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Title: Modelling Brain Metastasis: Standardized Analysis of Metastatic Colonization and Histological Growth Patterns by Stereotactic Intracortical Injection

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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**
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: 09

Number of Shots: 31

Introduction

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

Videographer's NOTE:

I shot the Interviews in 4K ProRes Log and the protocol in 1080 Log and added a conversion LUT for both Locations. I picked 25 fps because 30 fps would lead to light flickering in Europe.

During the Interviews we used a beamer in the Background and I noticed a little fan noise that is why included the enhanced audio files if needed.

I have also included two images of Dr. Raquel Blazquez in the interview and the laboratory setup.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Raquel Blazquez:** Our group investigates how tumor cells colonize the brain and how metastatic growth patterns influence clinical outcomes and treatment responses.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.2. **Raquel Blazquez:** We have demonstrated that brain metastasis growth patterns influence prognosis and neurological death, supporting their relevance for guiding clinical decision-making.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What research gap are you addressing with your protocol?~~

- 1.3. **Raquel Blazquez:** This protocol is particularly useful for studying late-stage metastatic colonization, addressing clinically relevant aspects like growth dynamics and secondary dissemination.

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

~~What advantage does your protocol offer compared to other techniques?~~

- 1.4. **Raquel Blazquez:** Unlike other methods, the stereotactic model ensures brain-specific colonization and preserves full system complexity, enabling long-term studies of tumor progression and therapy response.

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

~~How will your findings advance research in your field?~~

- 1.5. **Raquel Blazquez:** By providing a reproducible clinically relevant platform, our model contributes to understand the mechanisms of brain colonization, and explore new therapeutic strategies.

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 Government of Lower Franconia

Protocol

2. Mouse Scalp Preparation and Incision

Demonstrator: Raquel Blazquez

- 2.1. To begin, weigh each mouse using a precision scale to calculate the required volume of anesthetic based on its body weight [1]. After administering the anesthetic, place the mouse on a pre-warmed heating pad maintained at 37 to 38 degrees Celsius to support thermoregulation [2-TXT], then apply eye ointment to protect the cornea from drying [3].
 - 2.1.1. WIDE: Talent placing a mouse on a precision scale and recording the weight. **Videographer's NOTE:** For 2.1.1. we added an additional Closeup-Shot of the mouse and the scale for better visibility
 - 2.1.2. Talent placing the anesthetized mouse on a pre-warmed heating pad. **TXT: Anesthesia (IP): Ketamine 10% (100 mg/kg body weight) + Medetomidine hydrochloride (0.25 mg/kg body weight)**
 - 2.1.3. Talent applying eye ointment to the mouse.
- 2.2. Thoroughly disinfect the scalp using an iodine-based solution [1-TXT]. Stretch the skin taut [2] and make a midline incision of approximately 3 to 4 millimeters along the skull using a sterile scalpel [3]. Using the same scalpel, gently scrape away the periosteum to expose the skull surface [4].
 - 2.2.1. Talent disinfecting the mouse's scalp with an iodine-based solution. **TXT: Local anesthesia: 1% lidocaine solution (5 mg/mL); Inject ~0.05 - 0.1 mL subcutaneously along the planned incision line**
 - 2.2.2. Talent stretching the skin. **Videographer's NOTE:** Shots 2.2.2. and 2.2.4 were combined because they usually proceed in one step
 - 2.2.3. Talent making a midline incision with a sterile scalpel.
 - 2.2.4. Talent using the same scalpel to scrape away the periosteum and reveal the skull.
- 2.3. Now, secure the mouse's head in a stereotactic frame using ear bars [1]. Using the stereotactic arm, locate Bregma, then move the arm 2 millimeters anterior and 1 millimeter lateral to the right [2] and mark the target site in the frontal cortex with a surgical marker [3]. Carefully drill through the skull at the marked site using a precision dental drill, ensuring not to penetrate the dura mater [4]. Confirm the opening by gently

probing the site with sterile forceps to feel for breakthrough [5].

