle into the dermis
 - 3.6.3. Shot of the needle visible beneath the skin surface.
- 3.7. Very slowly inject all 30 microliters of cell suspension until a small, visible bubble forms under the skin [1]. Wait 5 to 10 seconds before removing the needle [2].
 - 3.7.1. Talent slowly injecting the cell suspension to form a visible bubble. NOTE: 3.7.1 and 3.7.2 are filmed with 3.6
 - 3.7.2. Talent holding the needle in place briefly before removal.
- 3.8. After completing the injections, discard the used syringe [1].
 - 3.8.1. Talent discarding a used syringe.
- 3.9. Monitor all mice until they have fully recovered from anesthesia [1]. ~~Monitor the mice for tumor establishment [1].~~ NOTE: The VO is edited for the deleted shot
 - 3.9.1. Talent observing mice in a recovery area.
 - 3.9.2. ~~Talent examining mice for visible tumor growth.~~ NOTE: This shot was not filmed

4. Intratumoral Treatment Administration

- 4.1. Prepare all treatment solutions in advance [1]. Keep them on ice or at room temperature as required by the specific treatment [2].
 - 4.1.1. Shot of the treatment solution in tubes. NOTE: 4.1.1 and 4.1.2. are filmed in a

single shot

- 4.1.2. Talent placing prepared solutions on ice.
- 4.2. Place the anesthetized mouse under a nose cone to maintain anesthesia [1]. Position the mouse so the tumor is exposed [2].
 - 4.2.1. Talent positioning the mouse under a nose cone.
 - 4.2.2. Talent adjusting the mouse to expose the tumor site.
- 4.3. Draw the required volume of treatment solution into a 0.3 milliliter insulin syringe with a 29-gauge, half-inch needle [1].
 - 4.3.1. Talent drawing treatment solution into the syringe.
- 4.4. After removing the air bubbles, insert the needle horizontally into the tumor and advance the needle toward the center of the tumor mass [1].
 - 4.4.1. Talent inserting the needle horizontally into the tumor and advancing the needle toward the tumor center.
- 4.5. Now, inject the treatment slowly and steadily into the tumor [1]. Pause for 10 seconds before slowly withdrawing the needle to minimize backflow or leakage [2]. Take care to prevent leakage from the injection site due to internal tumor pressure [3]
 - 4.5.1. Talent slowly injecting the treatment into the tumor. NOTE: This step is filmed with 4.4.
 - 4.5.2. Talent pausing briefly before withdrawing the needle.
 - 4.5.3. Shot of the injection site showing careful needle withdrawal to prevent leakage.
- 4.6. ~~Repeat the intratumoral injection procedure for each mouse [1]. Replace syringes after each cage and when switching treatment groups to prevent cross contamination [2].~~ NOTE: This step was not filmed
 - ~~4.6.1. Talent performing intratumoral injections on multiple mice sequentially.~~
 - ~~4.6.2. Talent replacing the syringe before continuing with another cage or treatment group.~~
- 4.7. Monitor mice after anesthesia until they regain mobility and normal respiration. Continue daily monitoring for treatment response and overall health [1].
 - 4.7.1. Talent observing mice after anesthesia for mobility and normal respiration.

Results

5. Results

- 5.1. Intradermal tumors were described as firm and fixed in the dermal layer [1], whereas subcutaneous tumors move freely within the dermal and hypodermal layers with less distinct borders [2].
 - 5.1.1. LAB MEDIA: Figure 2A, B. *Video editor: Show the tumors on the mice and focus on the areas where the skin looks tight and raised*
 - 5.1.2. LAB MEDIA: Figure 2C. *Video editor: Show the tumor area and emphasize the looser, less defined swelling under the skin*
- 5.2. Post-mortem analysis showed that intradermal tumors were confined within the skin layer [1], whereas subcutaneous tumors extended deeper into both the skin and underlying muscle tissue [2].
 - 5.2.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the tumor area that remains within the thin skin layer after it is pulled back*
 - 5.2.2. LAB MEDIA: Figure 2E,F. *Video editor: Highlight the tumor areas that extend deeper into the exposed tissue beneath the skin*
- 5.3. CT26 (*C-T-Twenty-Six*) and B16F10 (*B-Sixteen-F-Ten*) tumors showed noticeable differences in size approximately 7 days after intradermal injection [1].
 - 5.3.1. LAB MEDIA: Figure 3A and D
- 5.4. Tumors were distributed across groups to achieve similar average volumes for CT26 [1] and B16F10 [2].
 - 5.4.1. LAB MEDIA: Figure 3B.
 - 5.4.2. LAB MEDIA: Figure 3E.
- 5.5. After group balancing, treatments were administered and tumor growth was tracked over time for CT26 [1] and B16F10 [2].
 - 5.5.1. LAB MEDIA: Figure 3C.
 - 5.5.2. LAB MEDIA: Figure 3F.

1. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnesθəˌtaɪzd/
Phonetic Spelling: uh·नेस·thuh·tyzd
2. Intradermal
Pronunciation link: <https://www.merriam-webster.com/dictionary/intradermal>
IPA: /ˌɪntrəˈdɜːməl/
Phonetic Spelling: in·truh·dur·muhl
3. Eppendorf
Pronunciation link: <https://www.howtopronounce.com/eppendorf>
IPA: /ˈɛpənˌdɔːrf/
Phonetic Spelling: ep·uhn·dorf
4. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪkrəˌliːtər/
Phonetic Spelling: my·kruh·lee·tur
5. Insulin
Pronunciation link: <https://www.merriam-webster.com/dictionary/insulin>
IPA: /ˈɪnsəlɪn/
Phonetic Spelling: in·suh·lin
6. Gauge
Pronunciation link: <https://www.merriam-webster.com/dictionary/gauge>
IPA: /ɡeɪdʒ/
Phonetic Spelling: gayj
7. Dermis
Pronunciation link: <https://www.merriam-webster.com/dictionary/dermis>
IPA: /ˈdɜːmɪs/
Phonetic Spelling: dur·miss
8. Bevel
Pronunciation link: <https://www.merriam-webster.com/dictionary/bevel>
IPA: /ˈbevəl/
Phonetic Spelling: bev·uhl
9. Euthanize
Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>
IPA: /ˈjuːθəˌnaɪz/
Phonetic Spelling: yoo·thuh·nyz
10. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /ˌsentrəˈfjuːˈɡeɪʃən/
Phonetic Spelling: sen·truh·fyoo·gay·shuhn
11. Confluence
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /ˈkɒːnfluəns/
Phonetic Spelling: kon·floo·uhns

12. Roswell
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Roswell>
 IPA: /'rɑ:zweɪ/
 Phonetic Spelling: roz·wel
13. Memorial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/memorial>
 IPA: /mə'mɔ:riəl/
 Phonetic Spelling: muh·mor·ee·uhl
14. Institute
 Pronunciation link: <https://www.merriam-webster.com/dictionary/institute>
 IPA: /'ɪnstətu:t/
 Phonetic Spelling: in·stuh·toot
15. RPMI
 Pronunciation link: No confirmed link found
 IPA: /,ɑ:r pi: ɛm 'aɪ/
 Phonetic Spelling: ar·pee·em·eye
16. Intratumoral
 Pronunciation link: <https://www.howtopronounce.com/intratumoral>
 IPA: /,ɪntrətu:'mɔ:rəl/
 Phonetic Spelling: in·truh·too·mor·uhl
17. Subcutaneous
 Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneous>
 IPA: /,sʌbkju:'tɛniəs/
 Phonetic Spelling: sub·kyoo·tay·nee·uhs
18. Hypodermal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/hypodermic>
 IPA: /,haɪpə'dɜ:məl/
 Phonetic Spelling: hy·puh·dur·muhl
19. Post-mortem
 Pronunciation link: <https://www.merriam-webster.com/dictionary/postmortem>
 IPA: /,pəʊst'mɔ:rtəm/
 Phonetic Spelling: pohst·mor·tuhm
20. CT26
 Pronunciation link: No confirmed link found
 IPA: /,si: ti: 'twenti sɪks/
 Phonetic Spelling: see·tee·twen·tee·siks
21. B16F10
 Pronunciation link: No confirmed link found
 IPA: /bi: sɪk'sti:n ɛf tɛn/
 Phonetic Spelling: bee·sik·steen·ef·ten
22. Anesthesia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
 IPA: /,ænəs'θi:zə/, /,ænəs'θi:ziə/
 Phonetic Spelling: an·uhs·thee·zhuh; an·uhs·thee·zee·uh

23. Tumor

Pronunciation link: <https://www.merriam-webster.com/dictionary/tumor>

IPA: /'tu:mər/

Phonetic Spelling: too·mur

24. Intravenous

Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>

IPA: /,ɪntrə'veɪnəs/

Phonetic Spelling: in·truh·vee·nuhs

25. Respiration

Pronunciation link: <https://www.merriam-webster.com/dictionary/respiration>

IPA: /,respə'reɪʃən/

Phonetic Spelling: res·puh·ray·shuhn