- 2.3.1. Talent positioning and securing the mouse's head using ear bars in the stereotactic frame. **Videographer's NOTE:** Shot 2.3.1. has an additional close up because the head fixture was covered by the hand in the other shot. **The Author asked not to show any other preparations besides the fixation which aren't part of their usual procedure**
- 2.3.2. Talent using the stereotactic arm to locate Bregma, adjusting to the target coordinates. **Videographer's NOTE:** Shots 2.3.2. and 2.3.3 were combined into one
- 2.3.3. Talent marking the target site.
- 2.3.4. Talent drilling through the skull with a precision dental drill.
- 2.3.5. Talent probing the drilled site gently with sterile forceps.

3. Intracortical Tumor Cell Injection

- 3.1. Next, resuspend the tumor cell suspension by pipetting up and down to ensure even distribution [1]. Load 3 microliters of the suspension into a 10 microliter Hamilton syringe [2] and place it into the stereotactic frame using the appropriate holder [3]. Carefully position the syringe needle over the burr hole, ensuring that the bevel is facing to the left for injection consistency [4].
 - 3.1.1. Talent pipetting the tumor cell suspension repeatedly to resuspend the cells evenly.
 - 3.1.2. Talent loading 3 microliters of cell suspension into a 10 microliter Hamilton syringe.
 - 3.1.3. Talent securing the syringe into the stereotactic holder.
 - 3.1.4. Talent aligning the needle over the burr hole, ensuring the bevel faces to the left.
- 3.2. Insert the needle vertically into the brain tissue to a depth of 3.5 millimeters [1]. Once the required depth is reached, slowly withdraw the needle by 0.5 millimeters to help prevent reflux of the cell suspension [2] and inject 3 microliters of the tumor cell suspension slowly over 1 minute [3]. After injection, keep the needle in place for an additional 2 minutes to allow the cells and extracellular matrix to settle and minimize reflux [4].
 - 3.2.1. Talent inserting the needle vertically into the brain tissue to a depth of 3.5

millimeters.

- 3.2.2. Talent withdrawing the needle slightly by 0.5 millimeters after reaching the depth.
- 3.2.3. Talent injecting the tumor cell suspension slowly using the Hamilton syringe.
- 3.2.4. Shot of the needle in place. **Videographer's NOTE: Shots 3.2.4 and 3.3.1 were combined into one**

3.3. Then, carefully withdraw the syringe [1] and remove the mouse from the stereotactic frame [2].

- 3.3.1. Talent withdrawing the syringe.
- 3.3.2. Talent gently removing the mouse from the stereotactic frame.

4. Suturing and Recovery from Anesthesia

4.1. Now, gently rinse the skull with sterile 0.9 percent sodium chloride solution [1] and seal the burr hole using bone wax [2]. Close the scalp incision using 3 to 4 sutures, tying each suture with three tight knots placed close to the wound edges for optimal healing [3]. Mark the mouse's ears using an appropriate ear punch pattern corresponding to the designated identification number [4].

- 4.1.1. Talent rinsing the skull with sterile 0.9 percent sodium chloride solution.
- 4.1.2. Talent sealing the burr hole with bone wax.
- 4.1.3. Talent placing 3 to 4 sutures to close the scalp incision, tying each with three knots near the wound edges. **Videographer's NOTE: 4_1_3 has an additional wide shot**
- 4.1.4. Talent marking the mouse's ear with an ear punch tool.

4.2. Then, re-apply eye ointment if necessary to prevent corneal drying during recovery [1] and return the mouse to a pre-warmed heating pad maintained at 37 to 38 degrees Celsius to aid recovery from anesthesia [2]. Thoroughly clean and disinfect the Hamilton syringe by rinsing the needle and barrel three times [3] and store the Hamilton syringe on ice until its next use [4].

- 4.2.1. Talent applying eye ointment to the mouse before recovery.
- 4.2.2. Talent placing the mouse on a pre-warmed heating pad for recovery.

4.2.3. Talent rinsing the Hamilton syringe with distilled water. **TXT: Rinse with 3 solutions sequentially: Distilled water; 70% ethanol; Sterile 0.9% NaCl solution**

4.2.4. Talent placing the cleaned Hamilton syringe on ice.

4.3. Finally, administer analgesics subcutaneously while the mouse is still under anesthesia to ensure immediate postoperative pain relief **[1-TXT]**. Monitor mice closely every 24 hours during the first 48 hours post-surgery, evaluating motor function and behavior **[2]**. From day 3 onward, monitor mice at least every 3 days, including body weight measurements **[3]**.

4.3.1. Talent administering analgesics subcutaneously to the anesthetized mouse.

Added shots 4.3.3: Talent placing the mouse back to its cage for recovery.

Videographer's NOTE: This is slated as 4.3.3 because the author wanted to include the final step of placing the mouse back into their container and letting her wake up. There are two alterations of this shot.

Added shot: Shot of the animal waking up. Please check if this footage is available in the shot 4.3.3

Added step

4.4. Evaluate neurological function using the Hanging Wire Test to assess grip strength and coordination **[1-TXT]**. Suspend a mouse on a horizontal wire and record whether it reaches an escape platform within 60 seconds **[2-TXT]**. A non-healthy mouse fails to reach the platform and falls off the wire **[3]**. After euthanising and collecting the animal tissues, evaluate the histological growth pattern of the metastasis and associated features on digital tissue slides **[4]**.

- LAB MEDIA: Hanging-wire_Healthy.MOV 00:00-00:10
- LAB MEDIA: Hanging-wire_Healthy.MOV 00:10-00:16 **TXT: Healthy mouse**
- LAB MEDIA: Hanging-wire_Sick.MOV 00:14-00:20
- **4.4.1, and 4.4.2 Talent looking at the digital slides on the computer.**

Videographer's NOTE: Furthermore we also added 4.4.1 and 4.4.2 of the author showing a brain scan of the infected mouse on a laptop. because of a slight light change I have also included a conversion LUT for this scene

Results

5. Results

- 5.1. Macroscopic images confirmed the absence of tumor growth in ECM-injected control Balb/C (*Balb-C*) mice [1-TXT], while visible metastatic lesions were present in mice injected with tumor cell–ECM suspensions [2].
 - 5.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the image labeled "Control" showing no visible tumors.* **TXT: ECM: Extracellular Matrix**
 - 5.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the image labeled "Met" showing visible metastatic growths pointed by white arrow.*
- 5.2. Mice injected with either 4T1 or 410.4 (*four-ten point four*) breast cancer cells showed a significantly higher metastatic burden [1] compared to ECM-injected controls [2], with no significant difference between the two tumor cell lines [3].
 - 5.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the data points labeled 4T1 and 410.4.*
 - 5.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the data points for CTRL.*
 - 5.2.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the data points labeled 4T1 and 410.4.*
- 5.3. Kaplan–Meier survival analysis demonstrated that mice injected with 410.4 tumor cells had a significantly longer overall survival [1] compared to those injected with 4T1 cells [2].
 - 5.3.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the blue survival curve (410.4) .*
 - 5.3.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the orange line (4T1).*
- 5.4. Histological analysis showed that 4T1 and 410.4 brain metastases exhibited distinct growth patterns, with 4T1 showing cohort-like and 410.4 showing strand-like epithelial infiltrative growth [1].
 - 5.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the left images labeled 4T1*
 - 5.4.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the images labeled 410.4.*

5.5. Improper stereotactic injection led to tumor growth in the skull and meninges due to cell suspension leakage [1], while proper intracortical injection resulted in parenchymal metastasis with subsequent meningeal dissemination [2].

5.5.1. LAB MEDIA: Figure 3A-B.

5.5.2. LAB MEDIA: Figure 3C-D.

1. Anesthetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetic>
IPA: /ˌænəsˈθetɪk/
Phonetic Spelling: an·uhs·THEH·thik
2. Thermoregulation
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermoregulation>
IPA: /ˌθɜːmoʊˌrɛɡjəˈleɪʃən/
Phonetic Spelling: THUR·moh·reg·yuh·LAY·shuhn
3. Cornea
Pronunciation link: <https://www.merriam-webster.com/dictionary/cornea>
IPA: /ˈkɔrniə/
Phonetic Spelling: KOR·nee·uh
4. Periosteum
Pronunciation link: <https://www.merriam-webster.com/dictionary/periosteum>
IPA: /ˌperiˈɑstiəm/
Phonetic Spelling: peh·ree·OS·tee·uhm
5. Stereotactic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotactic>
IPA: /ˌsteriəʊˈtæktɪk/
Phonetic Spelling: STEHR·ee·oh·TAK·tik
6. Bregma
Pronunciation link: <https://www.merriam-webster.com/dictionary/bregma>
IPA: /ˈbrɛgmə/
Phonetic Spelling: BREG·muh
7. Dura mater
Pronunciation link: <https://www.merriam-webster.com/dictionary/dura%20mater>
IPA: /ˈdʊrəˈmeɪtər/
Phonetic Spelling: DUR·uh MAY·ter
8. Medetomidine
Pronunciation link: <https://www.merriam-webster.com/dictionary/medetomidine>
IPA: /ˌmɛdɪˈtoʊməˌdiːn/
Phonetic Spelling: meh·deh·TOH·muh·deen
9. Hydrochloride
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrochloride>

- IPA: /ˌhaɪdrəˈklɔːraɪd/
Phonetic Spelling: HY·druh·KLOR·ide
10. Ketamine
Pronunciation link: <https://www.merriam-webster.com/dictionary/ketamine>
IPA: /ˈkiːtəˌmiːn/
Phonetic Spelling: KEE·tuh·meen
11. Lidocaine
Pronunciation link: <https://www.merriam-webster.com/dictionary/lidocaine>
IPA: /ˈlɪdəˌkeɪn/
Phonetic Spelling: LIH·duh·kayn
12. Intracortical
Pronunciation link: <https://www.merriam-webster.com/dictionary/intracortical>
IPA: /ˌɪntrəˈkɔːrtɪkəl/
Phonetic Spelling: IN·truh·KOR·ti·kuhl
13. Reflux
Pronunciation link: <https://www.merriam-webster.com/dictionary/reflux>
IPA: /ˈriːˌflʌks/
Phonetic Spelling: REE·fluhks
14. Extracellular matrix
Pronunciation link: <https://www.merriam-webster.com/dictionary/extracellular%20matrix>
IPA: /ˌɛkstrəˈsɛljələr ˈmeɪtrɪks/
Phonetic Spelling: EK·struh·SEL·yuh·ler MAY·triks
15. Analgesics
Pronunciation link: <https://www.merriam-webster.com/dictionary/analgesic>
IPA: /ˌænəlˈdʒiːzɪk/
Phonetic Spelling: an·uhl·JEE·zik
16. Subcutaneously
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
IPA: /ˌsʌbkjuːˈtɛniəsli/
Phonetic Spelling: sub·kyoo·TAY·nee·uhs·lee
17. Neurological
Pronunciation link: <https://www.merriam-webster.com/dictionary/neurological>
IPA: /ˌnjʊərəˈləːdʒɪkəl/
Phonetic Spelling: nyur·uh·LOJ·i·kuhl
18. Euthanizing
Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>
IPA: /ˈjuːθəˌnaɪz/
Phonetic Spelling: YOO·thuh·nyze
19. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
IPA: /ˌhɪstəˈləːdʒɪkəl/
Phonetic Spelling: his·tuh·LOJ·i·kuhl
20. Metastasis
Pronunciation link: <https://www.merriam-webster.com/dictionary/metastasis>

IPA: /mə'tæstəsis/

Phonetic Spelling: muh·TAS·tuh·sis

21. Macroscopic

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

IPA: /ˌmækroʊ'skɑːpɪk/

Phonetic Spelling: mak·roh·SKAH·pik

22. Kaplan–Meier

Pronunciation link: No confirmed link found

IPA: /'kæplən 'maɪər/

Phonetic Spelling: KAP·lun MY·er

23. Epithelial

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

IPA: /ˌɛpɪ'thiːliəl/

Phonetic Spelling: eh·puh·THEE·lee·uhl

24. Parenchymal

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

IPA: /pə'reŋkɪməl/

Phonetic Spelling: puh·REN·kih·muhl

25. Meninges

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

IPA: /mə'nɪndʒiːz/

Phonetic Spelling: muh·NIN·jeez

26. Stereotactic frame

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

IPA: /ˌsteriəʊ'tæktɪk/

Phonetic Spelling: STEHR·ee·oh·TAK·tik